INFLAMMATION, SLEEP AND PERINATAL DEPRESSION
INFLAMMATION, SLEEP DISTURBANCES AND THEIR RELATIONSHIP TO DEPRESSIVE SYMPTOMS DURING THE PERINATAL PERIOD

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A thesis submitted to the School of Graduate Studies in partial fulfilment of the requirements for the Degree Doctor of Philosophy

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Descriptive Note

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**TITLE:** Inflammation, Circadian Rhythms and Perinatal Depressive Symptoms

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Lay Abstract

The biology behind depression during pregnancy and the postpartum period is not completely understood. New research in Major Depressive Disorder (MDD) suggests that inflammation and sleep disruptions are important factors that might be responsible for the development of the illness. The unique biology of pregnancy and the early postpartum period might make these factors especially important to the development of depression during this time. The goal of this thesis was to thoroughly examine both inflammation and sleep disruptions from late pregnancy into the early postpartum period and assess how they might be related the development of depressive symptoms.
Abstract

Introduction: The pathophysiology of perinatal depression is not completely understood. Advances in our understanding of Major Depressive Disorder (MDD) suggests that inflammation and sleep disruptions are important pathophysiological factors. The unique biological interactions between immune functioning, sleep disruptions and the Hypothalamic Pituitary Adrenal (HPA) axis may make these factors especially important for the development of depression during the perinatal period. The goal of this thesis was to thoroughly examine both inflammation and sleep disruptions from late pregnancy through the early postpartum period and assess how they might be related the development of depressive symptoms.

Results: Pregnancy IL-6 and IL-10 emerged as significant predictors of postpartum depressive symptoms. None of our other candidate inflammatory markers were associated with depressive symptoms during pregnancy, at 12 weeks postpartum or over time. Significant relationships were observed between mild depressive symptoms and disruptions in daily rhythms during pregnancy and at 3 months postpartum. We observed significant trait level differences in objective measures of sleep in women at higher risk for PPD (i.e those with a history of recurrent MDEs). Significant differences in the relationship between sleep and depressive symptoms across the perinatal period were also observed. For women with a history of recurrent MDEs, depressive symptoms during pregnancy appeared to be a trait rather than state phenomena. Our work is also the first to provided evidence linking objective (duration, efficiency and wake after sleep onset (WASO)) sleep parameters with small, but significant increases in IL-6 from late pregnancy to 12 weeks postpartum.
Conclusion: In sum, our results fail to show a state association between biomarkers of inflammation and perinatal depressive symptoms. Our work indicates that diagnostic history modulates the relationship between sleep and perinatal depressive symptoms. This diagnostic history also modulates the trajectory of these sleep parameters from late pregnancy to early postpartum. Finally, our results provide foundational evidence for an interaction between inflammation and sleep disturbances across the perinatal period.
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List of Abbreviations

3-HK
3-hydroxykynurenine

ACTH
Adrenocorticotropic hormone

BDNF
Brain Derived Neurotropic Factor

BMI
Body Mass Index

BRIAN
Biological Rhythms Interview of Assessment in Neuropsychiatry

CBT
Cognitive behavioural therapy

CES-D
Centre for Epidemiological Studies Depression Scale

CNS
Central Nervous System

CQ
Circadian Quotient

CRH
Corticotropin-releasing hormone

CRP
C-Reactive Protein

CTQ
Childhood Trauma Questionnaire
DLMO
Dim Light Melatonin Onset

DST
Dexamethasone Suppression Test

EEG
Electroencephalography

ELISA
Enzyme-Linked Immunosorbent Assay

EPDS
Edinburgh Perinatal Depression Scale

GVP
Global Validation Procedure

GWAS
Genome-wide Association Study

HPA
Hypothalamic Pituitary Adrenal

IDO
indoleamine 2,3-dioxygenase

IFN-γ
Interferon-γ

IL
Interleukin

IS
Inter-daily Stability

IV
Intra-daily Variability

LPS
Lipopolysaccharide
MAO
  Monoamine Oxidase

MDD
  Major Depressive Disorder

MDE
  Major Depressive Episode

MESOR
  Midline Statistic Of Rhythm

NCS
  National Comorbidity Survey

NCS-R
  National Comoribity Survey Replication

NESARC
  National Epidemiologic Survey on Alcoholism and Related Conditions

NF-κB
  nuclear factor kappa B

NPHS
  Canadian National Population Health Survey

PHQ-9
  Patient Health Questionnaire-9

POMS
  Profile of Mood States

PPD
  Postpartum Depression

PSQI
  Pittsburgh Sleep Quality Inventory

PVN
  paraventricular nucleus
REM
Rapid Eye Movement

SCN
suprachiasmatic nucleus

STAI
State Trait Anxiety Inventory

SWS
Slow Wave Sleep

Th
T-helper

TNF-α
Tumor Necrosis Factor-α

Tregs
T-regulatory cells

VIF
Variance Inflation Factor

WASO
Wake After Sleep Onset

WHO-PPGHC
World Health Organization Pathological Problems in General Health Care

ZDS
Zung Depression Scale
Declaration of Academic Achievement

Chapter 2: All data represented in this chapter was analyzed by W. Simpson. All biochemical assays were conducted by M. Coote. M. Steiner and B.N. Frey contributed to the composition of the text. Samples sent to D. Taylor were donated by M. Peer.

Chapter 3 and 4: All data represented in these chapters was collected and analyzed by W. Simpson. All biochemical assays were conducted by M. Coote. M. Steiner and B.N. Frey contributed to the initial research questions and the composition of the text.

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Chapter 7: All data represented in this chapter was collected and analyzed by W. Simpson. M. Steiner and B.N. Frey contributed to the initial research questions.

In addition to the work summarized in this thesis, collaborations between myself and Dr. B.N. Frey have resulted in two additional publications, both assessing the psychometric properties self-report rating scales during the perinatal period. The first examined the Mood Disorders Questionnaire and was done in collaboration with Lauren Wright. The second assessed the GAD-7 and was done in collaboration with Melanie Glazer and Natalie Michaelski. These works are not included herein, as they are outside the scope of this thesis. Citations for these publications are provided below:

1. Frey, Benicio N., William Simpson, Lauren Wright, and Meir Steiner. Sensitivity and specificity of the Mood Disorder Questionnaire as a screening tool for bipolar

Chapter 1

General Introduction

1.1 Major Depressive Disorder (MDD)

Major Depressive Disorder (MDD) is defined as recurrent episodes of depressed mood or a loss of interest or pleasure in daily activities which last for at least two weeks. These episodes are accompanied by impairments in social or occupational functioning and a cluster of specific symptoms including: weight loss or gain, disruptions in sleep (insomnia or hypersomnia), psychomotor agitation or retardation, fatigue, feelings of worthlessness or guilt, concentration difficulties and suicidality (American Psychiatric Association 2013).

1.1.1 Prevalence and Course

MDD is one of the most common mental disorders, with lifetime prevalence estimates for Major Depressive Episodes (MDE) ranging from 15-17% (Bijl, Ravelli, and Zessen 1998; Kessler et al. 1994; Kessler et al. 2005). While MDD is not a sex specific disorder, it does disproportionately affect more women than men. Lifetime MDE prevalence estimates for men range from 10.9-12.9% compared to 20.1-21.3% for women (Bijl, Ravelli, and Zessen 1998; Kessler et al. 1994), making women 1.7 times more likely to experience an MDE (Kessler et al. 1993; Kessler et al. 2003).

The concept of depression as a medical illness has existed since the time of Hippocrates' description of Melancholia. The diagnostic criteria for MDD as we know it
today was first published in DSM-III in 1980. After its diagnostic criteria was formally established, numerous large scale epidemiological studies were conducted to examine its prevalence and course. Some of the most widely cited include the National Comorbidity Survey (NCS) (Kessler et al. 1994) and its replication NCS-R (Kessler et al. 2005), the National Epidemiologic Survey on Alcoholism and Related Conditions (NESARC) (Hasin et al. 2005), and the World Health Organization Pathological Problems in General Health Care (WHO-PPGHC) study (Ormel et al. 1994). Collectively, these studies examined more than 85,000 individuals, forming the basis for our current epidemiological understanding of MDD.

Together, these studies found that risk for developing MDD is very low during childhood, before rising markedly from 12 to 16 years of age. After age 16, risk continues to increase linearly, thought at a much slower rate, until approximately age 40 when it begins a slow but linear decline (Hasin et al. 2005; Kessler et al. 2003). However, significant sex differences exist in patterns of risk across the lifespan. Compared to men, women experience a significantly increased risk of developing MDEs during the childbearing years (age 18-45, Figure 1.1) (Kessler et al. 1993) and again during the menopausal transition (Cohen et al. 2006).

Regardless of sex, MDD is a chronic and debilitating condition associated with high rates of comorbidity with other mental disorders. In the NESARC, individuals with MDD experienced an average of 4.7 MDEs in their lifetime, each lasting an average of 6 months (24.3 weeks). Comorbidity with other mental disorders was also high with approximately 40% of MDD individuals experiencing an anxiety or alcohol use disorder in their lifetime (Hasin et al. 2005). Comorbidity data from NCS-R was similar; 72.1% of individuals who met criteria for a lifetime MDE also met lifetime criteria for another mental disorder. Lifetime comorbidity rates in this sample were 59.2% for anxiety disorders, 24.0% for substance used disorders and 30.0% for impulse control disorders.
MDD is associated with marked disability and functional impairment. Examination of the WHO-PPGHC data indicate that MDD is associated with significant disability regardless of psychiatric/physical comorbidity or the disability measure used (Ormel et al. 1994). This disability impacts several domains of functioning. Data from NCS-R indicates that depression is responsible for an average of 35.2 days of missed work per person per year. Depressed individuals also have significantly lower quality of life, both during acute episodes and during remission. Social role functioning is also significantly reduced with almost half of all MDD individuals (43.4%) classified as severely or very severely impaired (Kessler et al. 2003).

Beyond psychiatric comorbidity and functional impairment, MDD is highly comorbid with other non-psychiatric illnesses. The incidence of MDD is higher in patients with diabetes, HIV, cancer, cardiovascular disease and stroke and Parkinson’s disease. Data
from the Canadian National Population Health Survey (NPHS) indicates that MDD is twice as prevalent in those with physical illnesses compared to those without (Patten 2001). This relationship between physical illness and MDD could be due to: 1) depression being a risk factor for the physical disease itself, 2) depression being secondary to a psychological reaction, complication or medication associated with the disease or 3) depression and the physical illness sharing some underlying pathophysiology (Katon 2003). While all three of these pathways are likely responsible for the observed association between MDD and physical illnesses, the relative contribution of each may be disease specific. For example, it has been suggested that the relationship between MDD and cardiovascular disease may be largely due to the shared pathophysiology of elevated systemic inflammation (Musselman, Evans, and Nemeroff 1998). Regardless of the mechanism of association, the comorbidity of MDD and physical illness has a significant impact on disease outcome. MDD itself decreases an individual's motivation and their adherence to positive self care behaviours (such as proper diet, abstaining from smoking and proper exercise). It also impacts the cognitive perception of physical symptoms, creating a more negative appraisal of the physical illness, further decreasing motivation and self-care and increasing the functional impairment associated with the illness (Katon 2003).

1.1.2 Pathophysiology

While the prevalence and negative sequelae of MDD have been well characterized, a complete understanding of its pathophysiology remains elusive. Classical views of MDD attribute the illness to a deficiency in central monoamines. However, more recent evidence suggests that MDD is a product of the interaction of several factors including, genetics, stressful life events, immune functioning and circadian rhythms.
The Monoamine Hypothesis

The monoamine hypothesis of depression can be traced back to the early 1950s and more specifically the anti-hypertensive drug reserpine. During clinical trials, a subset of hypertensive patients treated with reserpine developed depressive symptoms that were reversible with the discontinuation of the drug. Later animal studies indicated that reserpine caused a depletion of the monoamine serotonin in the Central Nervous System (CNS) and was associated with sedation and motor retardation (depressive-like behaviours). Around the same time, it was discovered that agents which inhibited Monoamine Oxidase (MAO) were effective in improving mood in depressed patients (Hirschfeld 2000), further supporting the role of monoamines in mood regulation. However, while these findings clearly showed that serotonin, norepinephrine and dopamine were important in mood regulation, there was no direct evidence indicating that depressed patients had pervasive deficiencies in these monoamines. Early reviews of the clinical and pre-clinical data reflected this viewpoint, but noted that while the evidence was inconclusive there was a strong therapeutic benefit to the use of these agents in depressed patients (Bunney and Davis 1965).

While early MAO inhibitors were effective, there were significant issues with tolerability and safety. The improved safety and efficacy of imiprimine, the first tricyclic antidepressant which inhibited the re-uptake of serotonin and norepinephrine, set the stage for all future development of antidepressive agents. Newer generations of antidepressants were designed for increased monamine/receptor specificity and greater tolerability and safety, but were based on the same indirect physiological evidence (Hirschfeld 2000).

Despite these agents being therapeutically effective, conclusive evidence for a monoamine imbalance being the core pathophysiological component of MDD has failed to mate-
rialize. While there is no doubt that monoamines play a role in mood regulation, more recent investigations suggest that the pathophysiology is not the deficiency of the monoamines themselves but in the downstream effects of their neurotransmission. Differences in serotonergic auto-receptor regulation, cyclic AMP second messenger systems, G-proteins and their transcription factors and numerous neurotrophic factors such as Brain Derived Neurotropic Factor (BDNF) have been observed in depressed patients and animal models of depression (Belmaker and Agam 2008; Werner and Coveñas 2010). However, extensive examination of these systems to date has produced few consistent biomarkers of disease.

**Genetic Factors**

Studies of monozygotic and dizygotic twins indicate that the heritability of MDD is approximately 37% (Sullivan, Neale, and Kendler 2000). These studies also indicate that individual, rather than shared environmental, factors play a more prominent role in the development of the illness (Sullivan, Neale, and Kendler 2000; Kendler et al. 2006). As such, it is not surprising that significant heterogeneity exists in the genes and loci implicated in the disease. In a large meta-analysis of 20 polymorphisms from 18 genes, Lopez-Leon and colleagues found five genes (APOE, GNB3, MTHFR, SLC6A3 and SLC6A4) associated with greater susceptibility to MDD. Many of these genes code for proteins in biological pathways implicated in MDD including: dopamine (SLC6A3) and serotonin (SLC6A4) neurotransmission and G-protein subunits (GNB3) (López-León et al. 2008). The significant association between MDD and APOE is notable as the protein is associated with lipoprotein metabolism, cardiovascular disease and immunoregulation as well as cognition and Alzheimer's disease (Mahley, Rall, and Rall Jr. 2000).

Older genetic studies relied on selecting candidate genes and polymorphisms for analysis. Advances in sequencing techniques and statistical methods have created a
surge in Genome-wide Association Studies (GWAS) attempting to confirm these previous findings while also identifying new genetic targets. Unfortunately, results from these large GWAS have been disappointing (Bosker et al. 2011). Despite large sample sizes and meta-analytic strategies, no loci (including previously significant targets) have emerged as robust predictors of MDD (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium 2013; Wray et al. 2012). This lack of concordance may be due to false-positives and publication bias of older genetic studies, a lack of sufficient sample size to overcome high threshold GWAS significance values ($p < 5 \times 10^{-8}$) or the heterogeneity of MDD as a phenotype (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium 2013; Wray et al. 2012; Bosker et al. 2011). Recent studies also suggest that some genes may be associated with psychiatric conditions in general and not simply confined to MDD (Smoller et al. 2013).

The importance of individual environmental factors, coupled with the lack of significant findings in GWAS suggests that genetic susceptibility to MDD may work through a gene-by-environment interaction. As detailed in the next section, there is a strong relationship between stress and the development of MDD. Evidence of a gene-by-environment interaction between stress and MDD was first published by Caspi and colleagues in 2003. In a prospective birth cohort, they found that the short allele of the serotonin transporter gene (5-HTT) modulated the relationship between stressful life events and MDD. Individuals with the short allele had a significantly higher incidence of MDD in relation to stressful life events compared to those with the long allele (Caspi et al. 2003). However, a subsequent meta-analysis has failed to replicate this association in humans (Risch et al. 2009), while another study of rhesus macaques suggests the differential methylation may explain the relationship rather than the genotype itself (Kinnally et al. 2010).
Stress and the Hypothalamic Pituitary Adrenal (HPA) axis

The Hypothalamic Pituitary Adrenal (HPA) axis is responsible for coordinating the physical response to environmental stressors or challenges through the release glucocorticoids (e.g., cortisol) and catecholamines (epinephrine and norepinephrine). Release of Corticotropin-releasing hormone (CRH) and vasopressin from the paraventricular nucleus (PVN) of the hypothalamus stimulates the release of Adrenocorticotropic hormone (ACTH) into circulation through the anterior pituitary. ACTH then stimulates the adrenal glands to secrete glucocorticoids which then feedback to limit the PVN production of CRH and vasopressin, primarily through activity in the hippocampus, amygdala and prefrontal cortex (Lupien et al. 2009).

The initial link between this stress control system and depression came from observations of individuals with Cushing’s syndrome, a disease characterized by an overproduction of glucocorticoids. Rates of depression in these patients were markedly higher than rates seen in the general population (Gillespie and Nemeroff 2005). Early examination of cortisol levels in depressed patients also indicated that these individuals often hypersecreted cortisol relative to healthy controls (Gibbons and McHugh 1962; Sachar et al. 1970). Furthermore, this hypercortisolism appeared to be state dependent, as remission of depressive symptoms was associated with a reduction in cortisol production (Carpenter and Bunney 1971). Studies using the Dexamethasone Suppression Test (DST) also found that dexamethasone often failed to suppress cortisol in depressed patients, suggesting that impairments in glucocorticoid regulation were central to the pathophysiology of depression. It was even suggested that the DST was powerful enough to be used as a diagnostic test (Gillespie and Nemeroff 2005). However, not all DST studies were positive (e.g., Butler and Besser 1968) and subsequent reviews of literature revealed that non-suppression of cortisol was much less specific than initially thought.
Despite this, hypercortisolemia has remained the most consistent biological correlate of MDD (Pariante and Lightman 2008).

This hypercortisolemia has significant effects on both the CNS and regulation of the HPA axis itself. Prolonged cortisol exposure is associated with reduced sensitivity of the negative feedback loop which regulates cortisol production as well as neurodegeneration and structural remodelling in brain areas which both regulate the HPA axis and are responsible for cognition and affect (i.e. hippocampus, amygdala and prefrontal cortex) (McEwen 2008). This is particularly evident for the hippocampus, where cortisol induced neurodegeneration is thought to be responsible for the dose-response relationship observed between reductions in hippocampal volume and the number of lifetime MDEs (McKinnon et al. 2009).

Subsequent examination of this relationship between stress, cortisol and depression revealed that repeated exposure to stressors, particularly early in life was most strongly associated with later development of MDD (Kessler and Magee 1993; Green et al. 2010). This led many researchers to suggest that this association may be mediated through programming effects of early life adversity on HPA axis functioning. Seminal studies by Meaney and colleagues found that rats stressed through repeated maternal separation during the first 14 postnatal days had persistently higher physiological reactions to stressors throughout the lifespan (Plotsky and Meaney 1993; Plotsky et al. 2005; Kalinichev et al. 2002). Studies in humans have also produced similar findings showing that a history of physical or sexual abuse in childhood results in significantly higher ACTH responses to stressors in adulthood (Heim and Nemeroff 2001; Heim et al. 2008). However, while many studies have shown interactions between stress, HPA axis physiology and subsequent MDD or depressive-like behaviours, the relationship is highly complex. Individual outcomes vary considerably and depend on interactions between developmental age, type and duration of the stressor and numerous environmental fac-

**Inflammation**

The investigation of inflammation as a biological correlate of MDD began with the examination of sickness behaviour in rats (Dantzer and Kelley 2007). Heightened levels of pro-inflammatory cytokines were found to reliably induce a cluster of characteristic sickness behaviours. These included: reductions in food/water intake, mobility, social interaction, sexual behaviour and an increase in preference for sleep (Hart 1988; Dantzer 2001).

While MDD cannot be considered a human analogue of animal sickness behaviour, the two are behaviourally similar. MDD is associated with significant changes in appetite, sleep, motivation and social activity, suggesting that heightened inflammation may play a role in the expression of these behaviours. Studies of cancer and hepatitis patients undergoing pro-inflammatory interferon-α therapy supported this hypothesis, as up to 40% of these patients develop depressed mood, fatigue and cognitive impairment which remits following treatment discontinuation (Myint et al. 2009). Clinical evidence for the association between heightened systemic inflammation and MDD was first reported in a series of studies by Maes (Maes et al. 1993; Maes, Meltzer, and Bosmans 1995). Numerous other researchers have since replicated these observations and to date, meta-analytic evidence indicates that there is a positive relationship between MDD and pro-inflammatory cytokine levels (Howren, Lamkin, and Suls 2009; Dowlati et al. 2010).

There are several mechanisms by which elevations in pro-inflammatory cytokines may influence the development of depressive symptoms. Immune challenges cause the release of pro-inflammatory cytokines into systemic circulation. These cytokines interact with the CNS through leaky regions in the blood-brain barrier and through activity
at afferent nerve fibers. Centrally, cytokines upregulate cortisol production through increases in CRH, while also altering metabolism of serotonin, dopamine and BDNF. This increased glucocorticoid activity feeds back to upregulate nuclear factor kappa B (NF-κB) which further enhances CRH production through stimulation of sympathetic nerve fibers by norepinephrine (Raison, Capuron, and Miller 2006). The presence of pro-inflammatory cytokines in the periphery also stimulates the activity of indoleamine 2,3-dioxygenase (IDO), the enzyme responsible for the oxidation of the serotonin precursor tryptophan to kynurenine. Increased activation of this pathway reduces levels of free tryptophan, decreasing its availability for active transport into the CNS and potentially reducing the rate of central serotonin synthesis. Furthermore, kynurenine is easily transported into the CNS and further metabolized to 3-hydroxykynurenine (3-HK) a potent free radical generator which may increase the degree of oxidative damage and lipid peroxidation of CNS tissue (Dantzer et al. 2008).

Preliminary evidence suggests that like hypercortisolemia, elevated inflammation may be a state phenomena. Animal studies suggest that the emergence of depressive-like behaviour overlaps closely with elevations in Interferon-γ (IFN-γ), Tumor Necrosis Factor-α (TNF-α) and IDO (Moreau et al. 2008; O’Connor et al. 2009). Human studies are similar, though controlling for known confounders such as Body Mass Index (BMI) significantly reduces the strength of this association (Stewart and Rand 2009; Duivis and Jonge 2011; Gimeno et al. 2009). However, while there are compelling reasons indicating that inflammation is a pathophysiological process (Dantzer et al. 2011), MDD cannot be considered an inflammatory disorder. Raison and Miller note that the evidence to date does not support MDD as an inflammatory disorder in the sense that autoimmune conditions are inflammatory disorders. Concentrations of pro-inflammatory cytokines in MDD patients are higher than healthy controls, but orders of magnitude lower than other inflammatory diseases. Furthermore, a high degree of systemic inflam-
information does not guarantee that an individual will develop depressive symptoms. This absence of causality suggests that the inflammatory cascade likely represents another pathway to depressive states in a subset of individuals with underlying vulnerabilities (Raison and Miller 2011).

**Sleep and Circadian Rhythms**

Sleep difficulties are one of most common symptoms seen in patients with MDD (Tsuno, Besset, and Ritchie 2005). Questions regarding sleep quality are universally present in all diagnostic criteria and in the majority of clinician and self rated severity measures of depression. Chronic sleep disruptions are also a strong risk factor for the development of MDD (Ford and Kamerow 1989) and Electroencephalography (EEG) studies of depressed patients indicate greater sleep latency, greater Wake After Sleep Onset (WASO), increased levels of non-restorative (Stage 1) sleep, shorter Rapid Eye Movement (REM) latency, and reduced levels of restorative Slow Wave Sleep (SWS) relative to controls (Armitage 2007).

These disruptions in the sleep wake cycle in MDD patients point to disruptions in the underlying circadian rhythms themselves (Turek 2007). Circadian rhythms are 24-hour biological rhythms which entrain homeostatic processes with the rest-activity cycle of the organism. In humans, these rhythms are governed by exposure to light (i.e. light/dark cycle) and are maintained centrally within the suprachiasmatic nucleus (SCN) of the hypothalamus. Rhythmicity is achieved through the activity of transcription factors CLOCK and BMAL1 and the expression of Period (Per1, Per2, Per3) and Cryptochrome (Cry1 and Cry2) timing proteins. The SCN conveys this circadian signal to slave oscillators in other tissues through the actions of hormones (e.g. melatonin) and the autonomic nervous system (Reppert and Weaver 2001).

The importance of these rhythms in the pathophysiology of MDD has become in-
creasingly apparent (McClung 2013). The hormone melatonin is tightly entrained to
the circadian cycle (Moore and Lenn 1972; Lewy et al. 1980) and is responsible for sign-
alling the onset of sleep (Brzezinski 1997). Changes in melatonin are known to induce
phase shifts in the circadian clock, suggesting that melatonin has bidirectional effects
of rhythmicity (Cajochen, Kräuchi, and Wirz-Justice 2003; Reppert et al. 1988). Early
studies of melatonin rhythms in MDD patients found reductions in rhythm strength
(amplitude) relative to controls (Beck-Friis, Rosen, and Kjellman 1984; Beck-Friis et al.
1985), suggesting that circadian rhythms were disrupted, potentially at the trait level
(Claustrat et al. 1984). Further improvements in our molecular understanding of these
rhythms (Reppert and Weaver 2002) revealed alterations in expression of CLOCK, Period
and BMAL1 in patients with a history of MDD relative to controls (Gouin et al. 2010).
Individual polymorphisms in Period genes have also been associated with depressive
symptoms (Kennaway 2010), though studies examining polymorphisms in Clock have
failed to show a consistent association with MDD (Kishi et al. 2011; Calati et al. 2010).
Analysis of postmortem brain tissue has also found weaker cyclic expression of timing
genes and a significant shift in peak gene activity patterns between MDD and healthy
controls (Li et al. 2013).

Circadian rhythms also have direct regulatory control of many of the pathophys-
iological components of MDD. Pre-clinical evidence suggests that the circadian clock
has a direct influence on central monoamines. Hampp and colleagues observed that
the MAO-A promoter is regulated directly by the circadian clock and that mutations in
Per2 were associated with reduced expression of MAO-A and an increase in dopamine
activity (Hampp et al. 2008). The circadian system also directly regulates the HPA
axis. In healthy individuals glucocorticoids follow a diurnal rhythm, reaching their
peak in the morning (around the time of awakening) and achieving their nadir in the
late evening (Kalsbeek et al. 2012; Nader, Chrousos, and Kino 2010). The maintenance
of this rhythm is dependent on the interaction between the central clock and the activity of the adrenal slave oscillator. Sympathetic projections from the SCN stimulate the production Per1 in the adrenal cortex which regulates corticosterone production by gating the adrenal sensitivity to ACTH (Oster et al. 2006; Ishida et al. 2005). Lesion of this sympathetic pathway alters phasic glucocorticoid expression, suggesting that this mechanism is essential for the maintenance of glucocorticoid rhythms (Ulrich-Lai and Herman 2009). The hypercortisolemia often observed in depressed patients is a direct result of differences in circadian glucocorticoid rhythms. Cortisol levels in depressed patients (relative to controls) are generally lower in the morning and higher in the evening, resulting in a flattening of the diurnal rhythm and hypercortisolemia over the duration of the circadian cycle (Young et al. 1994).

Disruption of normal sleep and circadian rhythms are also linked to significant immune disruptions. Many sleep disorders such as narcolepsy, obstructive sleep apnea and insomnia are associated with significant cytokine disruptions (Kapsimalis et al. 2008). Peripheral concentrations of numerous cytokines are known to follow a circadian rhythm (Lange, Dimitrov, and Born 2010) and both partial and complete sleep deprivation result in significant changes in cytokine levels (Born et al. 1997; Irwin et al. 1996; Irwin 2002).

1.1.3 The Role of Sex Differences

As previously noted, women are two times more likely than men to experience an MDE in their lifetime. This sex difference in prevalence may be due to psychosocial and biological differences, particularly in regards to stress (Chrousos, Torpy, and Gold 1998). In general, women have greater levels of neuroticism and are more likely to experience stressful or traumatic events during their lifetime (Kendler, Kuhn, and Prescott 2004).
Biological differences in HPA axis functioning may also contribute (Solomon and Herman 2009). Pre-clinical studies of mice and rats indicate that females consistently show a greater cortisol response to stressors relative to males (Kudielka and Kirschbaum 2005; Fernández-Guasti et al. 2012). This observed sex difference is likely mediated through interactions with gonadal hormones. Classically, estrogens were thought to enhance glucocorticoid production while androgens had the opposite effect (Handa et al. 1994). More recent studies suggest the effect of gonadal hormones on glucocorticoid production are more dynamic and may depend on HPA axis tone. When glucocorticoid levels are low, 17-β estradiol may blunt the stress response, however, when glucocorticoids are high 17-β estradiol may have opposing effects, augmenting the stress response (Walf and Frye 2005).

Significant sex differences in immune functioning may also contribute to the sex difference in MDD prevalence. Compared to men, women exhibit stronger immune responses. This sex difference is thought to explain the higher prevalence of autoimmune diseases in women (Whitacre 2001). This differential immune response is partially mediated by gonadal hormones. During the ovarian cycle, increasing estrogen levels are associated with increases in CD4+ T-cells and T regulatory cell populations. The nature of the immune response also shifts away from a T-helper (Th)-1 and cell-mediated response towards a Th2 and humoural response. Testosterone, in contrast, is largely immunosuppressive, decreasing T-cell proliferation and reducing cytokine production (Fish 2008). This higher lifetime inflammatory exposure may confer an increase risk for MDD. One small study has shown an association between Th1:Th2 ratio and depression (Myint et al. 2005), though to date, no large prospective studies have been conducted.

Subjective and objective sleep quality have also been shown to vary by sex and may contribute to the sex difference in MDD prevalence. Women are more likely to suffer from insomnia during their lifetime (Zhang and Wing 2006) and often report a greater
subjective need for sleep compared to men (Paul, Turek, and Kryger 2008). Studies of morningness-eveningness preference suggest that women show greater preference for the morning hours, while men tend to be shifted toward the evening (Adan and Natale 2002), though the effect size of this difference is small (Randler 2007). Studies of hormone replacement therapy in perimenopausal women suggest that estrogen and progesterone are associated with changes in subjective sleep quality and polysomnographic sleep architecture (Manber and Armitage 1999; Polo-Kantola 2011). Some studies also point to sex differences in the underlying circadian rhythms. Women have been shown to have a slightly shorter circadian period compared to men (Duffy et al. 2011) and circadian variations in melatonin secretion and core body temperature differ between sexes. Women show a higher peak in melatonin and a lower peak in core temperature over a complete cycle (Cain, Dennison, and Zeitzer 2010). Sex hormones may also directly regulate circadian functioning through the activity of estrogen receptors in the retina, intergeniculate leaflet, dorsal raphe nuclei (Mong et al. 2011) and the SCN itself (Kruijver and Swaab 2002).

1.2 Epidemiology of Perinatal Depression

1.2.1 History

The association between child-bearing and depressed or psychotic mental states is as old as the description of Melancholia itself (Demand 1994). While clinicians have observed this association since 400 BCE, scientific examination of this phenomenon did not begin until 1858 when psychiatrist Louis Victor Marce first published a categorization of psychiatric syndromes during pregnancy and the immediate postpartum (Marcé 1858). However, it would be another century before systematic epidemiological studies exam-
ining these syndromes were conducted (Paffenbarger et al. 1961; Paffenbarger 1964; Paffenbarger and McCabe 1966). While these studies helped to elucidate postpartum psychosis as a distinctive clinical syndrome, the classification of MDEs during pregnancy and the immediate postpartum as separate illnesses remained a point of contention (Hopkins, Marcus, and Campbell 1984). The publishing of DSM-IV in 1994 added a "with postpartum onset" specifier to the diagnosis of MDD, identifying the uniqueness of a postpartum MDE, but failing to distinguish pregnancy MDEs from those occurring outside of pregnancy (American Psychiatric Association 1994). With the introduction of DSM-5, the specifier was modified to "with peripartum onset" and broadened to include all mood disorders (Altemus et al. 2012; American Psychiatric Association 2013). While this change is welcome, some researchers suggest that this terminology fails to signify the known differences between mood disturbances occurring prenatally vs. postpartum (Sharma and Mazmanian 2014).

1.2.2 Prevalence

Regardless of onset period (prenatal or postpartum), perinatal MDEs are symptomatically similar to MDEs seen in non-pregnant populations (Wisner, Peindl, and Hanusa 1994). However, assessing the prevalence of these episodes requires the use of perinatal specific screening instruments. Screening tools used to assess depression in the general population often focus on the somatic symptoms of the illness, which can be masked by the normal somatic complaints associated with the perinatal period. Specific instruments such as the Edinburgh Perinatal Depression Scale (EPDS) (Cox, Holden, and Sagovsky 1987) have been designed to address this issue.

To date, three meta-analyses (1 pregnancy, 1 postpartum, 1 perinatal) have been conducted examining the prevalence of depression during the perinatal period. A meta-
analysis of 59 studies conducted by O’Hara and colleagues found an average postpartum depression prevalence of 13% (95%CI: 12.3-13.4) (O’Hara and Swain 1996). Bennett and colleagues examined 21 pregnancy studies and determined the prevalence rates to be 7.4% (95%CI: 2.2-12.6), 12.8% (95%CI: 10.7-14.8) and 12.0% (95%CI: 7.4-16.7) for first, second and third trimester respectively (Bennett et al. 2004). Gavin and colleagues examined 28 prospective studies and assessed point prevalence of depression across the perinatal period. Prevalence estimates were 11% (95%CI: 7.6-15.8), 8.5% (95%CI: 6.6-10.9) and 8.5% (95%CI: 6.5-11.0) for first, second and third trimester respectively. Postpartum depression prevalence was highest at 3 months (12.9% 95%CI: 10.6-15.8) and lowest at 12 months postpartum (6.5% 95%CI: 2.7-12.9) (Gavin et al. 2005).

1.2.3 Risk Factors

Several factors have been associated with risk for developing depression during the perinatal period. In a systematic review of 57 studies, multivariate analyses identified life stress, lack of social support and domestic violence as significant risk factors for the development of prenatal depression (Lancaster et al. 2010). Meta-analysis of risk factors for postpartum depression has produced similar findings. A meta-analysis of 59 studies conducted by O’Hara and colleagues identified a past history of psychopathology, psychological disruptions during pregnancy, poor marital relationships, low social support and life stress as significant contributors to postpartum depression (O’Hara and Swain 1996). Another meta-analysis of 84 studies by Beck identified self-esteem, childcare stress, infant temperament and maternity blues as additional risk factors (Beck 2001). In a follow up meta-analysis excluding studies from previous meta-analyses, Robertson and colleagues found depression or anxiety during pregnancy, stressful life events dur-
ing the perinatal period, low social support and a previous history of depression to be the strongest risk factors for developing postpartum depression (Robertson et al. 2004).

1.2.4 Maternal and Infant Outcomes

Several researchers have assessed how depression during the perinatal period may affect outcomes for both mother and infant. The majority of the literature examining infant outcomes has focused on cognitive functioning and risk for future psychopathology. In a systematic review of the literature, Grace and colleagues noted that depression during pregnancy was associated with cognitive impairments in the offspring. However these impairments appeared to be transient (remitting by 5 years of age) and more likely in male vs. female children (Grace, Evindar, and Stewart 2003). These impairments are thought to be due to suboptimal maternal-infant interactions as depressed mothers are frequently less attentive, less affirmative and more negative in their interactions with their children (Murray et al. 1996). Studies examining the effect of perinatal depression on infant psychological outcomes are limited. One study has shown that exposure to PPD is predictive of adolescent IQ (particularly in males), but not psychopathology (Hay et al. 2008). However, another study has shown that exposure to maternal depression (either prenatally or postnatally) is associated with a 4× increase in risk for psychopathology in adolescence, independent of sex (Pawlby et al. 2009).

While significant heterogeneity prevents researchers from drawing firm conclusions regarding the influence of maternal perinatal depression on infant cognitive/psychological outcomes (Alder et al. 2007), studies of maternal obstetric outcomes are more consistent. Depression during pregnancy has been associated with: preterm labour and delivery, low birth weight, operative deliveries, lower Apgar scores, intrauterine growth restriction, admission to neonatal care units and physical comorbidies such as diabetes.
and pre-eclampsia (Steer et al. 1992; Chung et al. 2001; Li, Liu, and Odouli 2009; Bansil et al. 2010; Berle et al. 2005). To date, two meta-analyses have examined the relationship between prenatal depression and these negative obstetric outcomes. Grote and colleagues examined 29 studies and found preterm birth (RR: 1.39, 95% CI: 1.19-1.61), low birth weight (RR: 1.49, 95% CI: 1.25-1.77) and intrauterine growth restriction (RR: 1.45, 95% CI: 1.05-2.02) to be positively associated with categorical (i.e. depressed vs. non-depressed) depression status. However, when depression was examined continuously, no significant associations were observed (Grote et al. 2010). A more recent meta-analysis of 30 studies conducted by Grigoriadis and colleagues found modest effects of prenatal depression on risk for preterm delivery (OR: 1.37, 95% CI: 1.04-1.81) and decreased likelihood of breastfeeding initiation (OR: 0.68, 95% CI: 0.61-0.76). Depression was not predictive of birth weight, neonatal care admission, Apgar scores or pre-eclampsia (Grigoriadis et al. 2013).

1.3 Pathophysiology of Perinatal Depression

As outlined in the previous sections, perinatal MDEs are often associated with unique risk factors which distinguish them from MDEs outside the perinatal period. Because of this, researchers have hypothesized that unique biological circumstances and the interaction between this biology and psychosocial risk factors may be the main driving force behind the development of perinatal depression (Steiner, Dunn, and Born 2003; Ross et al. 2004).

1.3.1 Estrogens and Progesterone

Classical etiological views of perinatal depression have centred around the importance of estrogens and progesterone. By the third trimester of pregnancy, circulating levels
of these hormones are 10-100× higher than those typically seen during the menstrual cycle. During the first few postpartum days, both undergo a steep decline which is often associated with the emergence of the postpartum blues. This has lead researchers to hypothesize that postpartum mood disturbances may be a consequence of rapid hormonal withdrawal (Bloch, Daly, and Rubinow 2003). However, studies investigating this withdrawal have often failed to observe robust associations. In a study of childbearing vs. non-childbearing women, estradiol was associated with depression in women with a diagnosis of PPD, but neither estradiol nor progesterone levels differed between depressed and non-depressed postpartum women (O’Hara et al. 1991). Harris and colleagues examined the relationship between progesterone and severity of postpartum blues. A small association was observed between pregnancy-to-postpartum change in progesterone and the peak severity of postpartum blues (Harris et al. 1994), though no direct association was observed between progesterone and postpartum depression (Harris 1994). Subsequent reviews of the literature have also failed to produce a consistent association between sex steroids and perinatal mood changes (Bloch, Daly, and Rubinow 2003; Brummelte and Galea 2010; Dennis, Ross, and Herxheimer 2010).

The most direct evidence to date linking estrogen and progesterone to changes in mood was published by Bloch and colleagues. Two groups of euthymic women (half with and half without a history of PPD) were placed into a hypogonadal state before being subjected to supraphysiologic doses of estradiol and progesterone for 8 weeks. Mood was then monitored daily as both steroids were withdrawn under double blind conditions. During withdrawal, mood worsened in 5 of 8 (62.5%) women with a history of PPD while mood remained unchanged in women without a history of PPD. This suggests that some (but not all) women who develop postpartum depression may do so because of a heightened sensitivity to the mood-destabilizing effects of gonadal steroid withdrawal (Bloch et al. 2000). Transdermal estrogen has been used as an effective
treatment for postpartum depression providing some clinical evidence for this hypothesis (Gregoire et al. 1996).

1.3.2 Cortisol and the HPA axis

The HPA axis and its hormones may play an important role in the pathophysiology of perinatal depression. Hypercortisolemia is a natural physiological component of pregnancy as the placenta is a potent producer of CRH (Majzoub and Karalis 1999). This placenta CRH stimulates the hypothalamic production of ACTH, which increases circulating cortisol levels. This up regulation of cortisol persists throughout pregnancy, returning to normal 1-4 days postpartum (Mastorakos and Ilias 2003). This progressive increase in CRH is essential, as the HPA axis is thought to act as a timekeeper signalling the onset of labour (McLean et al. 1995). This additional CRH production does not appear to alter the diurnal rhythm of ACTH or cortisol (Magiakou et al. 1996), however the resulting diurnal cortisol profile is similar to the blunted profile observed in depressed patients (Bloch, Daly, and Rubinow 2003).

As previously mentioned, estrogen may have opposing effects on HPA axis function, depending on the overall tone of the HPA axis (Walf and Frye 2005). This suggests that the naturally heightened levels of cortisol and estrogen present during pregnancy may interact, resulting in higher physiological responses to stressors during this time frame. However, large systematic reviews suggest that pregnancy is associated with an attenuation, rather than a sensitization of the stress response (Kajantie and Phillips 2006; Weerth and Buitelaar 2005). This finding suggests that heightened CRH secretion during pregnancy downregulates HPA axis sensitivity, blunting the stress response and producing a phenotype similar to that of depressed individuals.

While the interactions between gonadal and stress hormones during pregnancy are
not completely understood, the strong association between hypercortisolemia and MDD suggests that pregnancy hypercortisolemia may be relevant to the pathophysiology of perinatal depression (Kammerer, Taylor, and Glover 2006). However, to date, studies examining this hypothesis have been mixed. A longitudinal study by Yim and colleagues found placental CRH concentration at 25 weeks of pregnancy to be a strong predictor of subsequent postpartum depressive symptoms (Yim et al. 2009). One study of 25 women with postpartum depression found lower salivary cortisol levels relative to non-depressed postpartum women \((n=175)\) (Groer and Morgan 2007). Conversely, another study of 57 women found higher cortisol responses to a psychosocial stress test in women with probable depression \((n=16, \text{EPDS} \geq 9)\) vs. control women \((n=41)\) (Nierop et al. 2006). While these studies indicate the the HPA axis may play a role in the development of perinatal depression, larger longitudinal controlled studies are need to further examine this hypothesis.

1.3.3 Inflammation

As previously described, a strong sex difference in immunity is evident between men and women (Whitacre 2001; Fish 2008). This sex difference, coupled with the unique immunological conditions of the perinatal period suggest that inflammation may be an important factor in the development of perinatal depression.

T-helper (Th) cells are important for regulating the immune response. There are two main subtypes of these cells (Th1 and Th2) both of which are associated with different cytokines and different immune responses. Th1 cells are associated with cell-mediated (innate) immune responses and mainly pro-inflammatory cytokines such as IL-1 and IFN-\(\gamma\). In contrast, Th2 cells are associated with humoural (adaptive) immune responses and primarily anti-inflammatory cytokines such as IL-4 and IL-10. These dis-
Tinctions are not absolute as some inflammatory markers are common to both cell types (e.g. IL-3, TNF-α). Furthermore, Th2 responses are not strictly anti-inflammatory as pro-inflammatory IL-6 is produced by Th2 cells. A response to an immune challenge may be predominantly Th1 or Th2, however these responses generally work together to moderate the overall immune response (Mosmann and Sad 1996). This balance is mediated by sex, as men tend to have more Th1 predominant responses, while immune responses in women are predominantly Th2 (Fairweather, Frisancho-Kiss, and Rose 2008).

These differences in immunity have important implications for perinatal depression. Historically, a successful healthy pregnancy was conceptualized as an anti-inflammatory, Th2 phenomenon (Wegmann et al. 1993). More recent studies suggest that this is an oversimplification and rather than being considered immunosuppressive, it is now understood that the relative strengths of the innate vs. adaptive immune responses change depending on the phase of pregnancy (Kraus et al. 2012). However, it has been established that following delivery, the immune response becomes predominantly pro-inflammatory (Østensen et al. 2005), producing a heightened pro-inflammatory state which persists for at least 72 hours and possibly for up to 11 months following delivery (Corwin and Pajer 2008; Shimaoka et al. 2000).

Further evidence for the pathophysiological role of inflammation is derived from the bi-directional interactions between the immune system and HPA axis (Corwin and Pajer 2008). Increases in pro-inflammatory IL-1, IL-2, IL-6 and TNF-α directly upregulate cortisol production (McCann et al. 2000). Cortisol itself is a potent immunosuppressor and directly upregulates NF-κB to neutralize the secretion of pro-inflammatory cytokines (Dantzer et al. 2011). Thus, under normal circumstances the HPA axis and immune system exist at an equilibrium, each balancing the other. However, when the sensitivity of the HPA axis is blunted (e.g. under chronic stress or during pregnancy), there may be accompanying loss of immune regulation and an increase in circulating pro-inflammatory...
cytokines, which may further downregulate HPA axis sensitivity to negative feedback (Pace, Hu, and Miller 2007; Corwin and Pajer 2008).

Together, these findings suggest that depression during the perinatal period may be due in part to HPA axis mediated increase in inflammation (Christian 2014). Anecdotal evidence supports this hypothesis as perinatal depressive symptoms often co-occur with other inflammatory morbidities of pregnancy including: preeclampsia, gestational diabetes and preterm birth (Osborne and Monk 2013). A number of studies have examined this hypothesis directly, though results are mixed.

**Pregnancy**

Studies conducted during pregnancy differ considerably in trimester investigated, depression severity measure and inflammatory markers assessed. A prospective study of 27 women from first to second trimester found an association between depressive symptoms (measured using the Patient Health Questionnaire-9 (PHQ-9)) and IL-6, TNF-α and CRP (Azar and Mercer 2012). In a study of 187 women in their second trimester a positive screening for depression on the Centre for Epidemiological Studies Depression Scale (CES-D), was associated with increased IL-6 and IL-1β (Cassidy-Bushrow et al. 2012). Another study of 60 women in their second trimester found a significant association between CES-D score and IL-6. A marginally significant association was also observed for TNF-α (p=0.06) (Christian et al. 2009). Conversely, a study of 105 women during the second trimester found a significant inverse correlation between depressive symptoms (assessed using the Profile of Mood States (POMS)) and levels of IL-6 ($r=-0.23$) and TNF-α ($r=-0.25$) (Shelton, Schminkey, and Groer 2014). In another study of 200 women at 11-14 weeks gestation, a DSM-IV diagnosis of an MDE during pregnancy was associated with significantly higher IL-6 and TNF-α levels (Haeri, Baker, and Ruano 2013). Using a slightly different approach, one study of 22 pregnant women found
higher CES-D score to be associated with an increased immune response to influenza vaccination, suggesting that depression was associated with heightened immune sensitivity (Christian et al. 2010).

Contrary to these largely positive results, studies using perinatal specific depression measures (EPDS) have failed to observe an association between inflammatory markers and depressive symptoms. A prospective study of 145 women from second to third trimester found no significant association between EPDS score and IL-6 or TNF-α. TNF-α was however, associated with prior childhood trauma (Blackmore et al. 2011). Another study from the same group using a larger sample of the same cohort also failed to provide an association between depressive symptoms and IL-6 and TNF-α (Blackmore et al. 2014).

Postpartum

Studies of inflammatory markers during the postpartum period are also mixed. One study of 200 women using the POMS found significantly lower IFN-γ levels and IFN-γ/IL-10 ratio in depressed women relative to controls (Groer and Morgan 2007). Another study of 28 women found a modest association between urinary IL-6 levels at Day 14 postpartum and depressive symptoms (measured by the CES-D) at day 28 postpartum (Corwin, Johnston, and Pugh 2008). One study found an association between IL-6 (in both maternal serum and cord blood) and depressive symptoms in a sample of women who delivered prematurely using the Positive and Negative Affect Scale. This association was not observed in a comparison sample of full term women (Fransson et al. 2012).

Similar to studies in pregnancy, postpartum studies using the EPDS to quantify depressive symptoms are largely negative. One study of 56 women between 1-6 weeks postpartum found no relationship between serum IL-6 and depressive symptoms (Boufi-
dou et al. 2009). A prospective study of 347 women during the first 12 months post-partum found no association between IL-6 and a screening diagnosis for postpartum depression (EPDS>12) (Skalkidou et al. 2009). One positive study of 139 women at 12 weeks postpartum did observe a significant association between a score of >9 on the EPDS and increased breast milk levels of transforming growth factor beta (Kondo et al. 2011).

### Pregnancy to Postpartum

Prospective studies examining depressive symptoms and inflammatory markers from pregnancy to postpartum have also produced mixed results. Maes and colleagues have published 3 studies examining postpartum blues (day 1 and 3 postpartum) using the same cohort of up to 98 pregnant women and 22 non pregnant controls. In sum, these studies suggest some association between scores on the Zung Depression Scale (ZDS), State Trait Anxiety Inventory (STAI), diagnostic history of MDD and levels IL-6 and IL-1 receptor antagonist. However, these studies compared “reactors” to “non-reactors” which the authors defined by ranking participants based on “q25, median and q75 values from residualized STAI or ZDS scores” from pregnancy to postpartum (Maes et al. 1999, 2000; Maes et al. 2001), limiting the generalizability of these results. In a study of 27 women at risk for developing postpartum depression, Scrandis et al., found a significant association between prenatal CRP levels and prenatal depressive symptoms measured using the Hamilton Depression Rating Scale-Seasonal Affective Disorder. No relationship was observed between CRP and depressive symptoms at 5-6 weeks postpartum (Scrandis et al. 2008).
1.3.4 Sleep and Circadian Rhythms

Sleep and circadian rhythm disruptions are also potentially important pathophysiological components of perinatal depression (Okun et al. 2009b). As discussed in previous sections, circadian rhythms govern many of the physiological components of MDD and poor sleep is known to have a destabilizing effect on these rhythms. Disruptions in normal sleep and daily rhythms are a natural component of the perinatal period where women often experience significant disruptions in their sleep quality, daily rhythms, and day-to-day schedule (Mindell, Cook, and Nikolovski 2015; Leonhard and Randler 2009).

To date, several studies have examined how these disruptions are associated with perinatal depressive symptoms. These disruptions can be assessed subjectively, objectively or using both methods. Subjective sleep is assessed by quantifying the individual’s perception of their sleep. This is often done using a validated sleep quality scale such as the Pittsburgh Sleep Quality Inventory (PSQI) or through self-reported sleep diaries. Objective measures of sleep do not rely on an individual’s self-report. These measure can be obtained using polysomnography, or wrist worn actigraphy. The distinction between these two types of assessments is important as an individual’s subjective and objective measurements of sleep quality rarely correlate (Ravesteyn et al. 2014; Buysse et al. 2008)

Subjective Sleep

Previous studies examining perinatal sleep quality suggest that poor sleep quality is strongly correlated with the severity of depressive symptoms (reviewed in: Ross, Murray, and Steiner 2005; Bei, Coo, and Trinder 2015; Lawson et al. 2015). When assessed categorically (i.e. poor sleep yes/no and depression yes/no) women screening posi-
 Effective for depression are significantly more likely to have poorer sleep during pregnancy (Goyal, Gay, and Lee 2007; Jomeen and Martin 2007; Mellor, Chua, and Boyce 2014) and the postpartum period (Huang, Carter, and Guo 2004; Dørheim et al. 2009). Studies assessing sleep quality and depression as continuous measurements support this relationship (Skouteris et al. 2008), though some have found no relationship (Kamysheva et al. 2010) and others have found that the association can be accounted for by the co-occurrence of anxiety symptoms (Swanson et al. 2011).

**Objective Sleep**

When sleep is assessed objectively, the relationship between sleep disruptions and perinatal depressive symptoms is less clear. In a prospective study of 160 women from 10 to 20 weeks gestation, poor sleep quality (based on self-reported sleep diaries) was associated with a significantly greater depressive symptom severity. However, when sleep was assessed using actigraphy, no relationship was observed (Okun et al. 2013b). A study of 112 women at 12 weeks postpartum found that, after controlling for sociodemographic factors, prenatal depressive symptoms and infant temperament, subjective sleep quality and time awake between 12AM and 6AM were significant predictors of postpartum depressive symptoms (Goyal, Gay, and Lee 2009). In a study of 46 women between 6-26 weeks postpartum, those with PPD had greater Wake After Sleep Onset (WASO) and poorer sleep efficiency than those without PPD. Longer sleep latency, greater WASO and poorer sleep efficiency were also predictive of PPD severity (Posmontier 2008).

**Longitudinal Sleep Studies from Pregnancy to Postpartum**

Studies examining sleep quality and depressive symptoms longitudinally from pregnancy to postpartum have produced mixed results. A recent population-based study of 2088 women found pregnancy insomnia severity was predicative of postpartum de-
pressive symptoms, however this relationship was no longer significant after accounting for pregnancy EPDS score (Dørheim, Bjorvatn, and Eberhard-Gran 2014). One study of 51 euthymic women with a history of PPD found that poorer subjective sleep quality during the third trimester was associated with later recurrence (i.e. >4 weeks postpartum) of PPD (Okun et al. 2009a). In a follow up study, the same group examined 56 women with a history of MDD/PPD across the first 17 weeks postpartum and found that a single point increase (i.e. poorer sleep) on the PSQI was associated with a 25% increase in risk of PPD recurrence (Okun et al. 2011a). Another study of 44 pregnant women found that subjective but not objective measurements of sleep were associated with greater depressive symptoms during the third trimester. When examined longitudinally, third trimester subjective sleep parameters were significantly better than their objective counterparts at predicting depressive symptoms in the 1st week postpartum (Bei et al. 2010). Another study of 25 women from the third trimester of pregnancy to 14 weeks postpartum found that subjective sleep disruptions were highly and consistently correlated with depressive symptoms. Objectively measured sleep efficiency and fragmentation were also significant predictors of depressive symptom severity. However, the predictive power of these objective parameters was attenuated when subjective assessments were including in the model (Park and Friston 2013). A study of 29 healthy women in their third trimester of pregnancy found that poor subjective sleep was predictive of depressive symptom severity at 10-15 days and 10-12 weeks postpartum, but not during the third trimester. Objective sleep measurements did not predict depressive symptoms at either time point (Coo, Milgrom, and Trinder 2014). Lastly, a study of 33 healthy women within our own lab found that changes in objective sleep efficiency but not subjective sleep quality were predictive of increases in EPDS score from late pregnancy to early postpartum. This association appeared to be independent of lifetime psychiatric history (Krawczak et al., personal communication).
Circadian Rhythms

It is important to note that these sleep quality studies, whether objective or subjective, provide limited insight into the underlying circadian rhythmicity. Sleep is governed by the interaction between homeostatic and circadian processes (Borbély and Achermann 1999) and is only a reliable marker of circadian functioning when assessed using longitudinal polysomnography, cosinor based analysis of actigraphy (Cornelissen 2014) or hormonal sampling (eg. 6-sulphatoxymelatonin, Dim Light Melatonin Onset (DLMO)) (Lockley, Skene, and Arendt 1999).

Previous research using these methods to assess the relationship between circadian rhythms and perinatal depression is limited. In a small study comparing healthy (n=15) and depressed pregnant women (n=10), those with depression showed blunted melatonin secretion between 2:00am and 11:00am. However, in a sample of depressed postpartum women (n=13) the opposite was observed. Depressed women had higher melatonin secretion from 6:00am to 10:00 relative to non-depressed postpartum controls (n=11) (Parry et al. 2008). This finding during pregnancy was replicated in another study from the same group comparing depressed (n=11) and healthy (n=22) pregnant women (Posadas et al. 2012). In another study of 101 pregnant women, a diagnosis of depression was associated with significantly lower waking cortisol levels and a more modest decline in cortisol over the first 12hrs of wake time (O’Connor et al. 2014). A small study of 12 women found that advances in circadian phase (DLMO) from the third trimester of pregnancy to 6 weeks postpartum were associated with a greater severity of postpartum depressive symptoms (Sharkey, Pearlstein, and Carskadon 2013).
Perinatal Sleep and Inflammation

Given the strong link between sleep deprivation and inflammation (Born et al. 1997; Irwin et al. 1996; Irwin 2002), it is possible that poor sleep during pregnancy may be driving increases in cytokine secretion. As discussed in the previous section, this heightened inflammation may increase the likelihood of developing depressive symptoms.

To date, three studies (all conducted by Okun and colleagues during pregnancy) have examined how sleep disruptions may affect circulating cytokine levels. In a study of 35 pregnant women, greater disruptions on the PSQI, were correlated with higher levels of TNF-α during the first trimester. Longer sleep latency was also correlated with lower IL-4 during the second trimester. No significant relationships were observed between any sleep parameters and IL-6, IL-10 or CRP (Okun and Coussons-Read 2007). In another study using a subset of these participants (n=19), PSQI scores during the third trimester were associated with significantly higher circulating IL-6 and with Lipopolysaccharide (LPS) stimulated IL-6. Lower sleep efficiency and duration was also associated with increased LPS-stimulated production of IL-6 in second and third trimester (Okun, Hall, and Coussons-Read 2007). The final study by Okun and colleagues examined whether pregnancy sleep disruptions were associated with inflammatory cytokines and risk for adverse pregnancy outcomes. The authors also evaluated whether depression augmented the sleep-cytokine relationship (n=32 depressed, n=136 controls). Depressed women with short sleep duration (<7 hours) had greater increases in IL-8. Poor sleep efficiency (<85%) was also associated with increased IL-6 and daytime napping was associated with increased TNF-α. Depressed women with insomnia symptoms and shorter sleep duration had modestly smaller infants while greater IFN-γ was significantly associated with preterm birth (Okun et al. 2013a).
1.4 Summary and Rationale

The data presented above describes how disruptions in multiple homeostatic mechanisms increases risk for developing psychopathology. This interaction is best conceptualized by McEwen’s allostatic model wherein repeated exposure to stressors (both psychological and physical) places a load against normal homeostatic processes, pushing the system towards a state of allostasis or disorder and resulting in negative psychological outcomes (McEwen 1998b). HPA axis dysfunction, elevated inflammation and poor sleep can all be viewed as causes of this allostatic load (McEwen 1998a; McEwen 2006).

Whatever the underlying cause, MDD is a heterogeneous disorder affecting a significant proportion of the population, a disproportionate number of which are women. For women, risk of developing depression is particularly high during the childbearing years, suggesting that the unique biological and psychosocial circumstances of the perinatal period may predispose vulnerable individuals to develop depression. Perinatal depression is an important area of research, as it is associated with significant impairment and negative outcomes for both mother and child.

Depression during the perinatal period shares its underlying pathophysiology with non-perinatal depression, though the contribution of individual biological systems differs. In particular, immune functioning, sleep disruptions and circadian rhythms are emerging as pathophysiologically important systems. The physiological state of the HPA axis during pregnancy may increase the risk of immune system dysregulation, resulting in an imbalance in the cytokine milieu. Elevations in pro-inflammatory cytokines have been identified as important contributors to depressive symptomatology, suggesting that immune dysregulation during pregnancy may contribute to the emergence of perinatal depressive symptoms. Similarly, sleep and activity disruptions which are com-
monplace during the perinatal period are known to destabilize underlying circadian rhythms. These rhythms have become an increasingly important area of research as they regulate many of the biological systems implicated in the development of depression.

To date, studies examining immune activity and sleep disruptions during the perinatal period provide preliminary evidence for the importance of these systems in the development of perinatal depressive symptoms. However, results from these studies are mixed, providing little consensus for establishing the role of each of these systems in the pathophysiology of perinatal depression. Furthermore, these two domains do not exist in isolation, but interact with each other. To date, no study has assessed how the interaction between inflammation and sleep disturbances may contribute to the development of the depressive symptoms across late pregnancy into the early postpartum period.

1.5 Objectives

The objectives of this body of work are as follows:

1. To examine the relationship between biomarkers of inflammation and perinatal depression symptoms.

2. To examine the relationship between disruptions in sleep (both subjective and objective) and perinatal depressive symptoms.

3. To examine the interaction between inflammation and perinatal sleep disruptions and determine how this interaction may affect perinatal depressive symptoms.
1.6 Hypotheses

Based on the literature reviewed, the a priori hypotheses for each objective are as follows:

1. There will be a positive relationship between the severity of perinatal depressive symptoms and concentrations of IL-6 and TNF-α.

2. There will be a positive association between the severity of perinatal depressive symptoms and subjectively assessed sleep disruptions. In contrast, there will no relationship between objective sleep parameters and depressive symptoms.

3. There will be a positive association between sleep disruptions and concentrations of IL-6 and TNF-α. This increase in inflammation will be associated with greater severity of perinatal depressive symptoms.
Chapter 2

Quantifying Cytokines in Human Serum

Prior to examining inflammation and its relationship to perinatal depressive symptoms, we wanted to determine the best technique to measure our cytokines of interest. We knew of the traditional Enzyme-Linked Immunosorbent Assay (ELISA), but newer MULTIPLEX technologies were available. These technologies appeared to have an advantage over ELISA by offering a lower limit of detection while using lower sample volumes. However, we did not have previous experience using these assays. Therefore, we collaborated with Dr. Doris Taylor of the Texas Heart Institute and examined a broad spectrum of cytokines from third trimester pregnant women using a Bioplex MULTIPLEX panel. When we examined the data, the values for our cytokines of interest were far from concentrations previously reported within the literature. We hypothesized that the MULTIPLEX assay may not be appropriate to quantify our cytokines of interest and therefore compared MULTIPLEX immunoassays with the gold-standard ELISA in our own lab. Due to its exploratory nature, the contents of this chapter have not been published. This work was presented as a poster at 2013 Society for Biological Psychiatry Annual Meeting in New York, NY.
2.1 Abstract

**Introduction:** Heightened levels of pro-inflammatory cytokines have emerged as a key pathophysiological component of numerous diseases. The techniques used to measure these cytokines fall into two main categories: gold standard ELISAs or newer MULTIPLEX. Previous studies indicate good agreement between techniques, but results from physiologically representative samples are less consistent. The objective of our study was to compare the accuracy of MULTIPLEX assays to that of ELISAs for detecting IL-6, IL-10 and TNF-α in human serum.

**Method:** IL-6, IL-10 and TNF-α were assessed in serum of 31 individuals using two types of MULTIPLEX assay (non-magnetic and magnetic). Interassay variability was assessed through geometric means, correlations (Pearson and Spearman) and Bland-Altman plots.

**Results:** Despite good assay performance, we observed poor agreement between ELISA and MULTIPLEX assays when assessing the concentration of TNF-α, IL-10 and IL-6 in human serum. Overall, large differences between techniques were evident in both the magnitude of the observed concentration and its relative rank. Bland-Altman plots further confirmed the poor agreement between techniques.

**Conclusion:** Our results support others in the literature and indicate that MULTIPLEX may not be suitable for quantifying biomarkers present in low concentrations in human serum. Future biomarker studies should strongly consider these results and select the appropriate method for each candidate biomarker.
2.2 Introduction

Heighted systemic inflammation has emerged as a key pathophysiological component of numerous diseases. Research over the past two decades suggests that inflammation may also be central to the pathophysiology of major mental illnesses. Much of this research has focused on the inflammatory basis of Major Depressive Disorder (MDD) (Dantzer et al. 2008). Beginning with the work of Maes and colleagues (Maes et al. 1993; Maes, Meltzer, and Bosmans 1995), subsequent studies and meta-analyses have confirmed that some individuals with MDD have significantly higher levels of pro-inflammatory biomarkers (Dowlati et al. 2010; Howren, Lamkin, and Suls 2009; Miller, Maletic, and Raison 2009). While it is unlikely that this association is applicable to every individual with MDD (Raison and Miller 2011), it may be particularly relevant for specific sub-populations, such as pregnant and postpartum women (Osborne and Monk 2013).

Currently, the diagnosis of MDD is solely based on categorization of subjective symptoms through clinical interviews. However, evidence linking inflammation and MDD has created a surge in studies attempting to find biomarkers to objectively distinguish disorder from non-disorder. To date, these studies have focused mainly on blood-borne proteins as candidate biomarkers. This approach requires that the techniques used to quantify blood proteins be accurate, reproducible and cost effective (Vasan 2006). For these clinical studies, protein quantification in serum or plasma is done mainly through commercially available immunoassays. These assays generally fall into two categories: gold standard enzyme-linked immunosorbent assays (ELISA) or newer MULTIPLEX.

The traditional sandwich ELISA involves coating a microwell plate with the antibody of interest. When a sample is added this antibody physically captures the antigen, binding it to the plate. An antibody-enzyme complex is then added and serves to amplify the signal and induce a quantifiable change in a colourmetric substrate (Richens et al.
MULTIPLEX follows a similar principle though the quantification method differs significantly. In MULTIPLEX assays, fluorescently labeled, antibody coated microbeads are introduced into the sample, where they bind to the antigen of interest. Steptavidin, a bacterial protein with strong affinity and specificity for biotin, is then added to link a chromogen to the bound antigen of interest. Samples are then quantified through fluorogenic emissions detected using a modified flow cytometer (Leng et al. 2008).

Both techniques have their respective benefits and caveats however several factors have made MULTIPLEX technologies increasingly attractive for use in biomarker research. In contrast to ELISA, MULTIPLEX allows for the simultaneous quantification of several different proteins, increasing per sample efficiency and reducing the total amount of sample needed. Furthermore, MULTIPLEX assays tend to have a greater dynamic range and lower limit of detection relative to ELISA. Together these properties allow researchers to examine a greater number of candidate proteins, at a greater dynamic range of concentrations, at a lower cost per analyte (Leng et al. 2008; Elshal and McCoy 2006).

Some studies validating MULTIPLEX against ELISA report good to excellent concordance between techniques. However, these validation studies have primarily used purified cultured cell supernatants (Carson and Vignali 1999; Dupont et al. 2005), whole blood stimulated with endotoxin (Khan et al. 2004; Jager et al. 2005), enriched samples (Skogstrand et al. 2005) or synthetic samples of known concentration (Ray et al. 2005). On the other hand, studies examining more physiologically representative human samples are much more variable. A study by Kellar and colleagues observed excellent agreement between techniques in an 8-plex panel of cytokines in human serum (IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ, TNF-α) (n=28, r=0.86). However, this result was obtained by assaying both non-enriched and enriched human serum together on the same plate (Kellar et al. 2001). Liu and colleagues observed that MULTIPLEX
assessment of leptin, insulin and MCP-1 in obese individuals was also well correlated with ELISA ($r > 0.7$). Yet at the same time, correlations between IL-6, TNF-$\alpha$ and IL-8 were much poorer, ranging from -0.107 to 0.318 (Liu et al. 2005). Similarly, in a sample of healthy individuals Dossus and colleagues found a high inter assay correlation for CRP, IL-1ra and sCD40L ($r \geq 0.85$), but poor correlation for IL-6, IL-1$\beta$ and TNF-$\alpha$ ($r \leq 0.41$)(Dossus et al. 2009). A study of patients receiving hemodialysis treatment obtained similar findings, with good inter assay agreement for CRP ($r=0.62$) but poor agreement for IL-6 ($r=0.01$)(Ribeiro et al. 2012). Examination of IL-6, TNF-$\alpha$ IL-1$\beta$ and IFN-$\gamma$ in patients with both high (>5mg/L) and low (<2mg/L) CRP also suggests a lack of concordance between MULTIPLEX and ELISA. Intra assay variability, measured by the Coefficient of Variation (CV), was significantly higher for MULTIPLEX assays. Furthermore, compared to cytokine concentrations obtained by ELISA, those obtained through MULTIPLEX were markedly higher for IL-1$\beta$ IFN-$\gamma$ and TNF-$\alpha$ (25%, 181%, and 63% respectively) and significantly lower (40%) for IL-6 (Koning et al. 2012). Together these results indicate that MULTIPLEX assays are likely comparable in precision to ELISA for molecules present in higher concentrations (i.e ng/ml or greater), but may be significantly less accurate when examining molecules present in smaller concentrations (i.e. pg/ml) (Dossus et al. 2009; Wong et al. 2008).

These results from studies of physiologically representative human samples are notable, as many of the candidate biomarkers in psychiatric research including cytokines are present at relatively low physiological concentrations (Dowlati et al. 2010). Despite the potential advantages of the MULTIPLEX system (low costs, multiple analytes, low sample volume etc.), the above-mentioned literature suggests that this technique may not be sensitive enough to accurately quantify certain candidate biomarkers. The objective of our study was to assess whether MULTIPLEX immunoassays were comparable to gold-standard ELISA for quantifying serum concentrations of IL-6, IL-10 and TNF-$\alpha$
in a psychiatric population of women enrolled in a perinatal depression study.

2.3 Method

2.3.1 Participants

Venous blood was collected from thirty-one (31) physically healthy pregnant women. All participants were between 21 and 39 years of age and free of longstanding medical conditions, acute infections, or current treatment with prescription medication. All participants provided informed consent and this study was approved by the Hamilton Integrated Research Ethics Board.

2.3.2 Sampling Procedures

Blood was sampled from each participant between 10AM and 2:30PM. All samples were collected between April 2011 and August 2012. For each participant, blood was drawn into a single 10ml serum separator tube and left to clot at room temperature for 30 minutes. Samples were centrifuged at 20°C for 15 minutes at 3000 rpm. Serum was removed, aliquoted and immediately frozen at -80°C until analysis.

2.3.3 ELISA Kits

Commercial ELISA kits for IL-6, IL-10 and TNF-α were purchased from Ray Biotech Inc. (Norcross, GA, USA). On the day of assay, single aliquots of serum were thawed. To minimize the effects of multiple freeze/thaw cycles, fresh sample aliquots were used when available. All samples were assayed in duplicate. All analyses were performed according to the manufactures' protocols by the same experienced technician (M.C.) at St. Joseph's Healthcare Research Laboratory in Hamilton, ON, Canada.
2.3.4 MULTIPLEX Assays

Two types of commercial MULTIPLEX kits were used (non-magnetic and magnetic). These assays differed by both the wash method and size of the fluorescent microbeads. For non-magnetic MULTIPLEX assays, the plate was washed under vacuum by drawing the wash buffer through a filter below the plate. Under this vacuum filtration it is possible for the fluorescent microbeads to aggregate and become lodged within the filter plate, disrupting the quality of the cytometric analysis. In contrast, magnetic MULTIPLEX assays use larger magnetically charged fluorescent microbeads. This increases the surface area available for binding to the antigen and decreases the likelihood that beads would aggregate or be lost during filtration. For magnetic assays the plate is washed by placing a complementary magnetic plate underneath the assay; capturing the beads in a strong magnetic field. The plate is then inverted (rather than vacuumed) to remove wash buffer. Non-magnetic and magnetic MULTIPLEX assays were purchased from BioRad (Hercules, CA, USA) and Millipore (Billerica, MA, USA). Non-magnetic MULTIPLEX assays were 7-plex (IL1β, IL-4, IL-5, IL-6, IL-10, TNF-α, IFN-γ) while magnetic MULTIPLEX assays were 3-plex (IL-6, IL-10, TNF-α). All samples were assayed in duplicate. All analyses were performed according to the manufacturers' protocol by the same experienced research lab technician as the ELISAs. Prepared assay plates were read using the Bio-Plex 200 system. Prior to reading each plate, the system was calibrated using calibration microbeads and sheath fluid purchased from BioRad Inc. (Hercules, CA, USA).

2.3.5 Statistical Analysis

To remain conservative, concentration values falling below the limit of detection for each assay were considered missing values. Values >3 standard deviations from the
mean were considered outliers. One individual was excluded, resulting in a final sample size of 30. To examine the differences in the magnitude between cytokine concentrations obtained using ELISA and those obtained using MULTIPLEX, we computed the geometric mean. Interassay agreement was assessed using both Pearson (magnitude) and Spearman (rank/order) correlations. For Pearson correlations, observed concentrations were first standardized using a log transformation.

The use of correlation coefficients alone can distort the true relationship between techniques (Bland and Altman 2003). To account for this possibility we generated Bland-Altman plots (Martin Bland and Altman 1986) comparing ELISA and MULTIPLEX for all assays where values did not fall below the limit of detection or where there was a high level of correlation (r>0.7) between techniques. Bland-Altman plots were generated using non-transformed data. All analyses were conducted using the statistical package R (Version 3.0.2) (Vienna, Austria, http://www.R-project.org/)

2.4 Results

2.4.1 Assay Performance

All assays performed according to the manufacturer’s specifications. The correlation of residuals (R²) exceeded 0.9 for all ELISAs. For MULTIPLEX assays, all observed standard curve measurements were within the acceptable range of expected measurement (80-120%) as indicated by the manufacturer. Properties of each assay are shown in Table 2.1. Compared to ELISA, Multiplex assays had a wider range of detection and a lower limit of detection (LOD). Despite this, when compared to ELISA, the proportion of values falling below the LOD (N not detected) was higher for the majority of MULTIPLEX assays. The intra-assay coefficient of variation (%CV) differed considerably between
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assays, ranging for 2.3% for ELISA IL-10 to 62.5% for magnetic BioRad TNF-α.

Table 2.1: Individual assay properties for ELISA and MULTIPLEX assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Analyte</th>
<th>Min [ ]</th>
<th>Max [ ]</th>
<th>LOD</th>
<th>n ND</th>
<th>% ND</th>
<th>%CV</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td>IL-6</td>
<td>1.37</td>
<td>1000</td>
<td>3.0</td>
<td>0</td>
<td>0</td>
<td>3.5</td>
<td>09-13-13</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td>2.34</td>
<td>150</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>2.3</td>
<td>10-02-13</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>24.58</td>
<td>6000</td>
<td>30.0</td>
<td>7</td>
<td>22.6</td>
<td>32.5</td>
<td>09-24-13</td>
</tr>
<tr>
<td>Non-Magnetic BioRad</td>
<td>IL-6</td>
<td>5.64</td>
<td>22285.2</td>
<td>2.6</td>
<td>0</td>
<td>0</td>
<td>11.3</td>
<td>08-08-12</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td>1.62</td>
<td>1630.0</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>15.4</td>
<td>08-08-12</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>6.76</td>
<td>114200.1</td>
<td>0.07</td>
<td>0</td>
<td>0</td>
<td>11.3</td>
<td>08-08-12</td>
</tr>
<tr>
<td>Non-Magnetic Millipore</td>
<td>IL-6</td>
<td>3.19</td>
<td>10783.5</td>
<td>0.3</td>
<td>17</td>
<td>54.8</td>
<td>2.4</td>
<td>09-25-12</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td>3.02</td>
<td>9998.3</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>29.5</td>
<td>09-25-12</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>3.21</td>
<td>9995.4</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
<td>7.1</td>
<td>09-25-12</td>
</tr>
<tr>
<td>Magnetic BioRad</td>
<td>IL-6</td>
<td>1.67</td>
<td>6324.5</td>
<td>2.6</td>
<td>23</td>
<td>74.2</td>
<td>4.3</td>
<td>11-23-13</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td>2.12</td>
<td>8520.5</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>11.1</td>
<td>11-23-13</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>5.52</td>
<td>20963.8</td>
<td>0.07</td>
<td>13</td>
<td>41.9</td>
<td>62.5</td>
<td>11-23-13</td>
</tr>
<tr>
<td>Magnetic Millipore</td>
<td>IL-6</td>
<td>2.84</td>
<td>10141.0</td>
<td>0.2</td>
<td>31</td>
<td>100</td>
<td>49.5</td>
<td>10-23-13</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td>3.06</td>
<td>10097.2</td>
<td>0.48</td>
<td>24</td>
<td>77.4</td>
<td>37.5</td>
<td>10-23-13</td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>3.05</td>
<td>10146.9</td>
<td>0.07</td>
<td>3</td>
<td>9.7</td>
<td>15.1</td>
<td>10-23-13</td>
</tr>
</tbody>
</table>

2.4.2 Inter-assay Agreement

Geometric mean concentrations of each analyte are reported in Table 2.2. Observed concentrations varied considerably depending on technique and analyte. Compared to ELISA, IL-6, values obtained using BioRad assays were much higher, while values obtained using Millipore assays we much lower. For IL-10, values obtained using MULTIPLEX were consistently lower across all assays. For TNF-α, observed cytokine values were higher for non-magnetic BioRad MULTIPLEX but considerably lower for both Millipore and magnetic BioRad assays.

Strong Pearson correlations were observed between ELISA and non-magnetic Millipore MULTIPLEX for IL-6 (r=0.72). However, weak correlations between ELISA and MULTIPLEX were observed for all remaining analytes (all r<0.31, Table 2.3). Notably, IL-6 values obtained using the magnetic BioRad Multiplex were strongly anti-correlated
Table 2.2: Geometric mean of observed cytokine concentrations for each assay

<table>
<thead>
<tr>
<th>Analyte</th>
<th>ELISA</th>
<th>BioRad¹</th>
<th>Millipore¹</th>
<th>BioRad²</th>
<th>Millipore²</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>1.88</td>
<td>10.40</td>
<td>0.22</td>
<td>15.36</td>
<td>—</td>
</tr>
<tr>
<td>IL-10</td>
<td>11.44</td>
<td>7.29</td>
<td>2.23</td>
<td>2.60</td>
<td>0.91</td>
</tr>
<tr>
<td>TNF-α</td>
<td>49.34</td>
<td>61.46</td>
<td>5.83</td>
<td>2.05</td>
<td>0.08</td>
</tr>
</tbody>
</table>

¹ Non-Magnetic Assay
² Magnetic Assay

(r=-0.6), though there were only a small number of observations available for comparison (n=7). Interassay agreement between ELISA and MULTIPLEX did not appear to be affected by assay manufacturer (BioRad or Millipore) or filtration technique (non-magnetic or magnetic).

To examine the possibility that values obtained using MULTIPLEX differed in magnitude, but not in relative rank within the data we computed Spearman correlations between ELISA and MULTIPLEX assays for each analyte (Table 2.4). Strong Spearman correlations were observed between ELISA and non-magnetic Millipore multiplex for IL-6 (ρ=0.73). However, weaker correlations were observed between techniques for all remaining analytes (ρ<0.45).

Table 2.3: Pearson(r) interassay correlations for MULTIPLEX assays against gold-standard ELISA

<table>
<thead>
<tr>
<th>Analyte</th>
<th>n</th>
<th>BioRad¹</th>
<th>n</th>
<th>Millipore¹</th>
<th>n</th>
<th>Biorad²</th>
<th>n</th>
<th>Millipore²</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>30</td>
<td>0.08</td>
<td>13</td>
<td>0.72</td>
<td>7</td>
<td>-0.60</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>IL-10</td>
<td>30</td>
<td>0.13</td>
<td>30</td>
<td>0.03</td>
<td>30</td>
<td>0.22</td>
<td>7</td>
<td>0.31</td>
</tr>
<tr>
<td>TNF-α</td>
<td>23</td>
<td>0.29</td>
<td>23</td>
<td>0.20</td>
<td>12</td>
<td>0.20</td>
<td>22</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

¹ Non-Magnetic Assay
² Magnetic Assay

Five analytes satisfied the requirements for Bland-Altman analysis. These were non-magnetic BioRad IL-6 and IL-10, non-magnetic Millipore IL-6 and IL-10 and magnetic BioRad IL-10. For all of these analytes, the majority of values fell within the upper
Table 2.4: Spearman(\(\rho\)) interassay correlations for MULTIPLEX assays against gold-standard ELISA

<table>
<thead>
<tr>
<th>Analyte</th>
<th>n</th>
<th>BioRad(^1)</th>
<th>n</th>
<th>Millipore(^1)</th>
<th>n</th>
<th>Biorad(^2)</th>
<th>n</th>
<th>Millipore(^2)</th>
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</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>30</td>
<td>0.04</td>
<td>13</td>
<td>0.73</td>
<td>7</td>
<td>-0.48</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>IL-10</td>
<td>30</td>
<td>0.13</td>
<td>30</td>
<td>-0.01</td>
<td>30</td>
<td>0.23</td>
<td>7</td>
<td>0.08</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>23</td>
<td>0.40</td>
<td>23</td>
<td>0.20</td>
<td>12</td>
<td>0.45</td>
<td>22</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^1\) Non-Magnetic Assay  
\(^2\) Magnetic Assay

and lower limits of agreement (95% confidence intervals) (Figures 2.1-2.5, p.49-53). However, with the exception of non-magnetic BioRad IL-6 (Figure 2.2, 95% CI: -0.35 to +0.35), the magnitude of these confidence intervals was large, indicating poor agreement between techniques. Despite a relatively narrow confidence interval for non-magnetic BioRad IL-6, the magnitude of this difference was higher than expected for a reproducible biochemical assay. Furthermore, both Pearson (0.08) and Spearman (0.04) correlations were very low. Notably, while strong Pearson (0.72) and Spearman (0.73) correlations were observed between ELISA and non-magnetic Millipore IL-6, Bland-Altman analysis revealed poor agreement between techniques (Figure 2.4, 95% CI: -20 to +20).

### 2.5 Discussion

Similar to previous research (Liu et al. 2005; Dossus et al. 2009; Ribeiro et al. 2012; Koning et al. 2012), our results indicate poor agreement between ELISA and MULTIPLEX assays when assessing serum concentrations of TFN-\(\alpha\) and IL-10 in physiologically representative human samples. Excellent correlations (\(r>0.70\), both Pearson and Spearman) were observed between ELISA and MULTIPLEX for IL-6 however, this was only observed on 1 of the 4 MULTIPLEX assays. Furthermore, a Bland-Altman plot revealed a large
confidence interval (-20 to +20) indicating poor agreement between techniques. Overall our results highlight large differences between techniques in both the magnitude of the observed concentration and its relative rank within the dataset. These differences were further confirmed by Bland-Altman plots and did not appear to be affected by MULTIPLEX manufacturer (BioRad or Millipore) or assay filtration method (non-magnetic or magnetic).

Our overall lack of agreement between these techniques suggests that the underlying properties of the technique itself and not other factors (such as wash method or manufacturer) are likely responsible. However, it is possible that other factors may have contributed to the lack of agreement between ELISA and MULTIPLEX. For instance, errors made during the assay protocol or improper calibration of the assay equipment could significantly affect the results obtained from each assay. Furthermore, the number of analytes per assay (i.e 7-plex vs. 3-plex) could have resulted in increased microbead aggregation, further impairing accuracy. Examination of the standard curves for each assay however indicated that all assays performed adequately and within the manufactures’ specifications. Factors relating to storage of the samples prior to analysis may have also influenced our results. De Jager and colleagues examined the role of freezer time and freeze thaw cycles on the sensitivity of cytokine measurements using MULTIPLEX. Using LPS stimulated plasma, they observed a 50% reduction in IL-6 and TNF-\(\alpha\) levels within 2 years of storage and a near 100% reduction in IL-10 after 4 years. Repeated freeze thaw cycles also affected assay sensitivity. While IL-6 and IL-10 were relatively stable, observed concentrations of TNF-\(\alpha\) rose over sequential cycles (Jager et al. 2009). In our samples we attempted to minimize the number of freeze thaw cycles, though the total volume of available sample made repeated freeze thaws unavoidable. Time spent in the freezer also varied considerably with some samples being frozen for up to 3 years. MULTIPLEX assays performed at a later date (magnetic BioRad and Mil-
lipore), had a higher proportion of undetected values and higher %CV compared to MULTIPLEX assays completed earlier (non-magnetic Biorad and Millipore) suggesting that these factors may have influenced assay performance. Our results may have also been limited by our sample size. However, our sample size was similar to many clinical studies conducted in this area and our objective was limited to comparing the agreement between laboratory techniques using the same blood samples. Furthermore, our results are strengthened by the use of physiologically representative human samples and MULTIPLEX assays from different manufacturers with different filtration methods.

Overall our findings support previous evidence suggesting that MULTIPLEX is not suitable for quantifying some biomarkers present in lower concentrations in human serum. We believe this finding is an important consideration, particularly in biomarker studies using human samples. To obtain accurate and reproducible clinical results the techniques used to measure them must also be accurate and reproducible. For biomarkers present in higher concentrations (eg. CRP, insulin), MULTIPLEX has been shown to be a reliable technique. However, for biomarkers present in lower concentrations (such as most cytokines) the currently available MULTIPLEX assays have not yet shown an adequate level of agreement relative to gold standard ELISA. Ideally, future studies employing MULTIPLEX technology should first perform a similar validation trial against gold standard ELISA to ensure that the method is appropriate for the biomarkers in question. Future advances in MULTIPLEX technology should also attempt to address these technical shortcomings in order to advance the accuracy and utility of MULTIPLEX in biomarker research.
Figure 2.1: Bland Altman plot for ELISA vs. Magnetic BioRad IL-10 MULTIPLEX
Figure 2.2: Bland Altman plot for ELISA vs. Non-magnetic BioRad IL-6 MULTIPLEX
Figure 2.3: Bland Altman plot for ELISA vs. Non-magnetic BioRad IL-10 MULTIPLEX
Figure 2.4: Bland Altman plot for ELISA vs. Non-magnetic Millipore IL-6 MULTIPLEX
Figure 2.5: Bland Altman plot for ELISA vs. Non-magnetic Millipore IL-10 MULTIPLEX
References


Chapter 3

Inflammation and Depressive Symptoms in Late Pregnancy

Following our exploratory analysis of immunoassays in the previous chapter, we were confident in the use of ELISA over MULTIPLEX for quantifying our cytokines of interest. In this chapter, we begin our investigation into the relationship between inflammatory markers and perinatal depressive symptoms by examining a cross-sectional cohort of women in their third trimester. The contents of this chapter were peer reviewed, but rejected by the journal Psychiatric Research. We are in the process of integrating reviewer feedback and re-submitting to another journal.
Lack of association between inflammation and depressive symptoms during pregnancy: a replication study examining interleukin-6

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3.1 Abstract

Depression during the perinatal period is common with rates of 7.4% to 12.8% reported during pregnancy and 5.5% to 19.2% postpartum. The established relationship between inflammation and symptoms of depression may be particularly relevant for depression during pregnancy. To date, five studies have investigated this relationship, producing mixed results. The objective of our study was to further assess this relationship by investigating interleukin(IL)-6 and depressive symptoms in women in their third trimester of pregnancy. Forty-eight medication-free women were assessed using a structured clinical interview (MINI) and the Edinburgh Perinatal Depression Scale (EPDS). IL-6 concentrations were determined using ELISA immunoassays. We found no significant relationship between IL-6 and EPDS scores. Accounting for BMI using partial correlations and linear regression did not change this lack of association. We also failed to observe an association between IL-6 and depressive symptoms in a sub-analysis of individuals with the highest IL-6 concentrations. Overall, our study replicates findings from two previous studies using similar methodology and the EPDS to assess depressive symptoms. Future studies should continue to examine this potential relationship while accounting for important covariates such as smoking, BMI and medication effects.

**Keywords:** inflammation, perinatal, prenatal, cytokines
3.2 Introduction

Depression during the perinatal period is common with rates of 7.4% to 12.8% reported during pregnancy and 5.5% to 19.2% postpartum (Gavin et al. 2005; Bennett et al. 2004). In recent years, the inflammation or cytokine hypothesis of depression has received considerable attention (Dantzer et al. 2011). While it is unlikely that this association is applicable to every individual with major depression (Raison and Miller 2011), it may be particularly relevant to the etiology of depression during pregnancy. Prenatal depression is often associated with other inflammatory morbidities of pregnancy, such as preeclampsia, gestational diabetes and preterm birth suggesting that inflammation may serve as a common etiological pathway (Osborne and Monk 2013). To date, 5 studies have examined the relationship between inflammation and depression during pregnancy with mixed results.

In a prospective study, Azar and Mercer examined 27 pregnant women recruited from a community health centre. Depression was assessed using the Patient Health Questionnaire-9 (PHQ-9). Mean PHQ-9 scores were below clinical cutoffs, however regression analyses showed a significant association between these mild depressive symptoms and increased C-reactive protein (CRP), interleukin(IL)-6 and tumor necrosis factor alpha (TNF-α) (Azar and Mercer 2012).

In a cross-sectional study, Cassidy-Bushrow and colleagues examined high sensitivity CRP, IL-6, IL-10, IL-1β and TNF-α in a community sample of 187 African American women during the second trimester of pregnancy. Depressive symptoms were measured using the Centre for Epidemiological Studies Depression Scale (CES-D). History of depressive illness was extracted from each participant's medical record. In total 39.6% of women met screening criteria for current depression (CES-D ≥ 16) and linear regression revealed a significant association between CES-D score and levels of IL-6 and IL-1β. This
In another cross-sectional study, Christian and colleagues examined IL-6 and TNF-α in a community sample of 60 pregnant women in their second trimester. Depressive symptoms were assessed using the CES-D. After controlling for BMI, linear regression revealed a significant association between CES-D score and levels of IL-6. The relationship between depressive symptoms and TNF-α was marginally significant (p=0.06)(Christian et al. 2009).

Blackmore and colleagues conducted a prospective study examining IL-6 and TNF-α in a community sample of 145 pregnant women. Depressive symptoms were assessed using the Edinburgh Perinatal Depression Scale (EPDS) and a history of depression was assessed using the mood section of the Structured Clinical Interview for DSM (SCID). After controlling for BMI and other covariates, repeated measures ANOVA found no relationship between continuous EPDS score and IL-6 or TNF-α concentration. Categorical comparisons of these cytokines using clinical diagnoses were also non-significant. Of note, the authors did observe an association between heightened TNF-α levels and a history of childhood trauma (Blackmore et al. 2011).

Lastly, a more recent study by Blackmore and colleagues using the same prospective study cohort and statistical methodology compared IL-6 and TNF-α in 177 African American (AA) and non-African American pregnant women. Similar to their previous study, they observed no association between depressive symptoms and levels of IL-6 or TNF-α. Differences were observed between AA and non-AA women in IL-6 levels across pregnancy, though this difference was accounted for by differences in BMI (Blackmore et al. 2014).

Together these studies provide some evidence for an association between inflammation and prenatal depression. Despite significant methodological differences, IL-6 has
been most consistently associated with depressive symptoms across these studies. In light of these mixed results, we sought to examine the relationship between IL-6 and symptoms of depression in a group of women in their third trimester of pregnancy.

### 3.3 Method

#### 3.3.1 Participants

Fifty-six (56) women aged 18 to 45 in their third trimester of pregnancy (≥ 26 weeks gestation) were recruited from the Women’s Health Concerns Clinic (WHCC) at St. Joseph’s Healthcare in Hamilton, Ontario, Canada. The WHCC is a mental health clinic specializing in the assessment and treatment of female specific mood disorders. Women were also recruited through community advertising in midwifery and ultrasound clinics. Women were excluded if they were taking psychotropic medication, currently smoking or had a comorbid physical illness. Participants were recruited between April 2011 and July 2014. The study was reviewed and approved by the Hamilton Integrated Research Ethics Board and all participants provided signed consent before entering the study.

#### 3.3.2 Clinical Assessments

Demographics and medical history were obtained for each participant. Diagnosis of Major Depression was assessed using the MINI Neuropsychiatric Interview (Sheehan et al. 1997) Version 6.0. Severity of current depressive symptoms was assessed using the EDPS (Cox, Holden, and Sagovsky 1987).
3.3.3 Biological Samples and Immunoassays

Blood was sampled from each participant between 8AM and 2:30PM. For each participant, blood was drawn into a single 10ml serum separator tube and left to clot at room temperature for 30 minutes. Samples were centrifuged at 20°C for 15 minutes at 3000 rpm. Serum was removed, aliquoted and stored at -80°C. Commercial ELISA kits for IL-6 were purchased from R&D Systems (Minneapolis, MN). The published limit of detection for these assays was 0.7 pg/ml and published intra and inter-assay coefficients of variation (CV) were <7%. On the day of assay, single aliquots of serum were thawed. All samples were assayed in duplicate and all analyses were performed according to the manufactures’ protocols by an experienced technician (MC) at St. Joseph’s Healthcare Research Laboratory in Hamilton, ON, Canada.

3.3.4 Statistical Analysis

To remain conservative, assay values falling below the limit of detection were classified as missing data. Data points were classified as outliers if they were >3 standard deviations from the mean. In total, 5 IL-6 observations (8.9%) fell below the limit of detection and 3 were excluded as outliers (5.3%), resulting in a final sample of 48. Non-parametric correlations (Spearman \( \rho \)) were used to examine the relationship between depressive symptoms, IL-6 and other clinical factors including: age, gestational week, parity and blood sampling time. Participants were also grouped based on whether they met criteria for a lifetime Major Depressive Episode (MDE) (mood, \( n=20 \) vs. control, \( n=28 \)). Comparisons between group means were made using the Mann-Whitney U test. For multiple regression analysis, regression assumptions were verified using a Global Validation Procedure (GVP)(Pena and Slate 2006). Data did not require transformation to meet regression assumptions. All analyses were completed using R (Version 3.0.2,
3.4 Results

Demographic information, clinical characteristics and mean cytokine concentrations are presented in Table 3.1. The majority of women were married (72.9%), working full time (52.1%) and multiparous (57.5%). Mean scores on the EPDS were low (6.1 ± 5.5). While 20 women had experienced an MDE in their lifetime (41.6%) only 4 (8.3%) were currently depressed according to the MINI.

Spearman correlations between IL-6 and other clinical variables are reported in Table 3.2. IL-6 was not correlated with EPDS score, age, gestational week, parity or sampling time (all \( p > 0.05 \)). Consistent with previous studies, a significant correlation was observed between IL-6 and pre-pregnancy BMI (\( \rho = 0.36, p = 0.01 \)). Accounting for BMI through a partial correlation did not improve the association between IL-6 and EPDS score which remained negative and non-significant (IL-6, \( \rho = -0.22, p = 0.18 \)).

IL-6 concentrations followed a somewhat bimodal distribution, with a small subset of women \( n=5 \) having IL-6 values >3.0 pg/ml (Figure 3.1 p.68). To address the possibility that a relationship between inflammation and depressive symptoms may only be present in a subset of individuals with higher levels of inflammation, we examined this relationship in our small, enriched sample of “high inflammation” women \( n=5, \) IL-6>3.0 pg/ml). Spearman correlations between IL-6 and EPDS were near zero (\( \rho = -0.05 \)) and correlations between IL-6 and BMI were extremely strong (\( \rho = 0.97 \)).

To examine whether IL-6 differed according to diagnostic status, we classified women into two groups based on the presence of lifetime MDEs (mood=20, control=28). Mean IL-6 values did not differ significantly between groups (mood: 2.1 ± 1.1, control: 1.8 ± 1.0, \( p = 0.36 \), Figure 3.2 p.69). In our small high inflammation sub-sample \( n=5, \)
Table 3.1: Sample demographics and clinical characteristics (n=48)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>St.Dev</th>
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<tr>
<td>Presenting Age</td>
<td>29.4</td>
<td>4.9</td>
</tr>
<tr>
<td>Gestational Week</td>
<td>31.7</td>
<td>3.6</td>
</tr>
<tr>
<td>EPDS score</td>
<td>6.1</td>
<td>5.5</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>24.2</td>
<td>4.7</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>%</th>
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<tbody>
<tr>
<td>Parity</td>
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<tr>
<td>Primiparous</td>
<td>20</td>
<td>42.5</td>
</tr>
<tr>
<td>Multiparous</td>
<td>28</td>
<td>57.5</td>
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<td>Marital Status</td>
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<td></td>
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<tr>
<td>Legally Married</td>
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<td>72.9</td>
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<td>Separated</td>
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<td>18.8</td>
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<td>Single</td>
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<td>Maternity Leave</td>
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<td>Homemaker</td>
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<td>Other¹</td>
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<td>22.9</td>
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<tr>
<td>MDE status</td>
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<td>8.3</td>
</tr>
<tr>
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<td>16</td>
<td>33.3</td>
</tr>
<tr>
<td>Never</td>
<td>28</td>
<td>58.4</td>
</tr>
</tbody>
</table>

¹ Includes: Part time, disability, unemployed, student

Table 3.2: Spearman ($\rho$) correlations between IL-6 and other clinical variables

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Gest Wk</th>
<th>Parity</th>
<th>BMI</th>
<th>Sample Time</th>
<th>EPDS</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.00</td>
<td>0.14</td>
<td>0.70</td>
<td>0.10</td>
<td>-0.01</td>
<td>0.03</td>
<td>-0.09</td>
</tr>
<tr>
<td>Gest Wk</td>
<td>0.37</td>
<td>1.00</td>
<td>-0.27</td>
<td>0.03</td>
<td>0.54</td>
<td>0.15</td>
<td>0.02</td>
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<td>Parity</td>
<td></td>
<td></td>
<td>0.03</td>
<td>1.00</td>
<td>-0.18</td>
<td>0.16</td>
<td>-0.08</td>
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<tr>
<td>BMI</td>
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<td></td>
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<td></td>
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<td>0.36</td>
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<tr>
<td>Sample Time</td>
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<td></td>
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<td></td>
<td>0.23</td>
<td>0.36</td>
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<td>EPDS</td>
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<td></td>
<td>1.00</td>
<td>-0.17</td>
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<tr>
<td>IL-6</td>
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<td></td>
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<td>1.00</td>
</tr>
</tbody>
</table>

2 women had a prior history of an MDE and 3 had no MDE history. None of the women met diagnostic criteria for a current MDE. To examine differences between groups, while
accounting for the strong relationship between IL-6 and pre-pregnancy BMI we computed a multiple regression. A regression model with IL-6 concentration as the dependent variable and diagnostic group and BMI as independent factors met all regression assumptions. Following regression, a significant effect was observed for BMI ($t = 3.07$, $p = 0.003$), but not for diagnostic group ($t = 1.02$, $p = 0.31$). Overall the model accounted for 16.8% of the variance in IL-6 values.

### 3.5 Discussion

We failed to observe a significant relationship between mild depressive symptoms and concentrations of IL-6 in a sample of physically healthy, medication free pregnant women in their third trimester. In addition, no significant correlation was observed between depressive symptom severity and IL-6 concentration in our high inflammation subsample. Our findings replicate those of previous, larger studies using semi-structured clinical interviews and the EPDS to assess depressive symptoms (Blackmore et al. 2011, 2014). Our observed point prevalence of major depressive episodes (8.3% vs. 6.3-6.9%) and mean EPDS scores (6.1 vs. 5.9-7.2) are also highly comparable.

Our results are in contrast to three previous studies showing a significant association between mild depressive symptoms and IL-6 (Azar and Mercer 2012; Cassidy-Bushrow et al. 2012; Christian et al. 2009). This may be due to differences in study design (cross-sectional vs. prospective), trimester investigated, and tools used to assess depression severity and psychiatric history. Azar and colleagues examined women prospectively from first to second trimester, using the PHQ-9 to quantify depressive symptoms and a self-reported history of psychiatric issues. Both Cassidy-Bushrow and colleagues and Christian and colleagues examined women during the second trimester and used the CES-D to assess depressive symptom severity. Neither used clinical interviews to exam-
ine psychiatric history.

In our study, we examined depressive symptoms in women during the third trimester using the more specific EPDS and a semi-structured interview to assess lifetime MDEs. Previous meta-analyses have shown that rates of prenatal depression are similar in second vs. third trimester (12.8% vs. 12.0%) (Bennett et al. 2004). Furthermore IL-6 levels are known to increase throughout pregnancy (Opsjon et al. 1993). Together, this stable level of depressive symptoms and increasing IL-6 concentration between second and third trimester in this population may have decreased the probability of observing an association between IL-6 and depressive symptoms in our sample. Our use of the perinatal specific EPDS rather than the CES-D or PHQ-9 may have also influenced our results. In contrast to the CES-D and PHQ-9, the EPDS contains a validated anxiety subscale (EPDS-3A) (Matthey 2008; Simpson et al. 2014) which is composed of 3/10 EPDS items. Cytokines are known to influence many of the underlying neural circuits involved in anxiety disorders (Raison, Capuron, and Miller 2006), though to date, little data exists directly linking cytokine elevations with individual anxiety disorders. Given that EPDS scores in our sample were low, these EPDS-3A anxiety items may have accounted for a significant proportion of the total EPDS score, decreasing our ability to observe an association between purely affective symptoms and IL-6.

In our study, we observed a significant association between pre-pregnancy BMI and IL-6, though controlling for BMI did not significantly improve the association between depressive symptoms and IL-6. This relationship between IL-6 and BMI is consistent with a large body of literature identifying adiposity as a well established contributor to inflammation (Trayhurn and Wood 2004). Both Cassidy-Bushrow et al. and Christian et al. accounted for BMI within their statistical models (Cassidy-Bushrow et al. 2012; Christian et al. 2009), however Azar and colleagues found no association between BMI and IL-6, CRP or TNF-α levels (Azar and Mercer 2012) casting some doubt on the relia-
Some limitations of our study deserve attention. The proportion of our data set lost due to sub-threshold detection and outliers was high (n=8, 14.2%). In addition, while previous studies have examined other cytokines (such as IL-10, TNF-α and CRP) we chose to focus on IL-6 alone. It is possible that we may have observed an association between one of these other inflammatory markers and prenatal depressive symptoms. The severity of depressive symptoms in our sample was also below clinical cutoffs, potentially limiting the generalizability of our results.

Strengths of our study include the use of validated semi-structured interviews to assess diagnostic history and the use of the perinatal specific EPDS to assess depressive symptoms. Our exclusion of patients taking psychotropic medications is also unique to this literature. Meta-analysis suggests that antidepressant medications may reduce levels of pro-inflammatory cytokines such as IL-6 (Hannestad, DellaGioia, and Bloch 2011). Our exclusion of psychotropic medications allowed us to examine the relationship between depressive symptoms and inflammation without this confounding effect.

We did not find an association between IL-6 and depressive symptoms in a sample of psychotropic free, physically healthy pregnant women. Our findings replicate those of other studies using similar methodologies and add to a mixed literature examining the association between inflammatory markers and prenatal depressive symptoms. Future studies should continue to examine this relationship using samples with greater depression symptoms severity while accounting for confounding factors such as BMI and medication effects.
Figure 3.1: Distribution of IL-6 concentrations among pregnant women during the third trimester of pregnancy
Figure 3.2: Mean IL-6 concentration between women with (mood) and without (control) a lifetime history of MDEs
References


Chapter 4

Inflammation and Depressive Symptoms from Late Pregnancy to 3 Months Postpartum

Following our negative finding between IL-6 and depressive symptoms during pregnancy, we were interested in examining the longitudinal association between inflammation and perinatal depressive symptoms. This chapter describes the outcome of this investigation. The contents of this chapter was initially submitted to BMC Psychiatry, but was rejected. The comments from peer reviewers were integrated into the manuscript. It was re-submitted and has been accepted for publication in Revista Brasileira de Psiquiatria.
A longitudinal study of inflammatory markers and mild depressive symptoms in the late pregnancy and early postpartum period

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(1) McMaster Integrated Neuroscience Discovery and Study (MiNDS), McMaster University, Hamilton, ON, Canada. (2) Department of Psychiatry and Behavioural Neuroscience, McMaster University, Hamilton, ON, Canada. (3) Women’s Health Concerns Clinic, St. Joseph’s Healthcare Hamilton, Hamilton, ON, Canada. (4) Institute for Medical Sciences, University of Toronto, Toronto, ON, Canada.
4.1 Abstract

Objective: Perinatal depressive symptoms often co-occur with other inflammatory morbidities of pregnancy. The goal of our study was to assess the relationship between depressive symptoms and inflammation by examining these factors prospectively from the third trimester of pregnancy to 12 weeks postpartum.

Methods: Thirty-three (33) healthy pregnant women were recruited from the Women’s Health Concerns Clinic at St. Joseph’s Healthcare in Hamilton, Ontario. The impact of depressive symptoms on each biomarker during pregnancy, postpartum and across time was assessed using linear and mixed model regression.

Results: Regression analysis revealed no significant association between depressive symptoms and any of the candidate biomarkers during pregnancy, at 12 weeks postpartum, or from later pregnancy into early postpartum. However, greater pregnancy depressive symptoms (p<0.001), lower IL-6 (p=0.025) and lower IL-10 (p=0.006) did emerge predictors of postpartum EPDS score.

Conclusions: Our study supports others in the literature showing no relationship between inflammatory biomarkers and depressive symptoms during late pregnancy, early postpartum or across time. Our study is the first to observe an association between late pregnancy levels of IL-6 and IL-10 and postpartum depressive symptoms however, these results should be considered both exploratory further studies are required.

Keywords: perinatal, depression, cytokines, inflammation
4.2 Introduction

Between 7.4% and 12.8% of women suffer from depression during pregnancy while 5.5% to 19.2% develop depression during the postpartum period (Gavin et al. 2005; Bennett et al. 2004). Recent research examining the biological basis of Major Depressive Disorder (MDD) has focused on its potential inflammatory basis (Dantzer et al. 2011). Though it is generally accepted that this etiological pathway is applicable to only a sub-population of depressed patients (Raison and Miller 2011), it may be particularly relevant to the etiology of depression during the perinatal period. The perinatal period is a unique hormonal and immunological state and perinatal depressive symptoms often co-occur with other inflammatory morbidities of pregnancy including: preeclampsia, gestational diabetes and preterm birth (Osborne and Monk 2013). To date, a number of studies have examined this co-occurrence by assessing differences in cytokines and other inflammatory biomarkers during the perinatal period.

Cross-sectional studies conducted during pregnancy are mixed and differ considerably in trimester investigated, depression severity measure and inflammatory markers. Some studies show positive associations between prenatal depressive symptoms and Interleukin (IL)-6, Tumor Necrosis Factor-α (TNF-α) or C-Reactive Protein (CRP) (Christian et al. 2009; Cassidy-Bushrow et al. 2012; Azar and Mercer 2012), while others show a negative association (Shelton, Schminkey, and Groer 2014) (IL-6, TNF-α) or no association at all (Blackmore et al. 2011, 2014). Studies of inflammatory markers during the postpartum period have also produced mixed results. Two studies have shown a positive relationship between depressive symptoms and IL-6 (Corwin, Johnston, and Pugh 2008; Groer and Morgan 2007) and one has shown a negative association between depressive symptoms, Interferon-γ (IFN-γ) and IFN-γ/IL-10 ratio (Groer and Morgan 2007). One study has shown a positive relationship between depressive symptoms and transforming
growth factor β (Kondo et al. 2011), while two others have failed to observe any association between postpartum depressive symptoms and markers of inflammation (Boufidou et al. 2009; Skalkidou et al. 2009).

Prospective studies examining depressive symptoms and inflammatory markers from pregnancy to postpartum have also produced mixed results. Maes and colleagues have published three studies examining postpartum blues (symptoms of depression which occur between 24-48 hours after birth and 2 weeks postpartum). These studies suggest some association between symptoms of depression and anxiety, diagnostic history of MDD and levels IL-6 and IL-1 receptor antagonist. However, these studies compared “reactors” to “non-reactors” which the authors defined by ranking participants based on q25, median and q75 values from residualized symptom severity scale scores (Maes et al. 1999, 2000; Maes et al. 2001). This unconventional analysis limits the generalizability of these results. A small study of 27 women with a history of mood or anxiety issues (and therefore at high risk for developing postpartum depression) by Scrandis and colleagues found a significant association between prenatal CRP levels and prenatal depressive symptoms. No relationship was observed between CRP and depressive symptoms at 5-6 weeks postpartum (Scrandis et al. 2008).

Together these studies suggest that there may be some relationship between perinatal depressive symptoms and inflammatory status. The purpose of our longitudinal study was to explore this relationship during a period of high risk for developing perinatal depressive symptoms. The specific aims of our study were to: 1) examine whether pregnancy to postpartum changes in inflammatory markers were associated with changes in depressive symptoms, 2) examine whether third trimester inflammatory markers were predictive of postpartum depressive symptoms and 3) examine the relationship between inflammatory markers and depressive symptoms during the third trimester of pregnancy and at 3 months postpartum,
4.3 Method

4.3.1 Participants and Design

Participants selected from a larger study examining sleep quality, circadian rhythms and depressive symptoms across the perinatal period. This larger study (total 52 subjects) was a longitudinal design with two study visits, one during the third trimester of pregnancy (≥ 26 weeks gestation) and one 12 weeks postpartum as the prevalence of perinatal depressive symptoms is greatest between these two time points (Gavin et al. 2005). During the first visit, demographic information was collected and current and past psychiatric diagnoses were assessed. Women then completed a series of questionnaires examining current symptoms of depression, anxiety and history of childhood trauma and provided a blood sample. At 12 weeks postpartum, women returned for a second study visit where they repeated the depression and anxiety symptom questionnaires from visit 1 and provided another blood sample. Women were recruited from the Women’s Health Concerns Clinic at St. Joseph’s Healthcare in Hamilton, Ontario, Canada between February 2013 and October 2014. The WHCC is an outpatient clinical specializing in mood disruptions during the perinatal period that receives referrals mostly from family doctors in the community, but also from obstetric and midwifery clinics. Women referred to the WHCC are often currently experiencing a mood issue or have a past history of perinatal mood disturbances (and are therefore at risk for subsequent mood disruptions). Women were also recruited through community advertising in midwifery and ultrasound clinics. Women were included if they were aged 18-45, in their third trimester of pregnancy, free of major medical comorbidities (e.g. diabetes, hypertension, or other inflammatory conditions) and were non-smokers. This study was reviewed and approved by the Hamilton Integrated Research Ethics Board and all
participants provided informed consent.

Data from participants who were free of psychotropic medication and provided blood samples at both time points was used for this study, resulting in a sample size of thirty-three (33). Blood samples from these individuals were analyzed to determine levels of IL-6, IL-10, TNF-α, and CRP. Cytokines are known to follow a diurnal pattern (Lange, Dimitrov, and Born 2010), and while we attempted to collect blood samples at similar times for each visit, differences in availability made intra-individual variation in sampling time unavoidable. We therefore added time of blood sampling as covariate to control for this variation in our analyses.

4.3.2 Clinical Assessments

Psychiatric diagnoses were assessed using the MINI Neuropsychiatric Interview (Sheehan et al. 1997) version 6.0. Current symptoms of depression were assessed using the Edinburgh Perinatal Depression Scale (EPDS). The EPDS shows good specificity and is sensitive to change in depressive symptoms over time (Cox, Holden, and Sagovsky 1987). To assess anxiety symptoms, participants completed the State Trait Anxiety Inventory (STAI) (Form X and Y) (Spielberger 2010). To account for the potential interaction between inflammatory factors and prior trauma, participants also completed the Childhood Trauma Questionnaire (CTQ) (Bernstein et al. 1994).

4.3.3 Biological Samples and Immunoassays

Blood was sampled from each participant between 8:15AM and 3:30PM. For each participant, blood was drawn into a single 10ml serum separator tube and left to clot at room temperature for 30 minutes. Samples were centrifuged at 20°C for 15 minutes at 3000 rpm. Serum was removed, aliquoted and stored at -80°C. Commercial ELISA
kits for IL-6, IL-10, TNF-α and CRP were purchased from R&D Systems (Minneapolis, MN). The published limit of detection and intra/interassay coefficients of variation for these assays were as follows: IL-6: 0.7 pg/ml, <7%; IL-10: 3.9 pg/ml, <7.5%; TNF-α: 0.19 pg/ml, <10.4%; CRP: 0.022 ng/ml, <8.3%. On the day of assay, single aliquots of serum were thawed. All samples were assayed in duplicate and all analyses were performed according to the manufactures' protocols by an experienced technician (MC) at St. Joseph’s Healthcare Research Laboratory in Hamilton, ON, Canada.

4.3.4 Statistical Analysis

Data points were classified as outliers if they were >3 standard deviations from the mean. In total, 2 observations (6.1%) were excluded as outliers resulting in a final sample of 31. For each cytokine (IL-6, IL-10, TNF-α) sampling time and pre-pregnancy Body Mass Index (BMI) were used as covariates. Because CRP levels are more stable and do not exhibit a 24-hour variation (Meier-Ewert et al. 2001), only pre-pregnancy BMI was used as a covariate. While we did collect information examining childhood trauma (CTQ) and anxiety symptoms (STAI), we observed very low variability in these scores across the sample. Therefore to conserve power and in accordance with our modest sample size, we chose not to include these variables in the analyses.

To examine changes in inflammatory markers and depressive symptoms over time, we applied a mixed model regression analysis, using each biomarker as the dependent variable. The use of each biomarker as the dependent variable (rather than EPDS score) has been used previously to assess the relationship between prenatal depressive symptoms and IL-6 (Christian et al. 2009). The mixed model method of regression does not require adjustments for normality and homogeneity of variance. Main effects for each regression model were evaluated using Wald’s Chi-squared test. To assess the explana-
tory power of each model, the difference in deviance between the test and null model (a model containing only the dependent variable and within subjects factor) was dividing by the deviance of the null model (delta deviance). This results in a proportion that can be considered a pseudo adjusted $R^2$ value for mixed model regression analyses.

To determine whether levels of each biomarker during pregnancy were predictive of postpartum depressive symptoms, we computed linear regressions using postpartum EPDS scores as the dependent variable. Postpartum EPDS scores were skewed and required a log transformation to meet regression assumptions. Pregnancy EPDS score was strongly correlated with postpartum EPDS ($\rho=0.72$) and was included as a covariate in each model. Goodness of fit comparisons between linear models was assessed using an F-test.

To assess the impact of depressive symptoms on levels of each biomarker at each time point we computed linear regressions using the candidate biomarker as the dependent variable. For each model, regression assumptions were evaluated using a Global Validation Procedure (Pena and Slate 2006). To meet regression assumptions, pregnancy values of IL-10, TNF-$\alpha$ and CRP required a log transformation. Pregnancy values of IL-6 satisfied all regression assumptions. For postpartum regression models, values of IL-6, TNF-$\alpha$ and CRP required log transformation. Postpartum IL-10 values satisfied all regression assumptions.

All analyses were completed using the statistical package R (Version 3.1.3, http://R-project.org).

### 4.4 Results

Demographic and clinical characteristics are summarized in Table 4.1 and Table 4.2. Study participants were $30.7 \pm 4.0$ years old and $30.1 \pm 4.1$ weeks gestation. All par-
Participants were married (100%) and the majority were working full time (80.6%), multiparous (61.3%) and had a college or university education (87.1%). Third trimester mean scores on the EPDS were 5.2 ± 4.1. About one-third (35.5%) had experienced at least 1 major depressive episode in their lifetime, though none met current diagnostic criteria for a Major Depressive Episode (MDE). EPDS scores did not differ significantly between pregnancy and postpartum visits. At the postpartum visit, 2 individuals (6.4%) met screening criteria for postpartum depression (EPDS ≥ 13).

Table 4.1: Demographic and characteristics of the study sample (n=31)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>12</td>
<td>38.7</td>
</tr>
<tr>
<td>Multiparous</td>
<td>19</td>
<td>61.3</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legally Married</td>
<td>31</td>
<td>100</td>
</tr>
<tr>
<td>Work Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full Time</td>
<td>25</td>
<td>80.7</td>
</tr>
<tr>
<td>Maternity Leave</td>
<td>3</td>
<td>9.7</td>
</tr>
<tr>
<td>Part time</td>
<td>2</td>
<td>6.4</td>
</tr>
<tr>
<td>Student</td>
<td>1</td>
<td>3.2</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; High School</td>
<td>2</td>
<td>6.4</td>
</tr>
<tr>
<td>High School</td>
<td>2</td>
<td>6.4</td>
</tr>
<tr>
<td>College Degree</td>
<td>11</td>
<td>35.5</td>
</tr>
<tr>
<td>University Degree</td>
<td>16</td>
<td>51.7</td>
</tr>
<tr>
<td>MDE status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Past</td>
<td>11</td>
<td>35.5</td>
</tr>
<tr>
<td>Never</td>
<td>20</td>
<td>64.5</td>
</tr>
</tbody>
</table>
### Table 4.2: Demographic and Clinical characteristics of the study sample (n=31)

<table>
<thead>
<tr>
<th></th>
<th>Pregnancy Mean</th>
<th>Pregnancy St.Dev</th>
<th>Postpartum Mean</th>
<th>Postpartum St.Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>30.7</td>
<td>4.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gestational/Postpartum</td>
<td>30.1</td>
<td>4.1</td>
<td>13.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Postpartum Week</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>24.6</td>
<td>5.4</td>
<td>13.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Sampling Time</td>
<td>10.6</td>
<td>2.0</td>
<td>12.3</td>
<td>1.8</td>
</tr>
<tr>
<td>EPDS</td>
<td>5.2</td>
<td>4.1</td>
<td>4.5</td>
<td>5.1</td>
</tr>
<tr>
<td>STAI</td>
<td>31.8</td>
<td>8.3</td>
<td>32.8</td>
<td>11.1</td>
</tr>
<tr>
<td>CTQ</td>
<td>30.0</td>
<td>5.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.5</td>
<td>1.2</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>11.7</td>
<td>2.9</td>
<td>11.8</td>
<td>2.2</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.2</td>
<td>0.9</td>
<td>1.6</td>
<td>3.2</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>4.8</td>
<td>3.4</td>
<td>3.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

#### 4.4.1 Change in inflammatory status and depressive symptoms from third trimester to 12 weeks postpartum

Results of our mixed model regression analyses are summarized in Table 4.3. Values of IL-6, IL-10, and TNF-α did not change significantly from third trimester to 12 week postpartum (all $p>0.05$). CRP concentrations decreased significantly over time ($\beta = -1.58$, 95%CI: -2.71 to -0.46, $p=0.005$). Higher pre-pregnancy BMI was associated with higher CRP concentrations over time ($\beta = 0.30$, 95%CI: 0.15 to 0.46, $p<0.001$). EPDS score did not emerge as a significant predictor of any biomarker (all $p>0.05$). Delta deviance values were low (0.2-6.0%) suggesting that the parameters of time, EPDS, sampling time, and pre-pregnancy BMI made small to negligible contributions to the longitudinal trajectory of biomarker concentrations.
Table 4.3: Mixed model regression analysis examining change in inflammatory markers and depressive symptoms over time

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Parameter</th>
<th>( \beta )</th>
<th>95% CI</th>
<th>p</th>
<th>( \Delta \text{deviance} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>Time</td>
<td>0.43</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>EPDS</td>
<td>0.04</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sample Time</td>
<td>-0.07</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>-0.00</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td>IL-10</td>
<td>Time</td>
<td>0.04</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>EPDS</td>
<td>-0.04</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sample Time</td>
<td>0.01</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>0.01</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td>TNF-( \alpha )</td>
<td>Time</td>
<td>0.49</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>EPDS</td>
<td>-0.06</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Sample Time</td>
<td>-0.05</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>BMI</td>
<td>0.06</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td>CRP</td>
<td>Time*</td>
<td>-1.58</td>
<td>-2.71</td>
<td>-0.46</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>EPDS</td>
<td>0.01</td>
<td>—</td>
<td>—</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>BMI*</td>
<td>0.30</td>
<td>0.15</td>
<td>0.46</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*significant main effect for predictor/covariate (p<0.05). 95% CI are only reported for significant effects.

4.4.2 Pregnancy inflammatory markers and postpartum depressive symptoms

We examined whether third trimester inflammatory markers were predictive of depressive scores at 12 weeks postpartum. Results of these regression analyses are summarized in Table 4.4. Pregnancy EPDS (\( \beta = 0.14\) to 0.16, 95%CI: 0.08-0.19, p<0.001), IL-6 (\( \beta = -0.22\), 95%CI: -0.42 to -0.03, p=0.025) and IL-10 (\( \beta = -1.28\), 95%CI: -2.17 to -0.39, p=0.006) emerged as significant predictors of postpartum EPDS score. No relationship was observed between postpartum EPDS score and TNF-\( \alpha \) or CRP (p>0.05). Regression coefficients were negative for both IL-6 and IL-10 indicating that a lower level of both of these cytokines during the third trimester of pregnancy was predictive of a higher post-
partum EPDS score. To assess the strength of these effects we compared each model to a model containing pregnancy EPDS alone. Addition of either IL-6 or IL-10 resulted in a model with greater predictive power than a model containing pregnancy EPDS alone (IL-6: $F=5.62$, $p=0.025$, Adj-R$^2 +0.08$; IL-10: $F=8.61$, $p=0.006$, Adj-R$^2 +0.12$).

Table 4.4: Summary of regression models examining pregnancy inflammatory markers and postpartum EPDS score

<table>
<thead>
<tr>
<th>Model</th>
<th>Covariate</th>
<th>$\beta$ value</th>
<th>95% CI</th>
<th>Predictor $p$</th>
<th>Model $F$</th>
<th>Model $p$</th>
<th>Adj-R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IL-6*</td>
<td>-0.22</td>
<td>-0.42</td>
<td>-0.03</td>
<td>0.025</td>
<td>17.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Pregnancy EPDS*</td>
<td>0.16</td>
<td>0.10</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>IL-10*</td>
<td>-1.28</td>
<td>-2.17</td>
<td>-0.39</td>
<td>0.006</td>
<td>19.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Pregnancy EPDS*</td>
<td>0.14</td>
<td>0.08</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TNF-$\alpha$</td>
<td>-0.13</td>
<td>—</td>
<td>—</td>
<td>NS</td>
<td>12.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Pregnancy EPDS*</td>
<td>0.14</td>
<td>0.08</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>CRP</td>
<td>-0.14</td>
<td>—</td>
<td>—</td>
<td>NS</td>
<td>12.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Pregnancy EPDS*</td>
<td>0.14</td>
<td>0.08</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* significant main effect for predictor/covariate ($p<0.05$). 95% CI are only reported for significant effects

4.4.3 Inflammatory markers during pregnancy and the postpartum period

Regression analysis revealed no significant association between EPDS scores and any of the candidate biomarkers during pregnancy (Table 4.5). Despite sampling time being moderately correlated with IL-6, it was not a significant predictor following regression. Of note, pre-pregnancy BMI was also not correlated with IL-6 ($\rho=0.02$, $p=NS$). For IL-10, sampling time emerged as a significant predictor ($p=0.05$). TNF-$\alpha$ levels were not correlated with sampling time or pre-pregnancy BMI (all $p>0.05$). Pre-pregnancy BMI emerged as a significant predictor of CRP ($p=0.008$). Overall only the model for CRP achieved statistical significance, explaining 17% of the variance in CRP concentration.
Regression models examining inflammatory markers during the postpartum period are summarized in Table 4.6. Sampling time and pre-pregnancy BMI were not correlated with IL-6, IL-10 or TNF-α (all p>0.05). Pre-pregnancy BMI remained a positive predictor of CRP (p=0.009). Similar to pregnancy, no significant associations between EPDS scores and IL-6, IL-10, or CRP were observed. We were unable to examine the influence of EPDS scores on TNF-α as multiple data transformations did not result in an acceptable model which satisfied all regression assumptions. The low level of correlation between TNF-α and EPDS (ρ=0.07, p=NS) suggests that there was likely no significant relationship between them.

### Discussion

In our study we did not observe any significant associations between pregnancy to postpartum changes in biomarker concentrations and changes in depressive symptoms. We did however observe a significant association between pregnancy IL-6, IL-10 and post-
Table 4.6: Summary of regression models examining inflammatory markers and depressive symptoms at 12 weeks postpartum

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Predictors &amp; Covariates</th>
<th>( \beta )</th>
<th>Predictor</th>
<th>Predictor</th>
<th>Model</th>
<th>Model</th>
<th>Adj-R(^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>EPDS, Sampling Time, BMI</td>
<td>0.06, -0.19, -0.09</td>
<td>NS</td>
<td>NS</td>
<td>0.70</td>
<td>NS</td>
<td>0.00</td>
</tr>
<tr>
<td>IL-10</td>
<td>EPDS, Sampling Time, BMI</td>
<td>-0.25, -0.07, 0.11</td>
<td>NS</td>
<td>NS</td>
<td>0.52</td>
<td>NS</td>
<td>0.00</td>
</tr>
<tr>
<td>TNF-( \alpha )</td>
<td>EPDS, Sampling Time, BMI</td>
<td>0.07, -0.03, 0.19</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CRP</td>
<td>EPDS, BMI*</td>
<td>0.32, 0.44</td>
<td>NS</td>
<td>0.009</td>
<td>4.43</td>
<td>0.02</td>
<td>0.19</td>
</tr>
</tbody>
</table>

* significant main effect for predictor/covariate (p<0.05).

partum depressive symptoms. Similar to previous studies, we also failed to observe a cross-sectional association between any of our candidate biomarkers and depressive symptoms during the third trimester or at 12 weeks postpartum.

During normal pregnancy there is a rise in both IL-6 and IL-10 from second to third trimester (Thaxton and Sharma 2010; Opsjon et al. 1993). IL-6 and IL-10 are both associated with Th2 immune responses (Mosmann and Sad 1996) and successful healthy pregnancy is generally considered to be a Th2 dominant phenomenon (Wegmann et al. 1993). Our finding linking decreases in third trimester IL-6 and IL-10 with postpartum depressive symptoms suggests that late pregnancy reductions in Th2 immunity may affect the severity of postpartum depressive symptoms. Another possibility is that our observed reductions in IL-6 and IL-10 were indicative of a shift toward Th1 immunity. Previous studies have shown that disruptions in Th1:Th2 ratio during pregnancy are associated with negative obstetric outcomes (Saito et al. 1999), though no study has examined how Th1:Th2 ratio may relate to perinatal depressive symptoms. It is im-
important to note that other factors, particularly the diurnal variation in IL-6 and IL-10 may have influenced our findings. Larger, prospective and longitudinal studies which control sampling time and assess both Th1 (e.g. IFN-γ) and Th2 cytokines are required to further examine this hypothesis.

Our observed lack of cross-sectional association between inflammatory markers and depressive symptoms replicates findings from other studies of these biomarkers during pregnancy (Blackmore et al. 2011, 2014) and the postpartum period (Boufidou et al. 2009; Skalkidou et al. 2009) which use the EPDS to assess depressive symptoms. Our study is also consistent with the prospective study conducted by Scrandis and colleagues which found no association between pregnancy CRP levels and postpartum depressive symptoms (Scrandis et al. 2008).

Some limitations of our study are noteworthy. The mean level of depressive symptoms in our study population at both time points was in the subclinical range. The incidence of probable postpartum depression was also approximately half of what is observed in the general population (n=2, 6.4% vs. 10-15%) (Gavin et al. 2005). This lower level of depressive symptomatology may be due to our exclusion of women taking psychotropic medication. As such, we may have self-selected for a healthier or more resilient study population. The high degree of social support (100% of the sample was married), high level of education (>85% postsecondary) and absence of any current MDEs during pregnancy further supports this assumption. While this limits the generalizability of our results, our assessment of a physically and psychiatrically healthy, medication free perinatal population provides a unique opportunity to examine the role of these biomarkers in the development of perinatal depressive symptoms. A further limitation of our study was our use of a single postpartum time point (12 weeks). It is possible that other transient factors, such as delivery method, breastfeeding status or maternal infections at 12 weeks could have influenced our findings. It is also possi-
ble that the relationship between depressive symptoms and biomarkers of inflammation differs during the first few weeks postpartum as hormonal and immune responses normalize. Our results are also somewhat limited by our small sample size, as we were unable to assess the role of other important covariates, including: diagnostic history, childhood trauma and current anxiety symptoms. However, our sample size is similar to the only other perinatal study of similar design (Scrandis et al. 2008) and our use of semi-structured diagnostic interviews and the perinatal specific EPDS strengthen our results.

In conclusion, our study is the first to assess the change in perinatal inflammatory status over time and examine its relationship to perinatal depressive symptoms. It is also the first to observe an association between late pregnancy levels of IL-6 and IL-10 and postpartum depressive symptoms. Our study also supports others in the literature showing no relationship between inflammatory biomarkers and depressive symptoms during late pregnancy or early postpartum. These exploratory and preliminary results indicate that controlled longitudinal studies using larger, more depressed study populations, assessing both Th1 and Th2 cytokines are required to causally examine these associations.
References


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Chapter 5

Sleep and Daily Rhythm Disruptions in Late Pregnancy

Having completed our examination of inflammatory markers and perinatal depressive symptoms, our focus shifted to our second main objective: to examine the relationship between sleep disturbances and perinatal depressive symptoms. Similar to our investigation of inflammatory markers, we began by examining sleep disturbances and depressive symptoms in a cross-sectional cohort of pregnant women. The contents of the chapter are in press with the Journal of Women’s Health. Permissions to reprint this material have been obtained.
Mild depressive symptoms during the third trimester of pregnancy are associated with disruptions in daily rhythms but not subjective sleep quality

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5.1 Abstract

**Background:** Recent research in Major Depressive Disorder (MDD) suggests that dysregulation of the circadian system may be a core pathophysiological component. In pregnancy women often experience significant disruptions in their daily rhythms, including changes in day-to-day schedule and sleep habits. Current evidence suggests that these disruptions in daily rhythms may adversely affect underlying circadian rhythmicity. The purpose of our study was to examine whether subjectively rated daily rhythm disruptions were associated with a greater incidence of depressive symptoms during the third trimester.

**Method:** Our study was a cross-sectional design, assessing sleep quality, symptoms of depression and daily rhythm disruptions in 51 pregnant women in their third trimester.

**Results:** We observed a significant relationship between mild depressive symptoms and disruptions in daily rhythms. While we initially observed a strong correlation between subjective sleep quality and depressive symptoms, this was attenuated after accounting for daily rhythm disruptions. Disruptions in daily social rhythms, eating patterns and general activity were all significantly associated with depressive symptomatology.

**Conclusion:** Our findings point to a strong correlation between daily rhythm disruptions and prenatal depressive symptoms. Given that these daily rhythms are known to act as zeitgebers, longitudinal studies examining the directionality of this relationship between circadian rhythms and depressive symptoms during pregnancy are warranted.

Keywords: circadian rhythms, prenatal depression, sleep quality, zeitgebers
5.2 Introduction

Depression is a major concern for women of child bearing age, as mood disturbances during pregnancy range in prevalence from 7.4% to 12.8% depending on trimester (Bennett et al. 2004). The presence of depressive symptoms during pregnancy is also a strong risk factor for the development of postpartum depression (Robertson et al. 2004). While the prevalence and clinical outcomes of prenatal depression are well characterized, much less is known about its underlying etiology.

Recent research in Major Depressive Disorder (MDD) suggests that dysregulation of the circadian system may be a core pathophysiological component (McClung 2013). Sleep disturbances are highly prevalent in MDD patients (Tsuno, Besset, and Ritchie 2005) and the circadian clock regulates several other biological processes which are implicated in MDD (McClung 2013) including glucocorticoid secretion (Kalsbeek et al. 2012), central monoamine activity (Hampp et al. 2008) and immune functioning (Lange, Dimitrov, and Born 2010). Several studies indicate that sleep disruptions during pregnancy are important predictors of adverse pregnancy outcomes (Okun et al. 2009b) and recurrence of postpartum depression (Okun et al. 2009a, 2011a). Sleep disruptions may also be particularly relevant to the etiology of prenatal depression. Studies examining prenatal subjective sleep quality and depressive symptoms have shown that pregnant women who screen positive or meet diagnostic criteria for depression have significantly poorer perceived sleep quality (Ross, Murray, and Steiner 2005; Mellor, Chua, and Boyce 2014; Jomeen and Martin 2007; Goyal, Gay, and Lee 2007; Okun et al. 2011b). These results are consistent despite significant heterogeneity in the trimester investigated and the tools used to assessed sleep quality and depression. However, when prenatal depressive symptoms and subjective sleep quality are assessed as continuous measures, this relationship is more complex. One longitudinal study observed a positive
association between second trimester sleep quality scores and late third trimester depression (Skouteris et al. 2008). Another longitudinal study found that sleep deficient women (defined using self-rated sleep diaries) had significantly more depressive symptoms in early (10-12 weeks) and mid (18-20 weeks) pregnancy (Okun et al. 2013b). Other studies have failed to identify an association (Kamysheva et al. 2010) or found that the relationship was accounted for by co-occurring anxiety symptoms (Swanson et al. 2011). The relationship between prenatal depressive symptoms and sleep quality is further complicated by the established dissociation between subjective and objective (eg. polysomnography) measurements of sleep quality (Buysse et al. 2008). Studies examining objective prenatal sleep quality have failed to identify an association with prenatal depressive symptoms (Okun et al. 2013b; Bei et al. 2010).

It is important to note that these sleep quality studies, whether objective or subjective, provide limited insight into the underlying circadian rhythmicity. Sleep is governed by the interaction between homeostatic and circadian processes (Borbély and Achermann 1999) and is only a reliable marker of circadian functioning when assessed longitudinally and objectively (Lockley, Skene, and Arendt 1999). Circadian rhythmicity is commonly assessed using longitudinal polysomnography, cosinor based analysis of actigraphy (Cornelissen 2014) or the use of hormonal sampling (eg. 6-sulphatoxymelatonin, Dim Light Melatonin Onset (DLMO)) (Lockley, Skene, and Arendt 1999). Previous research using these methods to assess the relationship between circadian rhythms and prenatal depression is limited. In a small study comparing healthy (n=15) and depressed pregnant women (n=10), those with depression showed blunted melatonin secretion between 2:00am and 11:00am (Parry et al. 2008). This finding was replicated in another study from the same group comparing depressed (n=11) and healthy (n=22) pregnant women (Posadas et al. 2012). In another study of 101 pregnant women, a diagnosis of depression was associated with significantly lower waking cortisol levels and a
more modest decline in cortisol over the first 12hrs of wake time (O’Connor et al. 2014). A small study of 12 women found that advances in circadian phase (DLMO) from the third trimester of pregnancy to 6 weeks postpartum were associated with a greater severity of postpartum depressive symptoms (Sharkey, Pearlstein, and Carskadon 2013).

The lack of objective data examining the association between circadian rhythms and prenatal depressive symptoms may be partially due to the time consuming and complex nature of these assessments. It is possible, however, to use more pragmatic “behavioural proxies” to assess rhythmicity. Light is the most powerful zeitgeber (timekeeper) of the circadian system (Reppert and Weaver 2002) but several other external zeitgebers can modulate circadian rhythmicity. For example, feeding/eating patterns have been shown to act as zeitgebers, affecting corticosterone and core body temperature rhythms independent of the central clock (Stephan 2002). Ehlers and colleagues have also noted that social activity can act as a circadian zeitgeber. They have hypothesized that a disruption of normal social rhythms may be a central component in the onset and persistence of affective episodes (Ehlers, Frank, and Kupfer 1988). Studies of psychiatric populations support their hypothesis (Germain and Kupfer 2008), particularly in regards to the onset of manic episodes in patients with bipolar disorder (Malkoff-Schwartz et al. 2000).

In pregnancy women often experience significant disruptions in their daily rhythms, including changes in morning-eveningness preference, day-to-day schedule and sleep habits (Mindell, Cook, and Nikolovski 2015; Leonhard and Randler 2009). The evidence presented above suggests that disruptions in these daily zeitgebers may affect underlying circadian rhythmicity. To date, no studies have examined whether the intensity of these rhythm disruptions are associated with a greater severity of depressive symptoms. The objective of our study was to assess this relationship during the third trimester of pregnancy.
5.3 Materials and Methods

5.3.1 Participants

Women aged 18 to 45 in their third trimester of pregnancy (28-37 weeks gestation) were recruited from the Women's Health Concerns Clinic at St. Joseph’s Healthcare in Hamilton, Ontario, Canada. Women were also recruited through community advertising in midwifery and ultrasound clinics. In total, 51 women participated in our study. To control for potential medication effects on sleep quality or activity rhythms, women were excluded if they were taking psychotropic medication. Women were also excluded if they were currently smoking or had a comorbid physical illness. The study was reviewed and approved by the Hamilton Integrated Research Ethics Board.

5.3.2 Assessments

Demographics and medical history was obtained for each participant. Psychiatric diagnosis was assessed using the MINI Neuropsychiatric Interview (Sheehan et al. 1997) Version 6.0. The MINI is a validated structured clinical interview used to assess both current and past mood and anxiety disorders using DSM-IV criteria. Symptoms of depression were assessed using the Edinburgh Perinatal Depression Scale (EPDS). The EPDS is a self-report screening tool developed specifically for assessing depression in perinatal women (Cox, Holden, and Sagovsky 1987). Subjective sleep quality was assessed using the Pittsburgh Sleep Quality Inventory (PSQI) (Buysse et al. 1989). The PSQI is a self-report instrument developed for use in both clinical and research settings. It was shown to maintain its reliability, factor structure and utility when used to assess sleep quality in pregnant women (Jomeen and Martin 2007). Daily rhythms were assessed using the Biological Rhythms Interview of Assessment in Neuropsychiatry
(BRIAN) (Giglio et al. 2009). The BRIAN is an 18-item scale which subjectively assesses different behavioural proxies of daily rhythmicity. These include: sleep (5 questions), general activity (5 questions), social rhythms (4 questions) and eating patterns (4 questions). Each item (except #4 which is reverse scored) is scored on a Likert scale of 1-Not at all to 4- Often and summed to produce a total score. Lower scores are indicative of more stability in daily rhythms and activities, while higher scores are indicative of greater instability. The BRIAN can be assessed by examining the total score (range 18-72) or by examining total scores for individual subdomains. The BRIAN has been previously validated in bipolar disorder (Giglio et al. 2009).

5.3.3 Statistical Analysis

The relationship between EPDS, PSQI and BRIAN scores was first assessed using Pearson ($r$) and partial correlations. Partial correlations were as follows: EPDS and PSQI controlling for BRIAN, EPDS and BRIAN controlling for PSQI, and BRAIN and PSQI controlling for EPDS. To further examine the relationship between activity rhythms, sleep quality and depressive symptoms, clinical variables were subjected to linear regression using EPDS score as the dependent variable. To assess the presence of multicollinearity the Variance Inflation Factor (VIF) was calculated. The VIF quantifies the degree to which individual regression model coefficients increase due to the presence of collinear (i.e. highly correlated) independent variables. Higher values are indicative of greater co-linearity and conventional cutoff scores for are 5 or 10 (Craney and Surles 2002). We used the more conservative cutoff (VIF > 5) for our analysis. The regression assumptions were evaluated using a Global Validation Procedure (GVP) (Pena and Slate 2006). All statistical analyses were performed using R, version 3.0.2 (http://R-project.org).
5.4 Results

Demographic information is summarized in Table 5.1. Women were 30.0±4.9 years old and in mid-third trimester (mean gestational week = 31.9±3.7). The majority of women were married (76.5%), multiparous (60%) and working full time (50%). Approximately 43% (n=22) of women had experienced a major depressive episode (MDE) in their lifetime, though only 7.8% (n=4) met diagnostic criteria for a current MDE. Mean EPDS scores were low (mean=5.6±4.5), while mean PSQI scores exceeded the clinical cutoff score of 5, indicating the presence of significant sleep disruption (mean=7.2±3.5). No clinical cutoff scores exist for the BRIAN, however the mean score was within the lower half of the scale range (mean=35.6±10.2, scale range=18-72).

Overall, depressive symptom severity was strongly correlated with poorer sleep quality (r=0.55, p<0.0001) and greater disruptions in daily rhythms (r=0.67, p<0.0001) (Figure 5.1a p.106). Poor sleep quality alone was also highly associated with daily rhythm disruptions (r=0.68, p<0.0001). Given the multifaceted nature of depressive symptoms, we hypothesized that this high degree of correlation may be due to co-linearity between the scales themselves however, VIF was 1.88 for both PSQI and BRIAN indicating that both scales were independent. To account for any overlap between EPDS, PSQI and BRIAN we computed partial correlations (Figure 5.1b p.106). Using this method, the observed relationship between depressive symptom severity and sleep quality was no longer significant (r=0.17, p=0.22), while the significant correlation between depressive symptoms and activity rhythms was retained (r=0.49, p=0.0001). The strong relationship between sleep quality and activity rhythms was also retained (r=0.50, p<0.0001).

To further examine the relationship between depressive symptom severity and daily rhythm disruptions, we ran a linear regression. A model including EPDS as the depen-
Table 5.1: Sample demographics and mean symptom severity scores (n=51)

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Legally Married</td>
<td>39</td>
<td>76.5</td>
</tr>
<tr>
<td>Separated</td>
<td>7</td>
<td>13.7</td>
</tr>
<tr>
<td>Single</td>
<td>5</td>
<td>9.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parity</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Primiparous</td>
<td>20</td>
<td>40.0</td>
</tr>
<tr>
<td>Multiparous</td>
<td>31</td>
<td>60.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Work Status</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Time</td>
<td>26</td>
<td>50.9</td>
</tr>
<tr>
<td>Maternity Leave</td>
<td>9</td>
<td>17.6</td>
</tr>
<tr>
<td>Part Time</td>
<td>5</td>
<td>9.8</td>
</tr>
<tr>
<td>Other(^1)</td>
<td>11</td>
<td>21.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Psychiatric Diagnosis</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Current MDE</td>
<td>4</td>
<td>7.8</td>
</tr>
<tr>
<td>Past MDE</td>
<td>18</td>
<td>35.5</td>
</tr>
<tr>
<td>Anxiety Disorder</td>
<td>9</td>
<td>17.6</td>
</tr>
<tr>
<td>Bipolar Disorder</td>
<td>2</td>
<td>3.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>St.Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presenting Age</td>
<td>30.2</td>
<td>4.8</td>
</tr>
<tr>
<td>Gestational Week</td>
<td>31.9</td>
<td>3.7</td>
</tr>
<tr>
<td>EPDS</td>
<td>5.6</td>
<td>4.5</td>
</tr>
<tr>
<td>PSQI</td>
<td>7.2</td>
<td>3.5</td>
</tr>
<tr>
<td>BRIAN</td>
<td>35.9</td>
<td>10.2</td>
</tr>
</tbody>
</table>

\(^1\) Includes: Homemaker, disability, unemployed and student

\(^2\) MDE = Major Depressive Episode

dent variable with PSQI and BRIAN score and independent factors met all regression assumptions (GVP, p>0.05) and did not have significant multicolinearlity. Following regression, the effect of BRIAN was significant (t=3.877,p=0.0003), while the PSQI was not (t=1.22,p=0.23). This model accounted for 45% of the variance. Regression diagnostics revealed the presence of 3 potential outliers. After removal of these observations (n=48), the observed effects were maintained (BRAIN, t=5.81,p<0.0001; PSQI, t=0.034, p=0.973). The adjusted model accounted for 60% of the variance and had a
large effect size ($F^2=0.82$)

The BRIAN assesses four domains of daily rhythmicity; Sleep, General Activity, Social Activity and Eating Pattern. We hypothesized that one or more of these domains may be driving the observed association between BRIAN and EPDS. Correlations were strong between the EPDS and the 4 domains of the BRIAN and were also strong between the BRIAN domains themselves (Table 5.2). A linear model with EPDS as the dependent variable and each BRIAN domain as an independent factor met all regression assumptions (GVP, $p>0.05$) and did not have significant multicollinearity. Regression diagnostics identified the presence of 3 potential outliers. After removal, an adjusted model revealed significant effects of Social Activity ($t=3.34$, $p=0.002$), Eating Pattern ($t=2.63$, $p=0.02$) and General Activity ($t=2.01$, $p=0.05$) but not Sleep ($t=1.20$, $p=0.24$) on EPDS score. This adjusted model accounted for 64% of the variance and had a large effect size ($F^2=1.55$).

Table 5.2: Pearson ($r$) correlations between EPDS and the 4 BRIAN subscales

<table>
<thead>
<tr>
<th></th>
<th>EPDS</th>
<th>Sleep</th>
<th>Activity</th>
<th>Social</th>
<th>Eating</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPDS</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep</td>
<td>0.53**</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>0.50**</td>
<td>0.51**</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social</td>
<td>0.60**</td>
<td>0.53**</td>
<td>0.63**</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Eating</td>
<td>0.51**</td>
<td>0.48**</td>
<td>0.38*</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

* $p<0.01$  
** $p<0.001$

5.5 Discussion

In a group of women in their third trimester of pregnancy, we observed a significant relationship between mild depressive symptoms and disruptions in daily rhythms. While we initially observed a strong correlation between subjective sleep quality and depressive
symptoms, this was attenuated after accounting for daily rhythm disruptions. Analysis of the four BRIAN domains suggested that disruptions in social rhythms, eating patterns and general activity were significantly associated with depressive symptomatology. Our observed lack of association between depressive symptoms and subjective sleep is in contrast to the majority of previous studies (Mellor, Chua, and Boyce 2014; Jomeen and Martin 2007; Goyal, Gay, and Lee 2007; Okun et al. 2011b; Skouteris et al. 2008; Okun et al. 2013b), but is in line with one study which found that other psychological factors (such as anxiety) may mediate this relationship (Swanson et al. 2011).

To our knowledge, our study is the first to show a relationship between daily activity patterns (social, general and eating behaviours) and symptoms of depression during pregnancy. These findings are consistent with previous studies linking daily rhythms to changes in affect (Germain and Kupfer 2008) and the “social rhythm hypothesis” linking changes in social rhythms to changes in affect (Ehlers, Frank, and Kupfer 1988). Many polysomnographic studies of non-pregnant MDD patients indicate that sleep disruptions are state dependent (Tsuno, Besset, and Ritchie 2005), which suggests that prenatal depressive symptoms themselves may be the root cause of the daily rhythm disruptions observed in our study. This relationship may be bi-directional as disruptions in daily rhythms may further impair one’s ability to adhere to a normal daily rhythm, decreasing day-to-day functioning and potentially increasing the severity of depressive symptoms. A recent study of bipolar patients found that BRIAN score was a highly significant predictor of psychosocial functioning (Pinho et al. 2015), lending some preliminary support to this hypothesis. However, only a longitudinal study measuring depressive symptoms and daily rhythms at multiple time points can evaluate this hypothesis.

The results of our study must be interpreted within the context of our study’s limitations. We attempted to avoid any interference from medication effects by excluding patients taking psychotropic medication. This decision biased our sample in favour of
patients who were stable and euthymic or had lower levels of depressive symptoms. As such, scores on both the EPDS and BRIAN scale were biased towards the low to moderate level of symptom severity, limiting the generalizability of our results. While we did observe an association between daily rhythms and mild depressive symptoms, the cross-sectional design of our study limited our ability to ascertain the directionality of this relationship (i.e. whether depressive symptoms preceded daily rhythm disruptions or vice versa). It is also important to note that the behavioural proxies of circadian rhythmicity assessed by the BRIAN have not been validated against traditional gold standard circadian rhythm assessments. As such, we are unable to draw any conclusions about the relationship between prenatal depressive symptoms and underlying circadian rhythmicity. Furthermore, we did not measure morningness-eveningness preference (chronotype), an inheritable quality (Roenneberg and Merrow 2007) which can be altered during pregnancy (Leonhard and Randler 2009). We also did not examine the contributory effect of anxiety, perceived stress, family psychiatric history or the presence of sleep disorders, which may have also influenced our results (Okun et al. 2013b; Swanson et al. 2011).

5.6 Conclusions

Despite these limitations, our observed association between daily rhythm disruptions and symptoms of depression during the third trimester is unique. Furthermore, the effect size of our regression model incorporating EPDS, BRIAN and PSQI scores was large ($F^2=0.82$)(Cohen 1992), further suggesting that daily rhythm disruptions are associated with prenatal depressive symptoms. It is also possible that prenatal depressive symptoms themselves may lead to disruptions in daily rhythms, which in turn could affect day-to-day psychosocial functioning and further exacerbate depressive symptoms.
Given the importance of circadian rhythms in the pathophysiology of depression, prospective studies examining the relationship between subjective daily rhythm disruptions, objective measures of circadian rhythmicity and severity of prenatal depressive symptoms are warranted.
Figure 5.1: Full (a) and partial (b) Pearson ($r$) correlations between EPDS, PSQI and BRIAN scores
References


Chapter 6
Sleep Disruptions and Depressive Symptoms Across the Perinatal Period

Our finding that daily rhythm disruptions but not subjective sleep quality was associated with depressive symptoms during pregnancy was against our a priori hypothesis. These findings warranted a longitudinal investigation of these factors while also assessing objectively measured sleep parameters and exploring indices of circadian rhythmicity. This chapter describes the results of this investigation. The contents of the chapter was submitted to the Journal of Affective Disorders and is currently under review.
Trait differences in the relationship between sleep and depressive symptoms during the perinatal period

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6.1 Abstract

**Objective:** The relationship between sleep, circadian functioning and MDD may be particularly relevant to the etiology of perinatal depression. No studies to date have examined whether history of single vs. multiple depressive episodes influence the link between perinatal sleep parameters and perinatal mood. The objective of this study was to assess whether perinatal sleep disruptions differed by diagnostic history and whether a history of MDEs modulated the relationship between sleep disruptions and depressive symptoms across the perinatal period.

**Methods:** Fifty-two euthymic women participated in a longitudinal study with two visits, one during the third trimester of pregnancy and one at 12 weeks postpartum. Subjective sleep quality, daily rhythm disruptions and mood were evaluated with self-rated questionnaires, while objective sleep parameters were assessed using Actigraphy.

**Results:** We found trait level differences in the relationship between sleep and perinatal depressive symptoms. Euthymic women with a history of recurrent MDEs had more disrupted subjective and objective sleep and daily rhythms during pregnancy relative to euthymic controls. However, during the postpartum period, these parameters appeared to normalize or even strengthen. Longitudinal analysis from late pregnancy to 12 weeks postpartum supported this finding, suggesting that the coupling of external zeitgebers to internal rhythms improved and normalized over time.

**Conclusions:** Our study is the first to show that a history of recurrent MDEs is associated with trait level differences in sleep disruptions and depressive symptoms during the perinatal period. Our study highlights the potential importance of interventions meant to stabilize sleep and circadian rhythms during the perinatal period.

**Keywords:** perinatal; sleep; circadian rhythms; actigraphy
6.2 Introduction

Between 7.4% and 12.8% of women suffer from depression during pregnancy while 5.5% to 19.2% develop depression during the postpartum period (Gavin et al. 2005; Bennett et al. 2004). Electroencephalography (EEG) studies of depressed patients indicate greater sleep latency, greater Wake After Sleep Onset (WASO), increased levels of non-restorative (Stage 1) sleep, shorter Rapid Eye Movement (REM) latency, and reduced levels of restorative Slow Wave Sleep (SWS) relative to controls (Armitage 2007). These disruptions in the sleep wake cycle point to disruptions in the underlying circadian rhythms (Turek 2007). The importance of these rhythms in the pathophysiology of MDD has become increasingly apparent (McClung 2013). Beyond sleep, the circadian clock also regulates several biological processes that are implicated or disrupted in MDD including: glucocorticoid secretion (Kalsbeek et al. 2012), central monoamine activity (Hampp et al. 2008) and immune functioning (Lange, Dimitrov, and Born 2010). Individual polymorphisms in Period genes have also been associated with depressive symptoms (Kennaway 2010), though studies of Clock have failed to show a consistent association (Calati et al. 2010; Kishi et al. 2011). There is also evidence to suggest that patients with a history of MDD may have trait differences in expression of circadian timing proteins (CLOCK, Per and BMAL1) relative to healthy controls (Gouin et al. 2010).

This relationship between sleep, circadian functioning and MDD may be particularly relevant to the etiology of perinatal depression. Significant disruptions in sleep and daily rhythms are integral components of the perinatal period (Mindell, Cook, and Nikolovski 2015). Previous studies examining perinatal sleep quality suggest that poor sleep quality is strongly correlated with the severity of depressive symptoms (Ross, Murray, and Steiner 2005; Bei, Coo, and Trinder 2015; Lawson et al. 2015). Women screening posi-
tive for depression consistently show significantly greater disruptions in subjective sleep quality during both pregnancy (Goyal, Gay, and Lee 2007; Jomeen and Martin 2007; Mellor, Chua, and Boyce 2014) and the postpartum period (Huang, Carter, and Guo 2004; Dørheim et al. 2009). One study assessing sleep and depression as continuous measures supports this relationship (Skouteris et al. 2008), though some have found no relationship (Kamysheva et al. 2010) and others have found that the association can be accounted for by co-occurrence of anxiety symptoms (Swanson et al. 2011). When sleep is assessed objectively (e.g. using actigraphy), the relationship between sleep disruptions and perinatal depressive symptoms is less clear. Some studies have observed a positive relationship between depressive symptoms, total sleep time, sleep latency or WASO (Goyal, Gay, and Lee 2009; Posmontier 2008), while others have failed to show any association (Okun et al. 2013b).

Longitudinal studies examining sleep quality and depressive symptoms from pregnancy to postpartum have also produced mixed results. Subjectively rated insomnia severity during pregnancy has been associated with more severe postpartum depressive symptoms (Dørheim, Bjorvatn, and Eberhard-Gran 2014). Poor subjective sleep quality has been linked to later recurrence (ie. >4 weeks postpartum) (Okun et al. 2009a) and greater risk of recurrence of postpartum depression (PPD) (Okun et al. 2011a). Compared to objective sleep measurements, subjectively assessed sleep quality during pregnancy is consistently more predictive of depressive symptoms during the postpartum period (Bei et al. 2010; Park and Friston 2013; Coo, Milgrom, and Trinder 2014). However, a study from our own group found that changes in objective sleep efficiency but not subjective sleep quality were predictive of changes in depressive symptoms from late pregnancy to early postpartum. This association appeared to be independent of lifetime history of mood disorders (Krawczak et al. personal communication).

Together, the above studies suggest that the association between sleep disruptions
and perinatal depressive symptoms is strongest for subjective rather than objectively assessed sleep. A lifetime diagnosis of MDD is the strongest risk factor for the development of postpartum depression (Wisner, Parry, and Pointek 2002; Robertson et al. 2004) and previous studies point to trait level differences in circadian physiology between individuals with and without a history of MDD (Gouin et al. 2010). However, to date, no studies have examined whether a history of single vs. multiple depressive episodes influences the link between perinatal sleep parameters (objective and subjective) and perinatal mood. The objective of this prospective study was to assess whether subjective and objective perinatal sleep disruptions differed by diagnostic history and whether a history of Major Depressive Episodes (MDEs) modulated the relationship between sleep disruptions and depressive symptoms across the perinatal period.

6.3 Methods

6.3.1 Participants and Design

Fifty-two (52) healthy, euthymic women aged 18 to 45 in their third trimester of pregnancy (≥ 26 weeks gestation) were recruited from the Women’s Health Concerns Clinic at St. Joseph’s Healthcare in Hamilton, Ontario, Canada between February 2013 and October 2014. Women were also recruited through community advertising in midwifery and ultrasound clinics. Women were free of medical comorbidities (eg. gestational diabetes) and were non-smokers. Psychotropic medication use was not an exclusion criterion, provided that the dose had been stable for 12 weeks. This study was reviewed and approved by the Hamilton Integrated Research Ethics Board and all participants provided informed consent before study entry.

The study was a longitudinal design with two visits, one during the third trimester.
of pregnancy and one at approximately 12 weeks postpartum. During the first visit, demographic information was collected and current and past psychiatric diagnoses were assessed. Women completed a series of questionnaires examining symptoms of depression, subjective sleep quality and disruptions in biological daily rhythms (see below). Women were fitted with a wrist worn activity meter (actigraph) which they wore continuously for 72 hours. At approximately 12 weeks postpartum, women returned for the second study visit. During this visit, they repeated the battery of questionnaires from visit 1 and wore the actigraph for another continuous 72-hour period. In total, 23.1% of women (n=12) did not complete the postpartum follow up visit. Seven women were lost to follow up and 5 women were withdrawn due to pre-term delivery and obstetric complications.

### 6.3.2 Clinical Assessments

Psychiatric diagnoses was assessed using the MINI Neuropsychiatric Interview (Sheehan et al. 1997) version 6.0. Current symptoms of depression were assessed using the Edinburgh Perinatal Depression Scale (EPDS) (Cox, Holden, and Sagovsky 1987). Subjective sleep quality was assessed using the Pittsburgh Sleep Quality Inventory (PSQI) (Buysse et al. 1989). The PSQI has been shown to maintain its reliability, factor structure and utility when used in perinatal populations (Jomeen and Martin 2007).

To explore the potential role of underlying circadian rhythmicity, subjective disruptions in daily rhythms were assessed using the Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN) (Giglio et al. 2009). The BRIAN is an 18-item scale which subjectively assesses different behavioural proxies of daily rhythmicity. These include: sleep (5 questions), general activity (5 questions), social rhythms (4 questions) and eating patterns (4 questions). Each item (except #4 which is reverse scored) is
scored on a Likert scale of 1- Not at all to 4- Often and summed to produce a total score. Lower scores are indicative of more stability in daily rhythms and activities, while higher scores are indicative of greater instability. The BRIAN can be assessed by examining the total score (range 18-72) or by examining total scores for individual subdomains. The BRIAN has been previously used to assess subjective daily rhythmicity in patients with bipolar disorder (Giglio et al. 2009).

6.3.3 Actigraphy, Sleep and Circadian Variables

Actiwatch 2 monitors were purchased from Philips Respironics Inc (Murrysville, PA, USA). Previous perinatal actigraphy studies have used varying sampling windows (48 hrs-2 weeks) (Posmontier 2008; Goyal, Gay, and Lee 2009; Bei et al. 2010; Okun et al. 2013b; Park and Friston 2013). We chose to collect data collected in one-minute epochs continuously for 72 hours. Actigraph data was retrieved and processed using Philips Actiware Version 6.0. Sleep duration, onset latency, efficiency and WASO were generated by the Actiware software.

To explore the underlying circadian rhythmicity, the raw actigraphy data was analyzed using a Cosinor procedure. Briefly, this procedure fits activity data to a single cosine wave, generating 3 principle measurements: Midline Statistic Of Rhythm (MESOR), amplitude (a value indicating half the variation within the data) and acrophase (a measure of when peak activity occurs (i.e. phase advance/delay)) (Cornelissen 2014). MESOR and amplitude are further computed into the Circadian Quotient (CQ) (amplitude/mesor), a normalized measure of the strength of an individual rhythm (Levin et al. 2005).

Non-parametric indices of circadian functioning; Intra-daily Variability (IV) and Inter-daily Stability (IS) were also calculated. IV is a measure of rhythm fragmentation and
is calculated as a ratio of the mean square difference between successive measurements and the overall variance in the data. IS is a measure of the strength of coupling between activity rhythms and external zeitgebers (timekeepers). It is calculated as normalized ratio of the variance of the mean 24-hr rhythm and the overall variance in the assessment period (Van Someren et al. 1997).

6.3.4 Statistical Analysis

Subjects were grouped based on the number of MDEs experienced in their lifetime (controls = 0 lifetime episodes, single MDE = 1 lifetime episode, recurrent MDE = >1 lifetime episodes). To account for non-normality within the data, group comparisons for individual variables at each time point were made using the non-parametric Kruskal-Wallis Test. Post-hoc comparisons between groups were computed using Dunn’s Test. Bonferroni correction was applied to control for multiple comparisons.

The relationship between sleep disruptions and depressive symptoms during the third trimester and at 12 weeks postpartum was assessed using linear regression. Regression assumptions were verified using a Global Validation Procedure (Pena and Slate 2006). To meet regression assumptions, third trimester and postpartum EPDS scores required a log transformation. For each model, EPDS score was the dependent variable with group and individuals sleep/circadian variables as factors. To evaluate whether diagnostic history modulated the relationship, each regression model also contained a group × sleep/circadian variable interaction. Main effects were evaluated using a Type III Sums of Squares F-test. For reporting purposes, all β coefficients were transformed using an antilog (eβ) transformation. To assess the relationship between clinical, sleep and circadian variables across time, a mixed models regression procedure was used. Main effects for each regression model were evaluated using Wald’s Chi-squared test.
All statistical analyses were performed using R, version 3.1.3 (http://R-project.org).

6.4 Results

Demographic characteristics of the sample are presented in Table 6.1. Women were aged 31.2 ± 3.8 years and were most often married (88.5%), working full time (75.0%) and multiparous (66.4%). In total 36.6% of women had experienced at least one MDE in their lifetime. The incidence of current anxiety disorders varied by group. Recurrent MDE women had the highest rates (n=3, 37.5%) while single MDE women and euthymic controls were similar (n=1, 9.1% and n=4, 12.5% respectively). Two individuals (3.8%) were currently taking low doses of psychotropic medication (1: citalopram 20mg/day, 2: escitalopram 10mg/day). One individual in the recurrent MDE group had a diagnosis of Bipolar I Disorder. Removal of this individual did not alter our results and therefore, they were not excluded from our analysis. Of the women who completed the study (n=40), 12.5% (n=5) met screening criteria for postpartum depression (EPDS ≥ 13).

6.4.1 Third trimester of pregnancy

As per our entry criteria, all women were clinically stable and none met diagnostic criteria for an MDE according to the MINI. Group differences in clinical, sleep and circadian variables during the third trimester are reported in Table 6.2. Groups did not differ demographically (age, gestational week, parity, pre-pregnancy BMI, all p>0.05) or in subjective sleep quality (PSQI, p=0.324). However, significant group differences were observed in depressive symptoms (EPDS, p<0.001) and subjective disruption in daily rhythms (BRIAN, p=0.001). Post-hoc analysis indicated that women with recurrent MDE had significantly greater depressive symptoms than both single MDE (p=0.01)
Table 6.1: Demographic characteristics of the study sample (n=52)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>St.Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>31.2</td>
<td>3.8</td>
</tr>
<tr>
<td>Gestational Week</td>
<td>29.9</td>
<td>3.9</td>
</tr>
<tr>
<td>Postpartum Week</td>
<td>13.4</td>
<td>1.9</td>
</tr>
<tr>
<td>Pre-Pregnancy BMI</td>
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<td>6.8</td>
</tr>
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<table>
<thead>
<tr>
<th>Marital Status</th>
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<th>%</th>
</tr>
</thead>
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<tr>
<td>Married/Common Law</td>
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<td>88.5</td>
</tr>
<tr>
<td>Separated</td>
<td>4</td>
<td>7.7</td>
</tr>
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<td>Single</td>
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<thead>
<tr>
<th>Parity</th>
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<td>34.6</td>
</tr>
<tr>
<td>Multiparous</td>
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<td>66.4</td>
</tr>
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<td>Maternity Leave</td>
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</tr>
<tr>
<td>Part Time</td>
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</tr>
<tr>
<td>Other¹</td>
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<td>9.6</td>
</tr>
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<table>
<thead>
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<th>Diagnostic Group</th>
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<td>63.5</td>
</tr>
<tr>
<td>Single MDE</td>
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<td>21.2</td>
</tr>
<tr>
<td>Recurrent MDE²</td>
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<td>15.4</td>
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<table>
<thead>
<tr>
<th>Current Anxiety Disorders³</th>
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<th></th>
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<tr>
<td>Euthymic Control</td>
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<td>12.1</td>
</tr>
<tr>
<td>Single MDE</td>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td>Recurrent MDE</td>
<td>3</td>
<td>37.5</td>
</tr>
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<table>
<thead>
<tr>
<th>Dropouts</th>
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</thead>
<tbody>
<tr>
<td>Total</td>
<td>12</td>
<td>23.1</td>
</tr>
<tr>
<td>Euthymic Control</td>
<td>11</td>
<td>33.3</td>
</tr>
<tr>
<td>Single MDE</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Recurrent MDE</td>
<td>1</td>
<td>12.5</td>
</tr>
</tbody>
</table>

¹ Includes: Homemaker, disability, unemployed and student
² One (1) individual had a diagnosis of Bipolar I disorder
³ Includes: Social Anxiety Disorder, Obsessive Compulsive Disorder, Generalized Anxiety Disorder
and control women ($p=0.0003$). Control and single MDE women did not differ in EPDS score ($p=0.724$). Post-hoc analysis of BRIAN scores was similar, indicating that women with recurrent MDE scored significantly higher than both single MDE ($p=0.02$) and control women ($p=0.0008$). Again, no difference was observed between single MDE and control women ($p=0.745$).

No group differences were observed in sleep duration, onset latency or efficiency (all $p>0.05$). A significant difference was observed in WASO ($p=0.013$). Post-hoc comparisons revealed that control women had significantly lower WASO than women with any mood history (single MDE $p=0.04$, recurrent MDE $p=0.03$). No differences in WASO were observed between single MDE and recurrent MDE women. Circadian variables were also similar between groups with no differences observed in acrophase, CQ or IV values (all $p>0.05$). A significant group difference was observed in IS values ($p=0.011$). Post-hoc comparisons revealed that recurrent MDE women had significantly lower IS values than control women ($p=0.009$).

**Sleep and prenatal depressive symptoms**

Subjective sleep assessments were more predictive of prenatal depressive symptoms than objective measures (Table 6.3; Subjective, Adj-$R^2=0.49$; Objective, Adj-$R^2=0.19-0.27$). Subjective sleep quality was a significant predictor of depressive symptom severity (PSQI; $\beta=1.10$, 95%CI= 1.04 to 1.16, $p=0.001$), though a significant PSQI $\times$ group interaction was observed ($p=0.04$), suggesting that diagnostic group modulated the relationship. PSQI scores appeared to be positively associated with depressive symptoms in control and single MDE women, but not recurrent MDE women (Figure 6.1, panel: pregnancy, p.131). Greater subjective disruptions in daily rhythms were also predictive of higher EPDS scores (BRIAN; $\beta=1.05$, 95%CI= 1.02 to 1.07, $p<0.001$). This association did not differ significantly by group (BRIAN $\times$ group, $p=0.244$). Higher sleep
Table 6.2: Group differences in clinical, sleep and circadian variables in the third trimester of pregnancy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=33)</th>
<th>Single MDE (n=11)</th>
<th>Recurrent MDE (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age</td>
<td>30.9</td>
<td>3.9</td>
<td>31.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Gestational Week</td>
<td>29.0</td>
<td>3.4</td>
<td>30.5</td>
<td>3.7</td>
</tr>
<tr>
<td># of Pregnancies</td>
<td>2.4</td>
<td>2.0</td>
<td>2.0</td>
<td>0.6</td>
</tr>
<tr>
<td>BMI</td>
<td>25.3</td>
<td>5.4</td>
<td>24.2</td>
<td>6.5</td>
</tr>
<tr>
<td>EPDS</td>
<td>4.1</td>
<td>3.2</td>
<td>5.2</td>
<td>4.8</td>
</tr>
<tr>
<td>BRIAN</td>
<td>31.3</td>
<td>8.2</td>
<td>32.2</td>
<td>9.4</td>
</tr>
<tr>
<td>PSQI</td>
<td>6.1</td>
<td>3.3</td>
<td>5.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Duration</td>
<td>483.4</td>
<td>59.7</td>
<td>501.7</td>
<td>95.7</td>
</tr>
<tr>
<td>Onset Latency</td>
<td>20.5</td>
<td>22.9</td>
<td>19.9</td>
<td>18.6</td>
</tr>
<tr>
<td>Efficiency</td>
<td>85.1</td>
<td>5.6</td>
<td>81.1</td>
<td>6.6</td>
</tr>
<tr>
<td>WASO</td>
<td>45.0</td>
<td>22.7</td>
<td>65.4</td>
<td>28.3</td>
</tr>
<tr>
<td>Acrophase</td>
<td>0.68</td>
<td>0.35</td>
<td>0.79</td>
<td>0.33</td>
</tr>
<tr>
<td>CQ</td>
<td>0.85</td>
<td>0.15</td>
<td>0.85</td>
<td>0.15</td>
</tr>
<tr>
<td>IS</td>
<td>0.75</td>
<td>0.12</td>
<td>0.70</td>
<td>0.10</td>
</tr>
<tr>
<td>IV</td>
<td>0.86</td>
<td>0.22</td>
<td>0.76</td>
<td>0.19</td>
</tr>
</tbody>
</table>

duration was significantly associated with a lower EPDS score ($\beta = -1.00$, 95%CI= -1.01 to -1.00, p=0.049) while no significant relationship was observed for sleep efficiency, WASO and sleep onset latency (p>0.05). No additional group interactions were observed (all p>0.05).

### 6.4.2 12 weeks postpartum

Group differences in clinical and circadian variables at 12 weeks postpartum are reported in Table 6.4. No significant group differences were observed in PSQI (p=0.335) while BRIAN scores were marginally significant (p=0.06). Significant group differences were observed in depressive symptoms (EPDS, p=0.005). Post-hoc analysis indicated that women with recurrent MDE had significantly greater depressive symptoms than control women (p=0.004), but did not differ from women with single MDEs (p=0.316).
Table 6.3: Relationship between subjective and objective sleep disruptions and depressive symptoms (EPDS) during the third trimester of pregnancy

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Predictor</th>
<th>Model</th>
<th>Model</th>
<th>Adj-R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>PSQI</td>
<td>11.52</td>
<td>0.001</td>
<td>10.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PSQI × Group</td>
<td>3.46</td>
<td>0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRIAN</td>
<td>14.09</td>
<td>&lt;0.001</td>
<td>10.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BRIAN × Group</td>
<td>1.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>4.08</td>
<td>0.049</td>
<td>4.38</td>
<td>0.002</td>
</tr>
<tr>
<td>Duration × Group</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset Latency</td>
<td>0.38</td>
<td>NS</td>
<td>5.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Onset Latency × Group</td>
<td>2.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Efficiency</td>
<td>0.03</td>
<td>NS</td>
<td>3.35</td>
<td>0.011</td>
</tr>
<tr>
<td>Efficiency × Group</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WASO</td>
<td>1.17</td>
<td>NS</td>
<td>4.71</td>
<td>0.001</td>
</tr>
<tr>
<td>WASO × Group</td>
<td>1.23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Differences between single MDE and controls were not significant (p=0.08).

Table 6.4: Group differences in clinical, sleep and circadian variables at 12 weeks postpartum

<table>
<thead>
<tr>
<th></th>
<th>Control (n=22)</th>
<th>Single MDE (n=11)</th>
<th>Recurrent MDE (n=7)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>EPDS</td>
<td>3.7</td>
<td>4.3</td>
<td>7.6</td>
<td>6.8</td>
</tr>
<tr>
<td>BRIAN</td>
<td>35.2</td>
<td>8.9</td>
<td>36.9</td>
<td>13.4</td>
</tr>
<tr>
<td>PSQI</td>
<td>6.1</td>
<td>3.1</td>
<td>6.7</td>
<td>3.8</td>
</tr>
<tr>
<td>Duration</td>
<td>475.8</td>
<td>59.6</td>
<td>511.3</td>
<td>71.8</td>
</tr>
<tr>
<td>Onset Latency</td>
<td>23.6</td>
<td>20.4</td>
<td>15.6</td>
<td>17.3</td>
</tr>
<tr>
<td>Efficiency</td>
<td>84.8</td>
<td>4.4</td>
<td>82.4</td>
<td>5.2</td>
</tr>
<tr>
<td>WASO</td>
<td>43.4</td>
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<td>18.3</td>
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<tr>
<td>Acrophase</td>
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<td>0.30</td>
<td>0.72</td>
<td>0.34</td>
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<tr>
<td>CQ</td>
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</tr>
<tr>
<td>IS</td>
<td>0.75</td>
<td>0.10</td>
<td>0.71</td>
<td>0.10</td>
</tr>
<tr>
<td>IV</td>
<td>0.79</td>
<td>0.27</td>
<td>0.76</td>
<td>0.26</td>
</tr>
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</table>

No significant differences were observed in sleep duration. Marginal group differences were observed for both sleep efficiency and WASO (p=0.075, 0.074 respectively).

A significant difference was observed in onset latency with recurrent MDE women hav-
ing significantly lower values than control women (p=0.02). Values for single MDE women were between controls and recurrent MDE women and were not significantly different from either (p=0.251 and p=0.365 respectively). No group differences were observed in circadian variables (all p>0.05).

### Sleep and postpartum depressive symptoms

Similar to our results in late pregnancy, postpartum subjective sleep assessments were more predictive of postpartum depressive symptoms than objective sleep measures (Table 6.5; Subjective Adj-R²= 0.33-0.50, Objective Adj-R²=0.14-0.25). Subjective sleep quality was not significantly associated with postpartum EPDS (PSQI, p>0.05), while a greater degree of subjective daily rhythm disruption was associated with increased depressive symptom severity (BRIAN; β=1.03, 95%CI= 1.00 to 1.06, p<0.001). No significant group interaction was observed (BRIAN × group, p=0.570). A marginally significant association was observed between greater sleep onset latency and lower depressive symptom severity (p=0.052). Sleep efficiency, WASO and sleep duration were not significantly associated with postpartum EPDS score (p>0.05). No additional group interactions were observed (all p>0.05).

#### 6.4.3 Third Trimester to 12 weeks postpartum

The longitudinal trajectory of each clinical, sleep and circadian variable from third trimester to 12 weeks postpartum was assessed using mixed model regression. The trajectory of most clinical, sleep and circadian variables was flat: EPDS, PSQI, sleep duration, onset latency, WASO, CQ, IS, and IV scores did not change significantly over time (all p>0.05) or interact with diagnostic group (group × time, all p>0.05). BRIAN scores increased significantly (β=3.72, 95%CI 0.60 to 6.84, p<0.001) while acrophase
Table 6.5: Relationship between subjective and objective sleep disruptions and depressive symptoms (EPDS at 12 weeks postpartum)

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Predictor F</th>
<th>Predictor p</th>
<th>Model F</th>
<th>Model p</th>
<th>Adj-R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSQI</td>
<td>0.30</td>
<td>NS</td>
<td>4.91</td>
<td>0.002</td>
<td>0.33</td>
</tr>
<tr>
<td>PSQI × Group</td>
<td>1.69</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRIAN</td>
<td>4.59</td>
<td>&lt;0.001</td>
<td>8.39</td>
<td>&lt;0.001</td>
<td>0.50</td>
</tr>
<tr>
<td>BRIAN × Group</td>
<td>0.57</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration</td>
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<td>NS</td>
<td>3.57</td>
<td>0.010</td>
<td>0.25</td>
</tr>
<tr>
<td>Duration × Group</td>
<td>2.11</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>3.21</td>
<td>0.018</td>
<td>0.22</td>
</tr>
<tr>
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<td>NS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Efficiency</td>
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</tbody>
</table>

decreased significantly ( β=-0.05, 95% CI -0.18 to 0.08, p=0.041). No significant group × time interactions were observed for BRIAN or acrophase (p>0.05). While sleep efficiency did not change significantly over time (p=0.226), there was a marginally significant group × time interaction. For recurrent MDE women sleep efficiency increased, but for single MDE and control women it remained unchanged (Figure 6.2, p.132, p=0.070). Similarly, while no effect of time was observed for IS values (p=0.245) IS increased from pregnancy to postpartum for recurrent MDE women but remained unchanged in control and single MDE women. This group × time interaction also achieved a marginal level of significance (Figure 6.3, p.133, p=0.066).

**Sleep and depressive symptoms from pregnancy to postpartum**

To examine whether the relationship between sleep disruption and depressive symptoms differed over time by diagnostic group, we examined 3-way interactions (epds ~ time × group × sleep variable) for each subjective and objective sleep variable. No significant interaction was observed for sleep duration and sleep onset latency (p>0.05). However,
significant interactions were observed for the remaining 4 sleep variables. These interactions are depicted in Figures 6.1 (p.131) and Figures 6.4-6.6 (p.134-136). For subjective sleep quality, the positive association with depressive symptoms remained consistent from pregnancy to 12 weeks postpartum in single and control women. However, the lack of association observed in recurrent women during the third trimester became a positive association postpartum (Figure 6.1 p.131, panel: pregnancy; PSQI, p=0.005). A similar pattern was observed for subjective disruptions in daily rhythms, though it only achieved a marginal level of significance (Figure 6.4, p.134; BRIAN, p=0.052). A lack of association was observed between sleep efficiency and depressive symptoms for all diagnostic groups during pregnancy. However at 12 weeks postpartum, greater sleep efficiency was associated with lower depression symptom severity in single MDE and recurrent MDE women (Figure 6.5, p.135; Sleep Efficiency, p=0.039). Lastly, significant pregnancy-postpartum differences were observed in the relationship between WASO and depressive symptoms. During pregnancy, no relationship was observed between WASO and EPDS score. However at 12 weeks postpartum, increased WASO was associated with higher EPDS scores in recurrent MDE and single MDE women, but not controls (Figure 6.6, p.136; WASO p<0.001).

6.5 Discussion

Consistent with previous studies from our group (Krawczak et al, personal communication), we observed significant differences in subjective and objective measures of sleep in women at higher risk for PPD (i.e those with a history of recurrent MDEs). Our study is however, the first to investigate how diagnostic history may modulate changes in sleep from late pregnancy to early postpartum. We found that euthymic women with a history of recurrent MDEs appear to have more disrupted sleep and daily rhythms during
pregnancy relative to euthymic controls (greater WASO and lower IS). However, during the postpartum period, these parameters appear to normalize or even strengthen as recurrent MDE women showed improvement in sleep efficiency and reduced sleep onset latency relative to euthymic controls. Longitudinal analysis from late pregnancy to 12 weeks postpartum supports this finding. While these group × time interactions were marginally significant (p=0.076 and p=0.066 respectively) sleep efficiency and IS values increased from late pregnancy to 12 weeks postpartum for recurrent MDE women but not for euthymic controls or single MDE women, suggesting that the coupling of external zeitgebers to internal rhythms improved and normalized over time.

Two previous longitudinal studies using objective sleep parameters reported increases in sleep efficiency and decreases in WASO from late pregnancy to 6 weeks postpartum (Lee, Zaffke, and McEnany 2000; Signal et al. 2007), and one study has found the exact opposite (Matsumoto et al. 2003). However, these were relatively small studies (n=29, 19 and 10 respectively) and were conducted in healthy populations.

Consistent with the majority of previous research (Goyal, Gay, and Lee 2007; Jomeen and Martin 2007; Skouteris et al. 2009; Bei et al. 2010; Mellor, Chua, and Boyce 2014) we found that subjective sleep quality (PSQI) was a stronger predictor of depressive symptoms during the third trimester of pregnancy. In line with previous studies from our group we also found that subjective disruptions in daily rhythms were highly predictive of prenatal depressive symptoms (Simpson et al, personal communication). During the postpartum period, daily rhythm disruptions were a significant predictor of depressive symptoms, but in contrast to previous studies (Huang, Carter, and Guo 2004; Dørheim et al. 2009) no relationship was observed between postpartum depressive symptoms and subjective sleep quality. While objective sleep measures explained some of the variance in depressive symptom severity, no measure was a significant predictor of EPDS score at either time point. This is consistent with some (Bei et al. 2010; Okun et al. 2013b;
However, the presence of significant 3-way interactions between sleep and circadian parameters (subjective sleep, daily rhythms, sleep efficiency and WASO), diagnosis and time indicates that diagnostic history modules this relationship. Our preliminary results suggest that subjective (acrshortpsqi and BRIAN), but not objective (sleep efficiency and WASO) sleep is consistently associated with depressive symptoms in euthymic women from late pregnancy to early postpartum. In contrast, for women with a lifetime history of recurrent MDEs, depressive symptoms appear to be independent of both subjective and objective sleep disturbances during pregnancy. However during the postpartum period, this independence is not maintained, as a positive association is observed between sleep disturbances and depressive symptoms.

This lack of association between sleep and depressive symptoms during pregnancy for women with recurrent MDEs suggests that prenatal depressive symptoms may differ categorically by diagnostic group. Consistent with findings from large systematic reviews euthymic women with a history of recurrent MDEs had a greater incidence of subclinical depressive symptoms across the perinatal period (Robertson et al. 2004; Lancaster et al. 2010). The strong correlation observed between the EPDS and PSQI during pregnancy has led some researchers to suggest that prenatal depressive symptoms may be an artifact of sleep disturbances (Mellor, Chua, and Boyce 2014). While this is difficult to evaluate empirically, our results suggest that depressive symptoms experienced by euthymic control women may be have been state dependent (i.e. due to sleep disturbances or other psychological factors), while those experienced by women with a history of MDD may be a trait phenomena. This idea of trait depressive symptoms is consistent with the well-established persistence of residual depressive symptoms in remitted MDD patients (Fava 1999).
Our results suggest that disrupted sleep and rhythm patterns in late pregnancy normalize by 12 weeks postpartum in women with recurrent MDEs. This may be due to the strong zeitgeber effect of the newborn baby. During the early postpartum period, mother’s rhythms are significantly disrupted by the irregular rhythm of the newborn. However over time, maternal rhythms strengthen and synchronize with those of the newborn suggesting that the newborn may be acting as a strong zeitgeber. (Wulff and Siegmund 2000; Tsai et al. 2011; Thomas et al. 2014).

In our study, rates of probable postpartum depression (n=5, 12.5%) were similar to those seen in the general population, but lower than expected for the at risk psychiatric population we studied. This suggests that a normalization of sleep and daily rhythms by 3 months postpartum may be protective against the development of postpartum depressive symptoms. Previous studies have linked advances in circadian phase from the third trimester of pregnancy to 6 weeks postpartum with a greater severity of postpartum depressive symptoms (Sharkey, Pearlstein, and Carskadon 2013). In contrast, we observed a significant phase delay from pregnancy to postpartum, independent of diagnostic group.

Our results also point to subjective daily rhythmicity as an important predictor of perinatal depressive symptoms. Our measure of daily rhythmicity (BRIAN) was the only measure which was predictive of depressive symptoms at both time points. This predictive effect of the BRIAN scale is also consistent with other studies from our group (Krawczak et al. personal communication). Our previous assessment of the BRIAN during pregnancy (Simpson et al. personal communication) suggests that it does not share an overlapping factor structure with the PSQI and further studies are needed to examine its relationship with perinatal depressive symptoms.

Some limitations of our study are noteworthy. Our desire to examine the role of single-episode and recurrent MDEs required all women to be euthymic. As such, our
conclusions regarding the relationship between sleep and depressive symptoms are limited to the low-moderate levels of depressive symptomatology observed in our sample. However, our sample is highly comparable to other longitudinal investigations of sleep across the perinatal period, which strengthens our findings. The duration of actigraph sampling in our study is another limitation. While many previous studies have used similar short (<1 week) sampling windows to assess sleep parameters, assessment of circadian rhythms require longer (weeks-months) assessment intervals. Therefore, our examination of circadian rhythms variables must be considered preliminary and exploratory. Lastly, we chose to assess women at only 2 time points (late pregnancy and 12 weeks postpartum). Because of this, we were unable to observe how sleep and rhythm parameters changed over the first 11 weeks postpartum. Some of the strengths of our study include its longitudinal design, use of both subjective and objective sleep measures, the use of a standardized diagnostic interview and its sample size, which is larger than most prospective studies conducted to date.

In conclusion, our study is the first to show that a history of recurrent MDEs is associated with trait level differences in sleep disruptions and depressive symptoms during the perinatal period. Our results also indicate that diagnostic status modulates both the relationship between sleep and depressive symptoms and the trajectory of sleep parameters from late pregnancy to early postpartum. Our findings also suggest that normalization of sleep and daily rhythms within the first 3 months postpartum may be protective against the development of postpartum depressive symptoms. Our study highlights the potential importance of interventions meant to stabilize sleep and circadian rhythms during the perinatal period. Future studies should continue to examine the role of perinatal sleep and circadian rhythms using both subjective and objective measures, while also evaluating the importance of these disruptions for evaluating risk and managing perinatal depression.
Figure 6.1: The relationship between EPDS and PSQI scored by diagnostic group in the third trimester of pregnancy and at 12 weeks postpartum.

During pregnancy (left panel), a positive relationship between PSQI and EPDS score is evident for control and single MDE women, but not recurrent MDE women (psqi $\times$ group $p=0.04$). However, during the postpartum period (right panel), this interaction is no longer present (psqi $\times$ group, $p=NS$).
Figure 6.2: Changes in sleep efficiency from late pregnancy to 12 weeks postpartum by diagnostic group

Sleep efficiency did not change significantly over time ($p=0.226$) but there was a marginally significant group $\times$ time interaction. For recurrent MDE women sleep efficiency increased, but for single MDE and control women it remained unchanged ($p=0.070$).
No effect of time was observed for IS values ($p=0.245$), however IS increased from pregnancy to postpartum for recurrent MDE women but remained unchanged in control and single MDE women. This group $\times$ time interaction achieved a marginal level of significance ($p=0.066$).
Figure 6.4: 3-way interaction (time × group × variable) in the relationship between daily rhythm disruptions (BRIAN) and depressive symptoms (EPDS) from late pregnancy to 12 weeks postpartum.

For subjective disruptions in daily rhythms, the positive association with depressive symptoms remained consistent from pregnancy (left panel) to 12 weeks postpartum (right panel) in single MDE and control women. However, the lack of association observed in recurrent MDE women during the third trimester became a positive association postpartum though it only achieved a marginal level of significance ($p=0.052$).
Figure 6.5: 3-way interaction (time × group × variable) in the relationship between sleep efficiency and depressive symptoms (EPDS) from late pregnancy to 12 weeks postpartum

A lack of association was observed between sleep efficiency and depressive symptoms for all diagnostic groups during pregnancy (left panel). However at 12 weeks postpartum (right panel), greater sleep efficiency was associated with lower depression symptom severity in single MDE and recurrent MDE women (p=0.039).
Figure 6.6: 3-way interaction (time × group × variable) in the relationship between WASO and depressive symptoms (EPDS) from late pregnancy to 12 weeks postpartum

During pregnancy, no relationship was observed between WASO and EPDS score (left panel). However at 12 weeks postpartum, increased WASO was associated with higher EPDS scores in recurrent MDE and single MDE women, but not controls (p>0.001).
References


Chapter 7

The Sleep-Inflammation Interaction and its Relation to Depressive Symptoms

In this chapter we describe the investigation of our third objective; to bring together the two lines of work (inflammation and sleep disturbances) to assess how these two etiological components may interact. The content of this chapter has not been submitted for publication.
7.1 Introduction

Sleep and circadian rhythms are two of the core pathophysiological components of Major Depressive Disorder (MDD) (McClung 2013). Sleep disruptions are highly prevalent (Tsuno, Besset, and Ritchie 2005) and circadian rhythms regulate several other biological processes implicated in MDD including: glucocorticoid secretion (Kalsbeek et al. 2012), central monoamine activity (Hampp et al. 2008) and immune functioning (Lange, Dimitrov, and Born 2010). This relationship between sleep, circadian functioning and MDD may be particularly relevant to the etiology of perinatal depression. Significant disruptions in sleep and daily rhythms are integral components of the perinatal period (Mindell, Cook, and Nikolovski 2015) and previous studies examining perinatal sleep quality suggest that poor sleep is strongly correlated with the severity of depressive symptoms (Ross, Murray, and Steiner 2005; Bei, Coo, and Trinder 2015; Lawson et al. 2015).

Previous studies have shown the sleep deprivation has profound effects on immune activation and circulating cytokines (Born et al. 1997; Irwin 2002; Lange, Dimitrov, and Born 2010). This sleep-mediated immune activation may also be particularly relevant to the perinatal period. Okun and colleagues have proposed that sleep disruptions in early gestation may increase adverse pregnancy outcomes through increases in systemic inflammation (Okun et al. 2009b). This heightened inflammation may also increase risk for the development of depressive symptoms as perinatal depressive symptoms often co-occur with other inflammatory morbidities of pregnancy such as: preeclampsia, gestational diabetes and preterm birth (Osborne and Monk 2013). To date, cross-sectional studies examining the relationship between inflammation and perinatal depressive symptoms are mixed with some showing positive relationships (Azar and Mercer 2012; Cassidy-Bushrow et al. 2012; Christian et al. 2009; Shelton, Schminkey, and
To date, three studies (all conducted by Okun and colleagues) have examined how pregnancy-related sleep disruptions may affect circulating cytokine levels. In a study of 35 pregnant women, greater disruptions on the Pittsburgh Sleep Quality Index (PSQI), were correlated with higher levels TNF-α during the first trimester. Longer sleep latency was also correlated with lower IL-4 during the second trimester. No significant relationships were observed between any sleep parameters and IL-6, IL-10 or CRP (Okun and Coussons-Read 2007). In another study using a subset of these participants (n=19), PSQI scores during the third trimester were associated with significantly higher circulating IL-6 and with lipopolysaccharide (LPS) stimulated IL-6. Lower sleep efficiency and duration was also associated with increased LPS-stimulated production of IL-6 in second and third trimester (Okun, Hall, and Coussons-Read 2007). The final study by Okun and colleagues examined whether pregnancy sleep disruptions were associated with inflammatory cytokines and risk for adverse pregnancy outcomes. The authors also evaluated whether depression augmented the sleep-cytokine relationship (n=32 depressed, n=136 controls). Depressed women with short sleep duration (<7 hours) had greater increases in IL-8. Poor sleep efficiency (<85%) was also associated with increased IL-6 and daytime napping was associated with increased TNF-α. Depressed women with insomnia symptoms and shorter sleep duration had modestly smaller infants while greater interferon (IFN)-γ was significantly associated with preterm birth (Okun et al. 2013a).

The above research highlights the important relationship between sleep and depressive symptoms during the perinatal period and how this relationship may be affected by
systemic inflammation. Studies examining sleep and inflammation during the perinatal period, though limited, provide preliminary evidence of an association between pregnancy sleep disruptions and increases in inflammatory markers. One previous study by Okun and colleagues (Okun et al. 2013a) suggests that this relationship may be enhanced in depressed individuals. To date, studies examining this relationship have only used subjective assessments of sleep parameters. This is significant, given the established dissociation between subjective (eg. PSQI) and objective (eg. polysomnography) measurements of sleep quality (Buysse et al. 2008). Therefore the objective of our study was to examine whether perinatal subjective and objective sleep parameters were associated with changes in inflammatory markers (IL-6, IL-10, TNF-α and CRP) and how this relationship may affect perinatal depressive symptoms.

7.2 Method

7.2.1 Participants and Design

A subset of thirty-one (31) healthy pregnant women participating in a longitudinal study examining sleep disruptions and perinatal depressive symptoms (Chapter 4) were used for this analysis. Inclusion/exclusion criteria are described in detail elsewhere (pp.77). Briefly, the study was a longitudinal design with two study visits, one during the third trimester of pregnancy (≥ 26 weeks gestation) and one 12 weeks postpartum. During the first visit, demographic information and psychiatric diagnoses were assessed. Women completed a series of questionnaires examining symptoms of depression, subjective sleep quality and disruptions in biological daily rhythms (see below). Women were then fitted with a wrist worn activity meter (actigraph) which they wore continuously for 72 hours. A blood sample was taken to quantify levels of IL-6, IL-10, TNF-α
and CRP. At 12 weeks postpartum, women returned for a second study visit where they repeated the battery of questionnaires, provided another blood sample and wore the actigraph for another continuous 72-hour period.

### 7.2.2 Clinical Assessments

Psychiatric diagnoses were assessed using the MINI Neuropsychiatric Interview (Sheehan et al. 1997) version 6.0. Current symptoms of depression were assessed using the Edinburgh Perinatal Depression Scale (EDPS). The EPDS shows good specificity and is sensitive to change in depressive symptoms over time (Cox, Holden, and Sagovsky 1987). Subjective sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al. 1989). The PSQI has been shown to maintain its reliability, factor structure and utility when used in perinatal populations (Jomeen and Martin 2007).

To explore the potential role of underlying circadian rhythmicity, subjective disruptions in daily rhythms were assessed using the Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN) (Giglio et al. 2009). The BRIAN has been used previously to assess daily rhythm changes in patients with bipolar disorder. It assesses different behavioural proxies of daily rhythmicity including: sleep, general activity, social rhythms and eating patterns. It also assesses predominant chronotype (morning or eveningness preference).

### 7.2.3 Actigraphy, Sleep and Circadian Variables

Actiwatch 2 monitors were purchased from Philips Respironics Inc (Murrys ville, PA, USA). Previous perinatal actigraphy studies have used varying sampling windows (48 hrs-2 weeks) (Posmontier 2008; Goyal, Gay, and Lee 2009; Bei et al. 2010; Okun et al. 2013b; Park, Meltzer-Brody, and Stickgold 2013). We chose to collect data collected
in one-minute epochs continuously for 72 hours. Actigraph data was retrieved and processed using Philips Actiware Version 6.0. Sleep duration, onset latency, efficiency and wake after sleep onset (WASO) were generated by the Actiware software.

To explore the underlying circadian rhythmicity, the raw actigraphy data was analyzed using a Cosinor procedure. Briefly, this procedure fits activity data to a single cosine wave, generating 3 principle measurements: MESOR (Midline Statistic Of Rhythm), amplitude (a value indicating half the variation within the data) and acrophase (a measure of when peak activity occurs (i.e. phase advance/delay)) (Cornelissen 2014). MESOR and amplitude are further computed into the circadian quotient (amplitude/mesor), a normalized measure of the strength of an individual rhythm (Levin et al. 2005).

Non-parametric indices of circadian functioning; intra-daily variability (IV) and inter-daily stability (IS) were also calculated. IV is a measure of rhythm fragmentation and is calculated as a ratio of the mean square difference between successive measurements and the overall variance in the data. IS is a measure of the strength of coupling between activity rhythms and external zeitgebers (timekeepers). It is calculated as normalized ratio of the variance of the mean 24-hr rhythm and the overall variance in the assessment period (Van Someren et al. 1997).

7.2.4 Biological Samples and Immunoassays

Blood was sampled from each participant between 8:15AM and 3:30PM. For each participant, blood was drawn into a single 10ml serum separator tube and left to clot at room temperature for 30 minutes. Samples were centrifuged at 20°C for 15 minutes at 3000 rpm. Serum was removed, aliquoted and stored at -80°C. Commercial ELISA kits for IL-6, IL-10, TNF-α and CRP were purchased from R&D Systems (Minneapolis,
MN). The published limit of detection and intra/interassay coefficients of variation for these assays were as follows: IL-6: 0.7 pg/ml, <7%; IL-10: 3.9 pg/ml, <7.5%; TNF-α: 0.19 pg/ml, <10.4%; CRP: 0.022 ng/ml, <8.3%. On the day of assay, single aliquots of serum were thawed. All samples were assayed in duplicate and all analyses were performed according to the manufactures’ protocols by an experienced technician (MC) at St. Joseph’s Healthcare Research Laboratory in Hamilton, ON, Canada.

7.2.5 Statistical Analysis

To determine the appropriate covariates for use in our regression analyses, we computed non-parametric (Spearman) correlations between each inflammatory marker and other clinical variables including: age, sampling time, gestational/postpartum week and pre-pregnancy BMI. Any variable with a $\rho$ value $\geq 0.30$ (moderate) during pregnancy or postpartum was included as a covariate in our regression models.

Both biomarker and clinical data were non-normal. Rather than transform them to meet the assumptions for linear regression we chose to assess the impact of sleep and circadian variables on levels of each biomarker using a mixed model regression procedure. Main effects for each regression model were evaluated using Wald's Chi-squared test. Goodness-of-fit for each model was assessed by taking the difference in deviance between the test and null model and dividing by the deviance of the null model ($\Delta$deviance). In each case, the null model contained time and any covariates. Groups of subjective (PSQI, BRAIN), objective sleep (duration, onset, efficiency and WASO) and objective circadian (acrophase, CQ, IS, IV) variables were modelled separately for each cytokine. All statistical analyses were performed using R, version 3.1.3 (http://R-project.org).
7.3 Results

7.3.1 Demographic Characteristics

Demographic and clinical characteristics are summarized in Table 7.1 and Table 7.2 respectively. Study participants were 30.8 ± 4.0 years old and 30.5 ± 4.2 weeks gestation. The majority of participants were married (96.8%), working full time (80.6%), multiparous (58.1%) and had a college or university education (87.2%). About one-third (38.8%) had experienced at least 1 major depressive episode in their lifetime, though none met current diagnostic criteria for a major depressive episode (MDE). Mean EPDS scores were low and did not differ significantly between pregnancy and postpartum visits. At the postpartum visit, 2 individuals (6.4%) met screening criteria for postpartum depression (EPDS ≥ 13).

7.3.2 Sleep parameters and inflammatory makers

Covariates for each cytokine are summarized in Table 7.3. Inflammatory markers did not change significantly from late pregnancy to 12 weeks postpartum (time p > 0.05 for all models). Significant effects for each inflammatory marker are depicted in Table 7.4. Δdeviance values were very low (0.02-0.07) indicating that the contribution of sleep and rhythm parameters to the levels of each inflammatory marker was small.

No significant associations were observed between IL-10, TNF-α or CRP and subjective sleep quality (PSQI) and daily rhythm measures (BRIAN) (all p > 0.05). For IL-6, there was a significant effect of PSQI (β = 0.15, 95% CI: 0.03-0.26, p = 0.02) but not for BRIAN score. Examination of objective sleep parameters was similar. No significant associations were observed between IL-10, TNF-α or CRP and sleep duration, onset, efficiency or WASO (all p > 0.05). Sleep duration (β = 0.009 95% CI: 0.003-0.014, p = 0.002),
Table 7.1: Sample demographics and clinical characteristics (n=31)

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Table 7.2: Clinical characteristics of the study sample (n=31)

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<td></td>
<td>Mean</td>
<td>St.Dev</td>
</tr>
<tr>
<td>EPDS</td>
<td>5.7</td>
<td>4.3</td>
</tr>
<tr>
<td>PSQI</td>
<td>6.2</td>
<td>3.2</td>
</tr>
<tr>
<td>BRIAN</td>
<td>33.4</td>
<td>8.5</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>11.6</td>
<td>3.0</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>4.8</td>
<td>3.4</td>
</tr>
<tr>
<td>Sampling Time</td>
<td>10.6</td>
<td>2.1</td>
</tr>
</tbody>
</table>
Table 7.3: Covariates for each cytokine used in mixed model regression analysis

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>Gestational/Postpartum Week Sampling Time</td>
</tr>
<tr>
<td>IL-10</td>
<td>Sampling Time</td>
</tr>
<tr>
<td>TNF-α</td>
<td>—</td>
</tr>
<tr>
<td>CRP</td>
<td>Sampling Time Pre-pregnancy BMI</td>
</tr>
</tbody>
</table>

efficiency ($\beta=-0.12$, 95% CI:-0.21 to -0.04 ,p=0.003) and WASO ($\beta=-0.03$, 95% CI:-0.049 to -0.007,p=0.01) were all significantly associated with IL-6. No significant relationship was observed between IL-6 and IL-10 for any circadian variable (all p>0.05). A significant negative association was observed between both TNF-α and CRP and acrophase (TNF-α $\beta=-1.75$, 95% CI:-3.51 to -0.001,p=0.05; CRP $\beta=-2.05$, 95% CI:-3.85 to -0.25, p=0.02). A marginally significant association was observed between IL-6 and CRP and IS (both p=0.07).

Table 7.4: Significant main effects from mixed model regression analysis for subjective sleep, objective sleep and objective circadian parameters

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Parameter</th>
<th>$\beta$</th>
<th>95% CI 2.5%</th>
<th>95% CI 97.5%</th>
<th>p</th>
<th>$\Delta$deviance</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>PSQI</td>
<td>0.15</td>
<td>0.03</td>
<td>0.26</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>duration</td>
<td>0.009</td>
<td>0.003</td>
<td>0.014</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>efficiency</td>
<td>-0.12</td>
<td>-0.21</td>
<td>-0.04</td>
<td>0.003</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>WASO</td>
<td>-0.03</td>
<td>-0.049</td>
<td>-0.007</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>acrophase</td>
<td>-1.75</td>
<td>-3.51</td>
<td>-0.001</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>CRP</td>
<td>acrophase</td>
<td>-2.05</td>
<td>-3.85</td>
<td>-0.25</td>
<td>0.02</td>
<td>0.07</td>
</tr>
</tbody>
</table>

To determine whether subjective (PSQI) or objective (duration, efficiency and WASO) sleep parameters were most strongly associated with IL-6, we created a model combining these measures. $\Delta$deviance improved slightly to 0.07 and all objective measures retained their significance (duration $\beta=0.007$, 95% CI: 0.003-0.0125, p=0.001; efficiency $\beta=-0.09$, 95% CI: -0.15 to -0.03, p=0.003; WASO $\beta=-0.02$, 95% CI: -0.04 to -0.001).
0.005, \( p=0.01 \). In contrast, the association between PSQI and IL-6 was only marginally significant (\( \beta=0.08 \), 95% CI: -0.002-0.17, \( p=0.06 \).

### 7.3.3 Relationship with depressive symptoms

To examine whether the associations between individual inflammatory markers and sleep and rhythm parameters were independent of current depressive symptoms, we added EPDS score as a covariate into each of our significant regression models. EPDS score did not emerge as a significant predictor for any inflammatory marker (all \( p>0.05 \)). With the exception of the relationship between TNF-\( \alpha \) and acrophase (\( p=0.06 \)), all previously significant associations were retained and \( \Delta \)deviance values were unchanged.

### 7.4 Discussion

Our study is the first to show that both subjective (PSQI) and objective (duration, efficiency and WASO) sleep parameters have small, but significant associations with IL-6 from late pregnancy to 12 weeks postpartum. Further analysis suggests that this relationship is strongest for objective sleep parameters, as the PSQI failed to retain statistical significance in a combined model. Examination of our \( \Delta \)deviance values suggest that together, sleep duration, efficiency and WASO explain an additional 5% of the variance in IL-6 values. Our exploratory analysis of circadian rhythm variables suggest that reductions in acrophase (phase delay) are associated with increases in CRP and TNF-\( \alpha \) while subjectively assessed rhythmicity (BRIAN) does not appear to be associated with inflammatory status. Current depressive symptoms did not emerge as a significant predictor of any inflammatory marker and the addition of the EPDS as a covariate did not improve the \( \Delta \)deviance of any of our regression models.

Our finding linking IL-6 with poorer sleep is consistent with numerous other stud-
ies showing that IL-6 levels are abnormal following partial or complete sleep deprivation (Born et al. 1997; Irwin 2002; Lange, Dimitrov, and Born 2010). Our results are also consistent with previous perinatal studies showing a significant positive association between IL-6 and PSQI scores as well as a negative association between sleep efficiency, sleep duration and IL-6 values (Okun, Hall, and Coussons-Read 2007; Okun et al. 2013a). Notably, our findings differ in our use of circulating (not stimulated) IL-6 and our use of actigraphy rather than self-reported sleep diaries. In contrast to previous work, we failed observe an association between any of our sleep parameters and TNF-α. This may be due to the lower levels of TNF-α (1.0-1.5 pg/ml vs. 3.9-7.0 pg/ml) in our sample compared to previous studies (Okun and Coussons-Read 2007). We did however observe a marginal association between acrophase and TNF-α in our exploratory analysis of circadian variables. The negative β value indicates that reductions in acrophase (a phase delay) were associated with greater TNF-α levels. This may be similar to the previously observed association between daytime napping and TNF-α (Okun et al. 2013a), though this is largely speculative and further studies are needed.

We failed to observe an association between inflammatory markers and the severity of depressive symptoms from late pregnancy to 12 weeks postpartum. While this is in contrast to several previous studies, our results are consistent with other larger studies of perinatal women with low to moderate levels of depressive symptoms (Blackmore et al. 2011, 2014). Our results suggest that sleep disruptions are stronger contributors to inflammatory status than mild depressive symptoms during the perinatal period. However, this must be interpreted cautiously. While sleep duration, efficiency and WASO were statistically significant predictors of IL-6, they only improved the strength of the model by 5%. This indicates that the effect size of this relationship is likely small. It is possible that the strength of this association is greater in clinically depressed individuals (Okun et al. 2013a), however the mild-moderate level of depressive symptom severity
and low prevalence of probable postpartum depression (n=2, 6.4%) in our sample prevented us from assessing this possibility.

Some limitations of our study are noteworthy. As described, the mean level of depressive symptoms in our study population at both time points was in the subclinical range. The incidence of probable postpartum depression was also approximately half of what is observed in the general population (n=2, 6.4% vs. 10-15%) (Gavin et al. 2005). As such, our conclusions regarding the relationship between sleep and inflammatory markers are limited to the low-moderate levels of depressive symptomatology observed in our sample. The duration of actigraph sampling in our study is another limitation. While previous studies have used similar short (<1 week) sampling windows to assess sleep parameters, assessment of circadian rhythms require longer (weeks-months) assessment intervals. Therefore, our examination of circadian rhythms variables must be considered preliminary and exploratory. Some of the strengths of our study include its longitudinal design, use of both subjective and objective sleep measures and the use of a standardized diagnostic interview.

In summary, our study replicates and extends previous findings by showing a significant association between sleep disruptions and increase in inflammatory markers during the perinatal period. Our study is the first to use both subjective and objective sleep parameters and the first to explore variables of circadian rhythmicity and their relationship to perinatal inflammatory status. Further studies are needed to examine how this sleep-mediated increase in inflammation may affect pregnancy outcomes and risk for perinatal depression.
References


Chapter 8
General Discussion

8.1 Summary of Findings

Similar to other work, our examination of ELISA and MULTIPLEX immunoassays indicates that MULTIPLEX assays may not be sensitive enough to accurately quantify the low levels of cytokines in our samples. Though exploratory, our analysis suggests that this lack of concordance is not due to MULTIPLEX manufacturer or assay filtration method (i.e. magnetic vs. non-magnetic).

We failed to observe a relationship between mild depressive symptoms and concentrations of IL-6, IL-10, TNF-α or CRP during late pregnancy or the early postpartum period. We also did not observe a significant association between pregnancy to postpartum changes in biomarker concentrations and changes in depressive symptoms. We did however observe a significant association between pregnancy IL-6, IL-10 and postpartum depressive symptoms.

In our examination of perinatal sleep disruptions, we observed a strong and significant relationship between mild depressive symptoms and disruptions in daily rhythms during pregnancy and at 3 months postpartum. Examination of subjective sleep quality produced contrasting results. While we failed to observe an association between sleep quality and depressive symptoms in our cross-sectional study of pregnancy (Chapter 5), we did observe an association in our follow up prospective study (Chapter 6). However, in our prospective study we did not evaluate subjective sleep quality (PSQI) and daily
rhythm disruptions (BRIAN) in the same regression model. Regression statistics were very similar for both models (both Adj-R$^2$=0.49), suggesting that the effect of PSQI may have been attenuated in a combined model. Subjective sleep quality was not associated with depressive symptoms at 3 months postpartum. Of note, subjective daily rhythm disruptions (BRIAN) were consistently and strongly associated with depressive symptoms during pregnancy and early postpartum. No other measure was associated with depressive symptoms at both time points.

In our longitudinal study, we observed significant trait level differences in objective measures of sleep in women at higher risk for PPD (i.e those with a history of recurrent MDEs). These women had more disrupted sleep during pregnancy relative to euthymic controls. During the postpartum period, these parameters appeared to normalize or even strengthen as these high risk women showed significant increases in sleep efficiency and significant reductions in sleep onset latency relative to controls. Longitudinal, within-subjects analysis from late pregnancy to 12 weeks postpartum supports this group finding.

Our longitudinal study also revealed significant differences in the relationship between sleep and depressive symptoms across the perinatal period. For women with a history of recurrent MDEs, depressive symptoms during pregnancy appeared to be a trait rather than state phenomena. However, during the postpartum period, the state relationship between sleep and depressive symptoms was restored, as we did not observe any differences between women with different diagnostic histories.

Lastly, our work is the first to show that objective (duration, efficiency and WASO) sleep parameters have small, but significant associations with IL-6 from late pregnancy to 12 weeks postpartum. Examination of our $\Delta$deviance values suggest that together, sleep duration, efficiency and WASO explain an additional 5% of the variance in IL-6 values. Exploratory analysis of circadian rhythmicity suggests that phase delays are
associated with increases in CRP and TNF-α. Subjectively assessed rhythmicity (BRIAN) does not appear to be associated with inflammatory status.

8.2 Implications

Proximate implications of our findings have been discussed in the ‘Discussion’ section of each individual chapter. However, our results have broader implications for the relationship between inflammation, sleep disturbances and depressive symptoms both within and outside the perinatal period.

8.2.1 Inflammation and Perinatal Depressive Symptoms

First and foremost, our failure to observe an association between depressive symptoms and biomarkers of inflammation does not imply that inflammation is not an important pathophysiological component of perinatal depression. As discussed, it is generally accepted that the relationship between inflammation and depression is likely only relevant to a subset of individuals with MDD (Raison, Capuron, and Miller 2006). Furthermore, it is possible that inflammation is only associated with more severe depressive symptoms. Studies examining IL-6 in non-pregnant depressed patients show a broad range of concentrations (0.8-12.4 pg/ml, mean=4.9 pg/ml, Dowlati et al. 2010). By contrast, the mean concentration of IL-6 in our samples was 1.5-1.9 pg/ml. This mean concentration is highly comparable with the concentrations observed in the negative studies by Blackmore and colleagues, who also examined low to moderate levels of depressive symptoms using the EPDS (Blackmore et al. 2011, 2014). Together this suggests that the low to moderate severity of depressive symptoms in our sample may have prevented us from observing an association between inflammation and depressive symptoms.

Our positive findings linking reductions in IL-6 and IL-10 during pregnancy to greater
severity of depressive symptoms postpartum adds to a literature linking reductions in Th2 immunity with adverse pregnancy outcomes. Normal pregnancy is characterized by a decrease in Th1 cytokine production and an accompanying increase in Th2 cytokines. Examination of this Th1:Th2 ratio during pregnancy suggests that a shift in immunity towards Th1 is associated with poorer obstetric outcomes. One study of 50 women noted that increased production of IL-2 and IFN-\(\gamma\) and reduced production of IL-10 was associated with spontaneous abortion and birth of small for gestational age infants (Marzi et al. 1996). Other studies provide indirect evidence for this association. Maternal infections tend to increase the risk of preterm birth (Goldenberg et al. 2008; Pararas, Skevaki, and Kafetzis 2006), while higher BMI is often associated with adverse obstetric outcomes (Kabiru and Denise Raynor 2004). However, advances in our understanding of the immunology of the perinatal period indicate that the conceptualization of this period as a balance between Th1 and Th2 responses is likely an oversimplification (Chaouat et al. 2004; Saito et al. 2010). Researchers are now examining how modulation of immunity by T-regulatory cells (Tregs) may impact these associations. A recent study by Krause and colleagues prospectively examined Tregs in 100 women from 34 weeks gestation to 6 months postpartum. They found that the number of prenatal Tregs predicted EPDS score at 6 months postpartum. Specifically, a greater number of Tregs during pregnancy was positively associated with postpartum depressive symptoms (Krause et al. 2014). Unfortunately, the authors did not examine the effect of these elevated Tregs on IL-6 or IL-10 concentrations, preventing us from directly comparing their result to ours.
8.2.2 Sleep and Perinatal Depressive Symptoms

Our findings suggest that women with a history of MDD continue to experience sleep disruptions, despite remission of depressive symptoms. It is possible that these disruptions are truly trait phenomena, however it is also likely that they are a result of the residual sub-syndromal depressive symptoms observed in these patients (Fava 1999). Unfortunately, our results do not allow us to comment on the directionality of this relationship. Studies of REM sleep patterns in depressed patients suggest that sleep disruptions are state dependent as abnormalities frequently disappear following remission. However, some studies have shown the opposite, suggesting that some disturbances may be trait phenomena and represent a vulnerability in some MDD patients (Tsuno, Besset, and Ritchie 2005). The suggestion that sleep disruptions may cause the development of depressive symptoms has been contentious. Because of the strong association between the two, many researchers have avoided concluding that poor sleep causes depression (Van Moffaert 1994). Others have suggested that the consistent findings linking insomnia with increased risk for depression provide strong enough evidence (Turek 2005). Large studies, systematic reviews and meta-analyses indicate that a diagnosis of insomnia is associated with a 4 to 38 fold increase in risk of developing MDD (Ford and Kamerow 1989; Breslau et al. 1996; Taylor et al. 2005; Franzen and Buysse 2008; Baglioni et al. 2011), suggesting that sleep disruption may be a causal pathway to depression in a subset of individuals.

Our findings suggest that psychological perceptions of sleep quality (i.e. subjective sleep) have a stronger association with depressive symptoms than objective sleep parameters. This is consistent with several studies showing a dissociation between subjective sleep quality and objective sleep parameters (Armitage et al. 1997; Buysse et al. 2008) indicating that cognitive appraisal of sleep may be important in the treatment
of depressive symptoms. Cognitive behavioural therapy (CBT) for insomnia has been shown to improve outcomes in depressed patients (Manber, Blasey, and Allen 2008). One very positive pilot study has shown that CBT for insomnia normalized depression scores in patients with mild depressive symptoms (Taylor et al. 2007). Together, these studies suggest that a similar approach during the perinatal period may provide meaningful, non-pharmacological improvements in mild depressive symptoms. The strong association between daily rhythm disruptions and depressive symptoms provides modern evidence for the relationship between external zeitgebers and depressive symptoms. Social rhythms are of particular interest (Ehlers, Frank, and Kupfer 1988), as social rhythm therapy is efficacious in preventing subsequent mood fluctuations in bipolar patients (Frank et al. 2005). Adequate social support also has important protective effects against perinatal mood disturbances (Beck 2001), but to date no studies have examined social rhythm changes across the perinatal period.

8.2.3 Sleep × Inflammation Interaction and Perinatal Depressive Symptoms

While we found no reliable association between objective sleep disruptions and depressive symptoms, we did find a significant association between these objective parameters and heightened inflammation. Our objective assessments account for an additional 5% of the variance in IL-6 values. This suggests that physiologically poor sleep may drive increases in systemic inflammation, potentially increasing risk for developing depressive symptoms. While we did not observe this interaction in our data, we may have been hampered by our largely healthy population presenting with low-moderate levels of depressive symptoms. These preliminary findings support the conceptualization of perinatal sleep disruptions as significant contributors to allostatic load and warrant
further investigation of their relationship with inflammation and depressive symptoms.

### 8.3 Limitations

While limitations of our individual studies have been summarized, some are worth noting. As mentioned, the low to moderate levels of depressive symptoms observed in our studies is our single greatest limitation. While the incidence of probable postpartum depression in our longitudinal cohort was similar to that observed in the general population (12.5% vs. 10-15%) (Gavin et al. 2005), it was much lower (6.4%) in our subsamples used to examine inflammation. While this limits the generalizability of our results, our assessment of a physically and psychiatrically healthy, medication free perinatal population provided a unique opportunity to examine the role of these biomarkers in the development of perinatal depressive symptoms.

Another major limitation is our sample size. This is particularly important for our studies examining biomarkers of inflammation. Studies of MDD patients have failed to reveal what proportion of MDD patients present with elevated inflammation. Forty percent of hepatitis patients treated with interferon-α develop depressive symptoms (Myint et al. 2009). Coupled with the 8-15% point prevalence of perinatal depression, a community-based sample of approximately 165 individuals would be required to observe 10 individuals with ‘inflammatory depression.’ However, the sample size of our longitudinal assessment of perinatal sleep disruptions (n=52) is among the largest in the literature. Furthermore, we chose our statistical methods carefully, limiting the use of data transformations and using the most powerful analyses at our disposal.

The use of a single postpartum time point (12 weeks) is also a significant limitation. It is possible that the relationships we examined differed significantly depending on the postpartum week. The lack of a 1-2 week assessment point limited our ability to
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assess the interaction between sleep, inflammation and mood during the normalization of hormonal and immune responses. Furthermore, the lack of a third time point gave us limited ability to comment on the directionality of some of our significant findings. In particular, additional time points would have provided us with an opportunity to assess whether sleep disruptions preceded depressive symptoms, or vice versa.

Lastly, our decision to not examine stress, anxiety or HPA axis functioning is also a significant limitation. A high degree of comorbidity exists between depression and anxiety and the importance of the HPA axis in the underlying pathophysiology of perinatal depression cannot be overstated. However while assessments of cortisol rhythmicity would have added considerable depth to our analysis, our smaller sample size would have impaired our ability to form strong conclusions.

Despite these limitations, our studies have a number of strengths. The use of a largely medication free sample allowed us to examine our candidate biomarkers without the confounding medication effects (Hannestad, DellaGioia, and Bloch 2011). This sample also allowed us to assess sleep disruptions independently from the effects of antidepressant medications, which are known to influence sleep physiology (Rush et al. 1998; Armitage 2007). Our studies are also strengthened by longitudinal, within-subjects designs, use of subjective and objective sleep measures and a standardized diagnostic interview.

8.4 Emerging Questions and Future Works

Our work raises a number of questions. Our investigation of inflammatory biomarkers leads us to question the relationship between Th1:Th2 ratio and perinatal depressive symptoms. Preliminary evidence supports an association between Th1:Th2 balance and depression in non-pregnant populations (Myint et al. 2005). This ratio has also been
implicated as a risk factor for negative obstetric outcomes, which are frequently associated with depressive symptoms. A longitudinal study examining this ratio from the first trimester through 12 months postpartum would provide meaningful insights.

Our findings indicate that trait differences in sleep and circadian physiology may exist between women with and without a history of recurrent MDEs. This warrants further investigation in a large longitudinal study from early pregnancy through 6 months postpartum. Individuals should be assessed at more frequent intervals (e.g. monthly) while also assessing physiological markers of stress and circadian physiology (cortisol rhythm and DLMO). This study would provide more in depth information about how sleep disruptions may influence the underlying circadian rhythms and how this interaction may also influence depressive symptoms.

While we failed to observe an association between depressive symptoms and inflammation, these negative results warrant a more thorough investigation. In particular a study recruiting depressed pregnant women only would allow us to determine how changes in inflammatory markers may influence depressive symptoms through the postpartum period. Standardizing the time of blood sampling and limiting the number of different antidepressant compounds included in the sample would further strengthen these results. Examination of cortisol rhythms, estrogen and progesterone levels would also allow us to statistically model the interaction between these hormones, inflammation and depressive symptoms.

Our finding that sleep improvements in the early postpartum period may be protective against the worsening of depressive symptoms raises the question of whether an insomnia-specific CBT intervention would be therapeutically beneficial in a perinatal population. Small studies indicate the insomnia CBT may be an effective and medication free treatment for mild depressive symptoms. The lack of psychotropic medication makes this treatment particularly attractive, as many mothers are weary of the effects
of antidepressants on the unborn fetus.

Lastly, while not explicitly evaluated in our studies, the interactions between sex hormones, HPA axis functioning and inflammation during the perinatal period suggest that perinatal inflammation may be pathophysiologically important for women who are susceptible hormonal changes in mood (i.e. women with premenstrual syndrome/premenstrual dysphoric disorder). Previous studies have shown modest relationships between a history of premenstrual syndromes and PPD (Sylvén et al. 2013; Buttner et al. 2013), though no studies have assessed the role of inflammation.

8.5 Conclusions

In sum, our results fail to show a state association between biomarkers of inflammation and perinatal depressive symptoms, though future studies with more depressed populations are needed. Our work indicates that diagnostic history modulates the relationship between sleep and perinatal depressive symptoms. This diagnostic history also modules the trajectory of these sleep parameters from late pregnancy to early postpartum. Finally, the preliminary work presented in this thesis provides foundational evidence for an interaction between inflammation and sleep disturbances across the perinatal period. Future studies examining how these sleep mediated increases in inflammation may affect the development of depressive symptoms are encouraged.
General Introduction References


Maes, M., et al. 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 (1-3): 85–92.


General Discussion References


