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Poor Sleep Quality, Circulating Pro-Inflammatory Cytokines and Severity and Frequency of Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis (CFS/ME) Symptoms in Women

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UNIVERSITY OF MIAMI

POOR SLEEP QUALITY, CIRCULATING PRO-INFLAMMATORY CYTOKINES
AND SEVERITY AND FREQUENCY OF CHRONIC FATIGUE SYNDROME/
MYALGIC ENCEPHALOMYELITIS (CFS/ME) SYMPTOMS IN WOMEN

By

Sara F. Milrad

A THESIS

Submitted to the Faculty
of the University of Miami
in partial fulfillment of the requirements for
the degree of Master of Science

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August 2016

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Poor Sleep Quality, Circulating Pro-Inflammatory Cytokines and Severity and Frequency of Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis (CFS/ME) Symptoms in Women.

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Thesis supervised by Professor Michael Antoni.

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Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a debilitating illness that is over represented among women and characterized by extreme fatigue and myriad distressing somatic complaints, with no known etiology or putative biomarker, though recent research has shown evidence of immunological abnormalities in CFS/ME. One of the major symptoms of CFS/ME is experiencing unrefreshing sleep or poor subjective sleep quality, which as a construct, can encompass sleep duration, sleep efficiency, sleep latency, and sleep disturbances, among other aspects of sleep quality. The subjective account of poor sleep quality has been consistently shown in CFS/ME, though objective evidence of sleep-related abnormalities by polysomnography or multiple sleep latency tests are inconsistent. Despite evidence of increased fatigue, somatic symptoms, pro-inflammatory cytokines and poor subjective sleep quality in CFS/ME, the association among these variables has not yet been studied extensively in women with CFS/ME, even though the relationship between poor subjective and objective sleep quality and

inflammation, fatigue, and symptom severity has been established in other healthy and chronically ill populations.

Additionally, CFS/ME patients report increased prevalence of mood disorders such as depression. Depression has been linked to both inflammation and poor sleep quality in other contexts, but not yet in CFS/ME. Due to depression's known relationship to increased inflammation and poor sleep quality, I hypothesized that the relationship between poor sleep quality and inflammation would be more salient for more depressed CFS/ME women.

This study sought to examine the main effects of poor sleep quality on pro and anti-inflammatory cytokines, fatigue severity and interference, symptom severity and frequency in CFS/ME women and also measure the moderating effect of depression on the relationship between poor sleep quality and pro and anti-inflammatory cytokines. In total, 95 women with CFS/ME provided blood samples, as well as self-reported measures of poor sleep quality, depressive symptomatology, CFS/ME somatic symptoms and fatigue. On average patients scored in the clinically elevated range for poor sleep quality on the Pittsburgh Sleep Quality Index (PSQI) global score. Multiple regression analyses showed that worse sleep quality overall (PSQI global score) related to more severe and more frequent CFS (non-sleep) symptoms, more fatigue severity and interference in daily life, and greater levels of inflammation (interleukin-2 [IL-2], IL-6, and tumor necrosis factor-alpha [TNF- α]), when controlling for age and educational level.

When the analyses were repeated using sleep quality subscale scores, the poor sleep quality subscale score was positively related to increased pro-inflammatory (IL-2, IL-6, and TNF- α) and anti-inflammatory (IL-10) cytokine levels. Longer sleep latency was positively

related to increased pro-inflammatory and sleep regulating cytokines (IL-1 β and TNF- α). The sleep disturbance subscale score was positively related to increased pro-inflammatory IL-6, which is known to disrupt sleep at increased concentrations. Shorter sleep duration was positively related to TNF- α . Poor sleep efficiency was not related to any pro or anti-inflammatory cytokine studied.

When examining the moderating effect of depression, poor global sleep quality's relationship to IL-4 and IL-6 was positively moderated by depressive symptom severity such that poorer sleep related to greater IL-4 and IL-6 to a significantly greater degree in women reporting more depressive symptoms. Additionally, sleep duration relationship to IL-4 was also positively moderated by depression symptom status such that the association between shorter sleep duration and IL-4 was significantly greater for more depressed women. Importantly, depression, fatigue severity and interference, and CFS symptom severity and frequency were not related to any cytokine.

In conclusion, poorer sleep quality is associated with greater fatigue, CFS symptoms, and inflammation is significant in CFS/ME women, and may be more pronounced in CFS/ME women who are more depressed. These preliminary and novel findings deserve further research using longitudinal study designs to establish the temporality of these associations, and may shed light on the biopsychosocial mechanisms underlying this chronic condition. This work also suggests that subgroups within this heterogeneous population (e.g., depressed women) may respond in distinctive ways to sleep disruptions, which could inform intervention strategies.

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CHAPTER 1: INTRODUCTION

Chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) is a chronic unremitting condition with an estimated worldwide prevalence of 0.8-3.5% (Bhui et al., 2011), and is overrepresented among women (Klimas & Koneru, 2007). The disorder is a mysterious and debilitating inflammatory illness with no known etiology or cure. CFS/ME symptoms include debilitating fatigue, post-exertional malaise, sore throat, and unrefreshing sleep, among other varied somatic symptoms (Fukuda et al., 1994). Research has revealed physiological manifestations of the disease, such as dysregulated cortisol awakening response (CAR) and cytokine expression imbalance, which are associated with sleep disturbances in other contexts (Klimas & Koneru, 2007; A. N. Mariman et al., 2013; K. P. Wright et al., 2015). CFS/ME patients' sleep is typically reported as unrefreshing and/or frequently disturbed (A. N. Mariman et al., 2013). Recent research has identified subjective and objective accounts of poor sleep quality in CFS/ME—possibly identifying different sleep phenotypes (Gotts et al., 2013; A. N. Mariman et al., 2013). Other studies found that CFS/ME patients report poor sleep, even while demonstrating otherwise normal sleep by polysomnography, as compared to healthy age- and gender-matched controls (M. Maes, F. N. M. Twisk, M. Kubera, & K. Ringel, 2012; Neu et al., 2007a).

In addition to experiencing somatic symptoms and poor sleep, CFS/ME patients typically show increased pro-inflammatory cytokine levels when compared to healthy controls (Fletcher, Zeng, Barnes, Levis, & Klimas, 2009; Klimas & Koneru, 2007; Michael Maes, Frank N. M. Twisk, Marta Kubera, et al., 2012; Michael Maes, Twisk, & Ringel, 2012). Elevations in pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α), and relatively lower levels of anti-inflammatory cytokines

(including IL-13) were shown most consistently in CFS/ME patients vs. healthy controls (Fletcher et al., 2009; Gupta, Aggarwal, See, & Starr, 1997; Moss, Mercandetti, & Vojdani, 1999). Increased levels of IL-2 were also implicated in veterans with CFS/ME (Pandi-Perumal, Cardinali, & Chrousos, 2007). However, no individual cytokine, set of cytokine expression profiles, or biomarker has been consistently and conclusively found to be a diagnostic marker or known etiological factor in CFS/ME (Broderick et al., 2010). Discrepancies in the CFS/ME cytokine research may be due in part to cytokine measurement issues (Pandi-Perumal et al., 2007). Inflammatory cytokine levels can also differ by gender, in part because of estrogen's immunomodulatory effects, including in the context of CFS/ME (Klimas & Koneru, 2007; Smylie et al., 2013). The sleep and inflammation literature is not always stratified by gender, which may account for some inconsistencies in the literature.

Sleep Problems and Inflammation

Central to ongoing research in psychoneuroimmunology, including the role of inflammation in sleep, is the finding that inflammatory processes are subject to neural control, and furthermore, are influenced by psychological processes. In this homeostatic process, the brain acts on the thymus and immune cells in the bone marrow via neuroendocrine and autonomic pathways to stimulate T cells, macrophages, and other cells of the lymphoid system, which give rise to various changes in circulating levels of soluble factors such as pro- and anti-inflammatory cytokines (Pandi-Perumal et al., 2007).

Sleep difficulties are reported for at least a third of the United States population, and this is considered a health risk, as sleep problems are implicated in the detriment of

cognitive function, and physiological and psychological well-being (M. R. Irwin, Witarana, Caudill, Olmstead, & Breen, 2014; Weil et al., 2009). Sleep deprivation and loss results in an activation of the immune system that is evident on a cellular and genomic level (M. R. Irwin, Wang, Campomayor, Collado-Hidalgo, & Cole, 2006; M. R. Irwin et al., 2014).

Immune functioning can be disturbed in individuals who suffer from sleep disorders, such as insomnia (A. N. Vgontzas et al., 2002; Weil et al., 2009). Inflammatory cytokines IL-6 and TNF- α are typically elevated in sleep disorders that result in excessive daytime sleepiness, such as sleep apnea and narcolepsy (A. N. Vgontzas et al., 1999). Even in healthy adults, these cytokines are usually elevated after sleep deprivation and may mediate sleep propensity and fatigue the next day (A. N. Vgontzas et al., 1999).

Acute sleep deprivation can have deleterious consequences on health and well-being and inflammation is thought to mediate this outcome directly, and through interaction with glucocorticoids (Alexandros N. Vgontzas et al., 2003; Weil et al., 2009). Sleep loss was shown to activate leukocyte inflammatory gene expression (M. R. Irwin et al., 2008). After a night of experimental sleep deprivation, increased IL-6 and TNF- α and increased activation of various signal transducer and activator of transcription (STAT) proteins were seen in humans (M. R. Irwin et al., 2014). Elevation of these proteins and cytokines result in an activated inflammatory state that is possibly implicated in the pathogenesis of inflammatory disorders, cardiovascular disease, and some cancers (M. R. Irwin et al., 2014). It is reasonable that sleep disruptions in CFS/ME patients may therefore promote increased pro-inflammatory signaling and symptomology, yet little is known about the

precise relationship between aspects of sleep disruption and specific inflammatory and symptomological indicators in this population.

Circadian Nature of Sleep and Cytokines

Many bodily processes, not just the sleep-wake cycle, are subject to circadian entrainment. Tissues in the body operate in part under the rule of the suprachiasmatic nucleus (SCN), which is the master clock of the circadian cycle, unless they have their own tissue-specific circadian clocks, which function independently of the SCN (Logan & Sarkar, 2012). In both humoral and cellular immunity, immune activity is rhythmically regulated by circadian control (Coogan & Wyse, 2008). The concentration of and metabolism of circulating immune cells, such as lymphocytes and T cells, along with concentrations and types of cytokines and relevant hormones and receptor molecules are influenced by the circadian clock (Logan & Sarkar, 2012). For example, research has shown that plasma TNF release peaks during sleep (Krueger et al., 1998). Evidence suggests that immune competent cells are heavily influenced by the SCN, but can also act under circadian control independently of the SCN (Logan & Sarkar, 2012).

Research has also shown that a circadian clock protein regulates the expression of inflammatory cytokines (Narasimamurthy et al., 2012). There is mounting evidence that immune byproducts and pathways feed back onto the SCN and influence circadian gene expression (Logan & Sarkar, 2012). These processes are some of many mechanisms that are posited to explain how chronic circadian rhythm disruption can lead to chronic inflammatory illness (Coogan & Wyse, 2008).

The ever-changing concentration and composition of inflammatory markers in the circulation is of significant diagnostic importance in research and clinical medicine. Nocturnal changes in cytokine levels are difficult to measure because of their relatively low baseline levels (Zhou, Fragala, McElhaney, & Kuchel, 2010). Additionally, procuring blood samples throughout the night poses a methodological challenge in research protocols.

Because cytokine imbalance and unrefreshing sleep are typically shown to characterize CFS/ME, further research is needed to look at differences between circulating pro-inflammatory cytokines measured during the day versus during the night, specifically within a CFS/ME population that exhibits poor sleep quality and possibly aberrant sleep-wake behavior. Much of CFS/ME literature that uses serum samples collected in the daytime shows an upregulation of pro-inflammatory cytokines. However, when circulating cytokines were measured throughout the night in a small sample of “purely defined” CFS women (i.e. without comorbid depression), with and without fibromyalgia, only anti-inflammatory IL-10 was upregulated in women with CFS without comorbid fibromyalgia, as healthy controls and women with CFS and comorbid fibromyalgia (Nakamura et al., 2010). The authors mention that anti-inflammatory IL-10 is disruptive to sleep, and that increased IL-10 shows evidence for a shift towards Th2 cytokines that has been previously shown in CFS/ME (Nakamura et al., 2010). Additionally, the authors also note the small sample size and the fact that increased IL-10 was only significant for one of the three methods of measuring inflammatory status in this study (Nakamura et al., 2010). Therefore, these results may not be sufficiently conclusive and the use of a non-depressed sample limits the generalizability of the results in the broader CFS/ME patient population.

Cytokine Role in Sleep Regulation

There exists a wide variety of sleep regulatory substances (SRS), and their characterization goes beyond the scope of this review. The criteria for SRS include that SRSs enhance a sleep phenotype, that inhibition of the SRS should reduce spontaneous sleep, the level of the SRS should correlate with sleep propensity, that SRSs should act on sleep regulatory circuits, and that their levels should correlate with sleepiness during illness and pathology (Krueger, Rector, & Churchill, 2007). Following these criteria, cytokines IL-1 and TNF are considered SRSs since they have been shown to regulate sleep and are a part of the “sleep homeostat,” along with other regulatory molecules such as adenosine (Krueger et al., 1998; Krueger & Majde, 2003; Krueger et al., 2007).

Cytokines affect sleep duration and also sleep architecture, including the proportion of NREM and REM sleep (Krueger et al., 2007). IL-1 and TNF are implicated in increasing slow wave sleep (Krueger et al., 2007). Inhibiting IL-1 or TNF decreased slow wave sleep and injecting the cytokines increases slow wave sleep in duration and intensity—resembling rebound sleep after sleep deprivation and loss (Krueger et al., 2007). Cytokines are uniquely able to enact these changes because their biochemical nature allows them to cross the blood brain barrier (at times) or to be actively taken up by transport proteins. Cytokines are also able to act as neuropeptides and hormones—eliciting changes in signal transduction at the cellular level (Krueger et al., 2007).

In general, pro-inflammatory cytokines promote sleep, and in contrast, anti-inflammatory prevent sleep (Krueger et al., 2007). Specifically, pro-somnogenic cytokines include IL-1 β , IL-1 α , TNF- α , TNF- β , IL-2, IL-6, IL-15, IL-18, epidermal growth factor, acidic

fibroblast growth factor, nerve growth factor, brain-derived neurotrophic factor, neurotrophin 3, neurotrophin 4, glia-derived neurotrophic factor, interferon- α , interferon- γ , granulocyte-macrophage colony stimulating factor and granulocyte stimulating factor (Krueger & Majde, 2003). Anti-somnogenic cytokines include IL-4, IL-10, IL-13, transforming growth factor beta, soluble TNF receptor, soluble IL-1 receptor, and insulin-like growth factor (Krueger & Majde, 2003). Mostly, IL-1 and TNF- α are conclusively found to be directly somnogenic when administered centrally or peripherally (Krueger & Majde, 2003). In rats, IL-6 modulates NREM sleep and is known to contribute to sleepiness, but does not meet full criteria for a SRS (Hogan, Morrow, Smith, & Opp, 2003).

There is also evidence that immune factors act on the SCN to influence circadian rhythm (Coogan & Wyse, 2008). Cytokine receptors exist on cells in the SCN (Coogan & Wyse, 2008). In the context of CFS/ME, aberrant cytokine expression patterns might affect circadian rhythm and fatigue-related symptoms throughout the day. Also, alterations in circadian functioning either reflective of, or as a result of chronic poor sleep quality could affect the homeostatic release of cytokines under circadian influence; however, this remains unknown.

Cytokine Regulation of Sleep During Acute Infection

Deep, restorative sleep is critical during periods of increased wakefulness, stress, or acute illness. When neuronal activity is high (i.e. during increased arousal), glutamate is released (Pandi-Perumal et al., 2007). Excess glutamate increased the concentrations of cytokines such as IL-1 and TNF, which act on sleep homeostasis (Pandi-Perumal et al., 2007). During acute illness, cytokines, among other factors, facilitate sickness behavior

(Pandi-Perumal et al., 2007). Sickness behavior includes increased sleep propensity and fatigue (R. Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008). This process ensures that our bodies direct resources and energy to appropriate sources of infection, so that they are eradicated most efficiently.

During acute illness or infection, TNF- α and IL-1 act to increase the intensity and duration of slow wave sleep during the night (Pandi-Perumal et al., 2007). Because of this, sleep is more deep and restorative during periods of sickness (Pandi-Perumal et al., 2007). NREM sleep is thought to be upregulated instead of REM sleep, because REM sleep uses more energy (Krueger & Majde, 2003). Many other factors and cytokines act synergistically or antagonistically on this process. Fever, the state predominately orchestrated by IL-6, can disrupt sleep (Pandi-Perumal et al., 2007).

Histamine, IL-1 β and TNF- α are released in a state of peripheral inflammation (Pandi-Perumal et al., 2007). Cytokine IL-1 β is released by macrophages and microglial cells during inflammation, which act on T cells (Pandi-Perumal et al., 2007). Release of IL-1 β promotes “sickness behavior,” including sleepiness, loss of appetite, and fever (Kelley et al., 2003; Pandi-Perumal et al., 2007). The cytokine is very somnogenic when administered to the anterior preoptic area, which includes the ventrolateral pre-optic nucleus (VLPO) neurons that it activates (Pandi-Perumal et al., 2007). Inhibition of IL-1 β with antibodies inhibits NREM sleep in rats (Pandi-Perumal et al., 2007). These mechanisms are especially relevant in CFS/ME, where sleep, and cytokine balance are shown to be abnormal after an acute infection and/or trauma. Many of the characteristics of “sickness behavior” are evident in the somatic experience of CFS/ME, and the

relationship between aspects of sleep and inflammatory status may explain sickness behavior type symptoms seen in the context of CFS/ME.

Poor Sleep Quality in Healthy and Chronically Ill Individuals

Poor sleep quality has been implicated in worse health outcomes in various clinical populations and also contributes to diminished physical and psychological well-being in otherwise healthy individuals (Lorton et al., 2006; Okun, Luther, Wisniewski, & Wisner, 2013). In a variety of clinical populations, disrupted sleep has been linked to greater fatigue and poorer health (Lorton et al., 2006). Poor sleep quality can be ascertained objectively by overnight polysomnography and subjectively by questionnaires such as the Pittsburgh Sleep Quality Index (PSQI), which measures sleep quality overall and a number of subscales that measure various aspects of sleep quality (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989).

Sleep is commonly disrupted during the course of a chronic illnesses (Polo-Kantola et al., 2014) and can also be an important etiological, precipitating, or maintaining factor of disease (Lorton et al., 2006). As noted previously, sleep deprivation and loss results in an activation of the immune system, evident on a cellular and genomic level (M. R. Irwin et al., 2006). These effects may be due to physiological dysfunction due to the illness (Lorton et al., 2006). The numerous interactive ways in which various chronic disorders and sleep dysregulation exert bi-directional effects on one another is beyond the scope of this thesis.

In the context of inflammatory disorders such as ankylosing spondylitis, sleep quality overall and its composite parts (PSQI subscales) are positively correlated with

symptom severity and with circulating C-Reactive Protein (CRP) levels (Aydin et al., 2015). In a pediatric population of depressed teens with Crohn's disease, poor sleep quality was significantly worse than healthy controls, with 50% meeting criteria for insomnia (PSQI global > 5) (Benhayon et al., 2013a). Sleep disturbance was linked to abdominal pain, depression, and anxiety, but not inflammatory markers (Benhayon et al., 2013a). However, when the authors performed a 2-factor analysis of the PSQI, the Quantitative latent factor (comprised of habitual sleep efficiency and sleep latency subscales) showed a significant relationship to inflammatory markers (Benhayon et al., 2013a). However, the inflammatory markers measured were specific to inflammatory bowel disease and not necessarily generalizable to CFS/ME (Benhayon et al., 2013a). Nevertheless, CFS/ME is conceptualized as an inflammatory illness, therefore, similarly performed analyses using the PSQI global and subscale scores might also correlate with symptom severity and inflammatory biomarker levels.

Indeed, in a sample of chronically fatigued individuals who were unemployed due to their symptoms, improvement in insomnia (via Acceptance and Commitment Therapy) predicted improvement in fatigue, independently of variables such as pain, depression and anxiety (Kallestad, Jacobsen, Landro, Borchgrevink, & Stiles, 2015). Furthermore, improvement of insomnia predicted cortisol recovery after the Trier Social Stress Test for Groups, independently of its effect on fatigue (Kallestad et al., 2015). Inflammatory markers were not measured in this study, so that effect remains to be researched; however, the cortisol results lends support to the theory that inflammatory markers would be affected, perhaps by way of changes in neuroendocrine regulation. Cortisol dysregulation has been shown in CFS/ME (Nater et al., 2008), which is significant in the context of a

psychoneuroimmunological model of CFS/ME because cortisol is immunosuppressive and chronically dysregulated cortisol can result in altered cytokine profile due to changes in the sensitivity of immune cell glucocorticoid receptors (Bamberger, Schulte, & Chrousos, 1996; Hermann et al., 2006). These results highlight how poor sleep quality and insomnia severity can perpetuate characteristics of CFS/ME on a symptom and biomarker level (Kallestad et al., 2015). The authors note that participants were not evaluated clinically (to establish a CFS/ME diagnosis), therefore, the results of the study may not be applicable to CFS/ME (Kallestad et al., 2015).

Psychoneuroimmunology of Sleep in the Context of Psychological Disorders

As evidenced throughout research mentioned earlier, there exist multi-factorial, multi-modal, and multi-directional relationships between brain, behavior, and immunity, especially in the context of sleep. Sleep is simultaneously a behavior, as well as a physiological state. Health behaviors, psychological states, and emotions affect sleep, which is evidenced at the biochemical level (Okun et al., 2011).

Health promoting or damaging behaviors can modulate both sleep and inflammation (Okun et al., 2011). Moderate and regular practice of health behaviors was associated with lower inflammation in older adults (Okun et al., 2011). Drug and alcohol abuse can affect sleep and immune activation concurrently. In depressed and/or alcoholic subjects, the extent of disordered sleep correlated with altered innate and cellular immune functions (M. Irwin, 2002).

Psychological disorders are frequently comorbid with sleep problems, which is also evidenced by dysregulated immune functioning (Pandi-Perumal et al., 2007). A prime

example is depression, which has been well established as a state that is associated with sleep disruption and immune abnormalities (R. Dantzer et al., 2008). Roughly three quarters of depressed individuals experience problems with sleep (Staner, 2010). However, the causal mechanisms of these connections have not yet been elucidated (Pandi-Perumal et al., 2007). Cytokines have been heavily implicated in the experience of depression (R. Dantzer et al., 2008). Exogenous administration of inflammatory cytokines, for example by cytokine immunotherapy, can cause depressive symptoms (Prather, Rabinovitz, Pollock, & Lotrich, 2009). Additionally, despite successful treatment of depression, abnormal cytokine patterns remain in previously depressed individuals, which suggests that altered cytokine patterns may be trait-specific to depression (Pandi-Perumal et al., 2007). In laboratory studies using animals, very high doses of cytokines induce depressive symptoms and sleep disruption or increases of slow wave sleep, depending on the dose (Pandi-Perumal et al., 2007).

Periods of increased perceived stress have deleterious effects on sleep and immune functioning (K. P. Wright et al., 2015). This process is achieved by autonomic and neuroendocrine means. Heavily cited in psychoneuroendocrinology and psychoneuroimmunology is the concept of allostatic load, which was put forth by Bruce McEwen and colleagues (McEwen, 2000). One of the concepts in allostatic load is the idea that cortisol is immunosuppressive under short term, acute conditions. However, with chronic increased cortisol secretion, the cells and tissues of the body become less responsive to cortisol (i.e., glucocorticoid resistance) (McEwen, 2000). This process is akin to dysregulation seen in other homeostatically regulated bodily processes, such as insulin or leptin resistance. Once glucocorticoid resistance occurs, immune cells are less

responsive to the immunosuppressive actions of cortisol; therefore, cytokine production is relatively uninhibited (McEwen, 2000).

Altered cortisol secretion has been implicated in dysregulated sleep (Juster & McEwen, 2015). Chronic sleep deprivation can be conceptualized as a chronic stressor (K. P. Wright et al., 2015). In this way, sleep and the HPA axis interact to affect immune activation, and this process is further modulated by psychological states and disorders (Juster & McEwen, 2015). Psychological treatment protocols, such as mindfulness meditation, have been developed and tested to help alleviate these comorbid and interacting problems (Black, O'Reilly, Olmstead, Breen, & Irwin, 2015).

Psychological distress, in general, is very common in CFS/ME. CFS/ME is commonly comorbid with depression and/or insomnia. In a study comparing CFS, fibromyalgia and irritable bowel syndrome, CFS/ME sufferers experienced more mood and anxiety disorders (ORs=2.00-4.08 and 1.63-2.32, respectively) than did individuals in the other groups (Janssens, Zijlema, Joustra, & Rosmalen, 2015). The interacting factors consisting of poor sleep quality, cortisol dysregulation, depression, and inflammation may directly and indirectly perpetuate each other, and may be related to sickness behavior in the context of CFS/ME (R. Dantzer et al., 2008).

Depression and Inflammation

Recent research has implicated the role of pro-inflammatory cytokines in the experience of depressive symptomatology. Specifically, pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α mediate “sickness behavior” processes that resemble the somatic experience of depression (R. Dantzer et al., 2008; Robert Dantzer, O’Connor, Freund,

Johnson, & Kelley, 2008; Kelley et al., 2003). These cytokines affect the body systemically and also affect the brain, even if they are produced peripherally (R. Dantzer et al., 2008). Sickness behavior that is induced by circulating peripheral cytokines includes fever, changes in sleep architecture, HPA activation, reduction of food intake, and behavioral inactivation/ withdrawal (R. Dantzer, 2009). These symptoms are similar to what is experienced during CFS/ME and depression. Experimentally, lipopolysaccharide (LPS)-induced secretion of pro-inflammatory cytokines causes symptoms of sickness behavior and depression in animals and humans (R. Dantzer, 2009). When pro-inflammatory IL-2 (Capuron, Ravaut, Miller, & Dantzer, 2004) or IFN- α therapy is administered to patients (e.g., to treat cancer), which induces IL-6 and TNF- α , “psychic misery” or depressive symptoms follow suit (Prather et al., 2009; Raison & Miller, 2011). Circulating IL-6 is especially salient in these processes, as the cytokine acts like a hormone and can cross the blood-brain barrier, thereby linking the mind and body (R. Dantzer, 2009). There is substantial evidence that depressed individuals show increased inflammation. Pro-inflammatory TNF- α (Dannehl et al., 2014; Himmerich et al., 2008) and IL-6 (Alesci et al., 2005) among other cytokines are elevated in depressed individuals, and can change in response to pharmacological agents like anti-depressants (Liu, Ho, & Mak, 2012; Raedler, 2011); however, this is not always consistently shown (Marques-Deak et al., 2007).

Sleep-Related Abnormalities in CFS/ME

A very high prevalence (87-95%) of people suffering from CFS/ME report non-restorative sleep. The symptoms of CFS/ME and insomnia overlap, as fatigue is a core feature of both disorders (Kallestad et al., 2015). Research has shown that many sufferers of CFS/ME have undiagnosed sleep disorders and psychiatric disorders (A. Mariman et al.,

2013). In one study of “pure” CFS/ME patients (i.e. without already diagnosed comorbid sleep disorders), 30.3% were identified with a primary sleep disorder such as sleep apnea or periodic limb movement disorder (A. Mariman et al., 2013). Of the remaining recruits, 89.1% met criteria for insomnia or hypersomnolence and four sleep-specific phenotypes were recognized by hierarchical cluster analysis (Gotts et al., 2013). These results point to the heterogeneity of the experience of CFS/ME, even just within a certain symptom (i.e. unrefreshing sleep). Further research is needed to identify the etiological and mechanistic implications of sleep-specific phenotypes in the context of CFS/ME and to tailor interventions aimed at alleviating sleep and possibly related symptom and biomarker abnormalities shown in these subtypes.

Objective data from polysomnography (PSG) and multiple sleep latency tests (MSLTs) show inconsistent differences between CFS/ME participants and healthy controls. Some studies reported discrepancies in sleep architecture (i.e. increased or decreased slow wave sleep), alpha-intrusions of delta sleep and more microarousals with respect to controls (Jackson & Bruck, 2012; A. N. Mariman et al., 2013; F. Togo et al., 2008). A small sample of pure CFS/ME patients showed worse subjective sleep quality, increased microarousals, and slow wave sleep when compared to healthy controls (D. Neu et al., 2014). When comparing pure CFS/ME patients with sleep apnea patients and healthy controls, CFS/ME patients experienced less sleep duration, longer sleep latencies, worse sleep efficiency, and more microarousals by PSG (Neu et al., 2009). Additionally, when compared to people with sleep apnea, people with CFS/ME exhibited increased evidence of periodic limb movements during sleep and microarousals (Neu et al., 2009). Theoretically, any one of these discrepancies can explain unrefreshing sleep seen in

CFS/ME, however, microarousal index (MAI) was not correlated with subjective poor sleep quality (Neu et al., 2007a). Objective sleep-related differences between CFS/ME and healthy controls are in general inconsistent and do not seem to convincingly explain the severity of subjective poor sleep quality (A. N. Mariman et al., 2013; Neu et al., 2007a). However, this literature measuring objective sleep quality uses CFS/ME samples that vary with respect to exclusion criteria and what objective variables are measured (i.e. microarousal index versus alpha intrusion during delta sleep). Additionally, there exist myriad other variables germane to sleep that are not always analyzed by treatment-as-usual overnight PSG (such as heart rate variability), that may be important in explaining these discrepancies. For example, autonomic functioning is thought to be dysregulated in CFS/ME and heart rate variability measured during overnight predicted subjective sleepiness in CFS/ME (Rahman, Burton, Galbraith, Lloyd, & Vollmer-Conna, 2011; Fumiharu Togo & Natelson, 2013).

There is evidence of a sleep-quality misperception in CFS/ME, such that people with CFS/ME feel as though they have poor sleep, despite experiencing sleep that was sufficient and not significantly different from healthy controls (Neu et al., 2007a). For example, monozygotic twins discordant for CFS/ME did not differ in any sleep parameters by PSG, but reported significantly worse subjective poor sleep quality (Watson et al., 2003). However, unlike insomniacs who tend to underestimate total sleep time, CFS/ME twins accurately perceive total sleep time; the difference lies in sleep *quality* perception. The authors also point out that the study did not measure alpha-intrusions, which might explain the feeling of inadequate sleep (Watson et al., 2003).

Poor subjective sleep quality is commonly assessed using the Pittsburgh Sleep Quality Index (PSQI), which calculates a global composite sleep quality score that is comprised of seven indicators: sleep quality, sleep duration, sleep latency, habitual sleep efficiency, sleep disturbances, daily disturbances, and use of sleep medication (Buysse et al., 1989). By using this measure, subjective sleep quality can be assessed globally or using one or more of the subscales. An exploratory and subsequent confirmatory factor analysis (EFA and CFA) was performed on the PSQI in a large sample of healthy depressed and non-depressed older adults, and a 3-factor model fit the data and included sleep efficiency, sleep quality, and daily disturbances (Cole et al., 2006). A confirmatory factor analysis (CFA) using the factors proposed by Cole et al. was performed on a sample of 413 with CFS/ME, and that model fit the data (Mariman, Vogelaers, Hanouille, Delesie, Tobback, et al., 2012). The global PSQI Cronbach's α for this sample was 0.66, as compared to 0.83 shown in the original healthy sample (Buysse et al., 1989; Mariman, Vogelaers, Hanouille, Delesie, Tobback, et al., 2012). These results highlight that the global PSQI score might be less indicative or relevant to CFS/ME-specific sleep pathology and that the factors sleep efficiency, sleep quality and daily disturbances might be more applicable. However, the authors only performed a CFA of Cole et al.'s model and not an EFA first. Therefore, other factors than those proposed by Cole et al. could have been identified that might be more appropriate in assessing subjective sleep quality components in CFS/ME. Nevertheless, these findings highlight the need for further research in the area of operationalizing sleep-related measures (i.e. at the global, factor and subscale level). It is plausible that different etiological mechanisms or phenotypes can be identified by regressing certain components of the PSQI onto CFS/ME-specific pathological variables.

There is more reliable evidence of poor subjective sleep quality in CFS/ME, as compared to poor objective sleep quality. Some research has suggested an under diagnosis of sleep disorders in CFS/ME and provides evidence that a primary sleep disorder can account for CFS/ME symptoms (Kallestad et al., 2015); however, people suffering from CFS/ME exhibit clinically significant differences in subjective sleep parameters vs. people suffering from primary sleep disorders such as sleep apnea. For example, people with CFS exhibited increased depression and anxiety, higher subjective fatigue, but lower subjective and objective sleepiness (by MSLT), as compared to those with sleep apnea (Neu et al., 2008). Even though there is overlap between the two sleep-related conditions, CFS/ME possesses its own clinically significant differences with respect to fatigue and sleep-related variables. More research is needed to identify differences between various CFS/ME phenotypes and other primary sleep disorders such as insomnia and periodic limb movement disorder.

When compared to healthy controls, a pure CFS/ME sample (with no sleep disorder diagnosis) had worse perceived sleep quality, depression, anxiety, and fatigue, but not sleepiness when compared to healthy controls (D. Neu et al., 2014). Poor sleep quality was significantly worse in a “pure” CFS/ME sample when compared to controls, and this was not explained by any objective differences in PSG or MSLT parameters (Neu et al., 2007a). Another study showed that people with CFS/ME exhibited more subjective sleepiness and worse poor sleep quality as compared to controls, however there was no MSLT to confirm the subjective account of sleepiness (Mariman, Vogelaers, Hanouille, Delesie, & Pevernagie, 2012). Therefore, even among a “pure” sample (i.e. where sleep disorder cannot explain symptoms), sufferers of CFS/ME exhibit more psychological distress,

worse sleep quality, and fatigue as compared to controls. Subjective and objective sleepiness is not always worse in CFS/ME, which may suggest that there are different sleep-specific phenotypes in CFS/ME, even just at the sleep quality -perception level.

Summary: Synthesis of Literature and Directions for Further Research

The robust and consistent finding that subjective sleep quality is worse in CFS/ME as compared to healthy controls highlights the salience of addressing this commonly experienced symptom in CFS/ME. Further research is needed to optimize the assessment and operationalization of poor sleep quality, especially examining different phenotypes within a heterogeneous and ill-defined disorder like CFS/ME. Additionally, poor sleep quality should be linked to other symptoms and variations in neuroimmune biomarkers in CFS/ME. Understanding the associations among these variables may elucidate underlying mechanisms involved in the predisposing, precipitating, and perpetuating aspects of CFS/ME and to design interventions aimed at improving these symptoms synergistically.

CFS/ME is a heterogeneous disorder, with an ever-changing definition; however, research using samples derived from varyingly stringent exclusion criteria ranging from “pure” CFS/ME samples to those who are defined as “chronically fatigued” show most consistently that there exists evidence of significantly worse subjective sleep quality, more psychological distress, and cytokine expression profile imbalance, in favor of pro-inflammatory cytokines. CFS/ME lends itself to being conceptualized as a psychoneuroimmunological and psychoneuroendocrinological disorder, especially with increasing evidence of high prevalence of psychological distress, sleep disturbance and neuroendocrine and immunologic abnormalities in this patient population. Past research in

healthy and chronically ill individuals, as well as experimental animal research, lends support to significant bi-directional relationships between poor sleep quality and pathology on a symptom and biomarker level in general, that would also be plausible in the context of CFS/ME (i.e. linking poor subjective sleep quality, depression, fatigue and pro-inflammatory cytokines).

The variance among sample characteristics and study methodologies in the literature pose challenges in interpreting results, especially with respect to internal and external validity. Sample sizes are typically small (i.e. 15 per group), especially in sleep-related studies of CFS/ME, probably due to the prohibitive cost of polysomnography, among other tests. Furthermore, there lacks consensus on an adequate control group for CFS/ME (i.e. normal or sedentary healthy controls), and therefore, confounding variables may mask a clinically meaningful difference that distinguishes CFS/ME and reflects underlying etiological factors. Operationalization and assessment of variables of interest is inconsistent in this population. Lastly, there is a paucity of research in this area aimed at identifying causal, mediating mechanisms in general, and especially with respect to poor subjective sleep quality, CFS/ME symptoms and inflammation.

The interactive, bi-directional relationships between sleep, inflammation, and pathology have not yet been fully elucidated in healthy individuals, and especially not in a relatively low prevalent, heterogeneous, possibly multi-phenotype disorder such as CFS/ME. However, the importance of examining these interacting factors in this population is crucial, due to the disorder's debilitating nature and the overwhelming evidence of the influence of poor sleep quality in CFS/ME. By further investigating these mechanisms in this inflammatory disorder, causal factors may be identified that generalize

to other somatic, chronic illnesses, and that possibly also apply to the impact of sleep disruptions in healthy individuals. The findings from future studies in this area that identify causal, predisposing, precipitating, and perpetuating mechanisms might improve quality of life in CFS/ME and other comorbid disorders and inform preventive, public health research and policy.

Proposed Study

Given the association between sleep disruptions and illness severity on the one hand and inflammation on the other, I hypothesized that among women with CFS/ME poor sleep quality (higher PSQI global scores) will be associated with greater circulating pro-inflammatory cytokine levels, and more severe and frequent CFS/ME-related symptoms. Specifically I hypothesize that poor sleep quality overall and certain subscales (i.e. sleep disturbances, sleep duration, and sleep latency) will relate to (a) greater levels of circulating pro-inflammatory cytokines, including IL-1 β , IL-2, IL-6 and TNF- α , and (b) greater CFS/ME symptom burden including Centers for Disease Control and Prevention (CDC) core CFS/ME symptom severity and frequency, and greater fatigue severity and fatigue-related interference in daily life.

Aims.

Aim 1a. Main effects of poor sleep quality on CFS/ME symptoms and inflammation

Poor sleep quality has been shown to contribute to greater inflammation in healthy, and in acutely and chronically ill individuals, and in a bi-directional manner, pro- and anti-inflammatory markers themselves affect various elements of sleep quality (Foley, Ancoli-Israel, Britz, & Walsh, 2004; M. Irwin, 2002). Disturbed sleep can be an important

etiological, precipitating, or maintaining factor of many chronic diseases (Lorton et al., 2006). Sleep deprivation and loss results in an activation of the immune system, which is evident on a cellular and genomic level (M. R. Irwin et al., 2014). Immune functioning can be disturbed in individuals who suffer from primary sleep disorders, such as insomnia (A. N. Vgontzas et al., 2002; Weil et al., 2009).

Given the association between sleep disruptions and chronic illness symptom exacerbations on the one hand and inflammation on the other in many disease conditions, I hypothesize that among women with CFS/ME, poor subjective sleep quality (PSQI global scores and subscale scores) (Buysse et al., 1989) will be associated with greater circulating pro-inflammatory cytokine levels, and more severe and frequent fatigue and CFS/ME-related symptoms (Donovan, Jacobsen, Small, Munster, & Andrykowski, 2008; Wagner et al., 2005). Poor sleep quality's relationship to anti-inflammatory IL-4 and IL-10 will be ascertained in exploratory analyses. Data analyses will be confined to women, as CFS/ME is most prevalent in women (Polo-Kantola et al., 2014), and gender exerts effects on sleep and inflammatory biomarker parameters (Suarez, 2008; Vitiello, Larsen, & Moe, 2004).

Aim 1b. Depression as a moderator of main effects of poor sleep quality on CFS/ME disorder variables

Depression is typically comorbid in CFS/ME and in insomnia (A. N. Mariman et al., 2013; Staner, 2010). Additionally, depressed individuals show increased circulating pro-inflammatory cytokines (e.g. interleukin-2 [IL-2], IL-1 β , IL-6 and tumor necrosis factor-alpha [TNF- α]) levels (Himmerich et al., 2008). Depression is also associated with dampened immune cell glucocorticoid receptor sensitivity and theoretically, poorer neuroendocrine regulatory control over pro-inflammatory signaling (Alesci et al., 2005;

Pace, Hu, & Miller, 2007). Thus the impact of disrupted sleep on pro-inflammatory cytokines may be less well governed by glucocorticoid-mediated anti-inflammatory signaling in persons with depression. Though there is considerable overlap in the somatic symptoms and biomarkers in both CFS/ME and depression (Anderson, Berk, & Maes, 2014), there are distinctive, clinically and mechanistically-relevant differences between the two disorders (Griffith & Zarrouf, 2008). Therefore, I plan to test whether the relationship between poor sleep quality and greater circulating pro-inflammatory IL-1 β , IL-2, IL-6, and TNF- α levels varies as a function of the severity of depressive symptoms endorsed by women with CFS/ME. Specifically, I predict that the association between poor sleep quality and inflammation will be significantly higher for women with more severe, clinically-significant depressive symptoms (higher CES-D scores) (Radloff, 1977). Depressive status effects on the relationship between poor sleep quality and anti-inflammatory IL-4 and IL-10 will also be explored.

CHAPTER 2: METHODS

Participants and Procedures

Female participants in this study were recruited from a larger study of stress and coping processes in CFS patients and study findings have been previously published (Hall et al., 2014; Lattie et al., 2012). All participants received a physician-determined CFS diagnosis, as defined by the CDC criteria (Fukuda et al., 1994). Recruitment methods included physician referral, support groups, CFS conferences and advertisements in CFS-related websites. Participants were eligible if they were fluent in English, lived within the study area, and were between the ages of 21 and 75 years.

Potential participants were excluded from the study if they met criteria for schizophrenia, bipolar disorder, or substance abuse or if they were actively suicidal, as assessed by a brief screening measure adapted from the Structured Clinical Interview for the DSM-IV (First, Gibbon, & Spitzer, 1997). Participants were also excluded if they showed markedly diminished cognitive capabilities, as evidenced by making four or more errors on the Short Portable Mental Status Questionnaire (Pfeiffer, 1975). Presence of another condition (e.g. AIDS, lupus, rheumatoid arthritis) that might influence biological processes associated with CFS symptomatology, or taking medications that would modulate immune or neuroendocrine functioning excluded participants from the study. Potential participants were also excluded from the study if they were suffering from untreated obstructive sleep apnea (OSA).

Participants who met criteria signed an informed consent form and scheduled a home visit between the hours of 11 am and 3 pm, where study personnel administered a battery of measures, and a blood sample was drawn by a certified phlebotomist. After completing survey answers and providing blood samples, participants were compensated with \$50.

Measures

Pittsburgh Sleep Quality Index (PSQI). The 19-item PSQI (Buysse et al., 1989) was used to assess 7 components of sleep difficulties for the past 30 days, including subjective sleep quality, sleep latency sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medication, and daytime dysfunction. The seven component scores are rated from 0-3, with 0 being no difficulty and 3 indicating severe difficulty. The composite score added these seven subscale scores to provide a global score ranging from 0-21, where higher numbers indicated poorer sleep quality.

Fatigue Symptom Inventory (FSI). The 14-item FSI assessed fatigue intensity using a 4- item subscale and fatigue interference using a 7-item subscale (Hann et al., 1998). Both subscales were scored on an 11-point scale, where 0 indicated feeling “not at all fatigued” and 10 indicated feeling “as fatigued as I could be” for the 4 fatigue intensity items. For the 7 fatigue interference items, 0 indicated “no interference” and 10 indicated “extreme interference.”

Center for Disease Control and Prevention (CDC) CFS Symptom Inventory. The 21-item CDC CFS Symptom Inventory was used to assess the frequency and severity of

CFS symptoms over the last 30 days (Wagner et al., 2005). Participants were asked yes or no questions about specific symptoms, and if the symptom was present, the symptom was rated based on how often the symptom was present, with 1 indicating “a little of the time” and 5 indicating “all the time.” Then, the participants rated the severity of the symptom on a 5-point scale, with 1 indicating “very mild” and 5 indicating “very severe.”

Center for Epidemiologic Survey Depression Scale (CES-D). The CES-D (Radloff, 1977) is a 20 item measure that assesses depressive symptomatology over the past week. Participants were asked questions such as “I felt sad” and responded on a 4 point scale ranging from “Rarely or none of the time (<1 day)” to “Most or All of the Time (5-7 days).” A score of 16 or above indicates clinically significant depressive symptoms.

Circulating pro-inflammatory cytokines. Blood was centrifuged and plasma stored at -80°C within 4 hours of collection until the samples were assayed in batches and in duplicate. Circulating cytokines IL-1 β , IL-2, IL-4, IL-6, IL-10 and TNF- α were measured from blood plasma as previously described (Fletcher et al., 2009) using the an ELISA-based test (Q-Plex™ Human Cytokine –Screen, Quansys Biosciences Logan, Utah). Images were captured using Quansys Imager, driven by an 8.4 megapixel Canon 20D digital SLR camera, and analyzed using Quansys Software. In order to assure compatibility with measurements of cytokines in previously published studies in the field (Chiswick, Duffy, Japp, & Remick, 2012; Trune, Larrain, Hausman, Kempton, & MacArthur, 2011; Wong et al., 2008), the antigen standard concentrations used by Quansys (R&D) were referenced to “gold standard” for each cytokine represented on the multiplex plate as previously described (Lattie et al., 2012).

Statistical Analyses

Statistical analyses were performed using SPSS version 22.0. Fatigue interference data were winsorized and cytokine data were log- transformed (i.e. $\ln(\text{IL-6}+1)$) and then winsorized. The CDC CFS Symptom variables (severity and frequency) were calculated with the two items related to sleep quality removed. We used multiple regression on completed cases to test our study hypotheses that higher global PSQI scores (indicating worse sleep quality) were associated with greater levels of circulating pro-inflammatory cytokines, more severe and frequent CFS symptoms, and more severe and interfering fatigue. As post-hoc analyses, we repeated the multiple regression analyses using PSQI subscales including: subjective sleep quality, sleep disturbances, sleep latency, sleep efficiency, and sleep duration. Participants' age and educational level were used as covariates, as sleep quality and inflammation are associated with those variables, differentially by gender (O'Connor et al., 2009; Vitiello et al., 2004).

Moderation Analyses

We also hypothesized that the relationship between poor sleep quality and inflammation would be moderated by depressive symptoms, such that worse sleep quality predicted higher levels of inflammation most strongly in persons with increasing depressive symptoms. A CES-D variable was calculated that did not include the item pertaining to sleep quality. For moderation analyses, poor sleep quality and the CES-D depression variables were centered on the mean. An interaction term was created by multiplying poor sleep quality and CES-D scores that were both centered on their means. Models including the depression term (along with sleep quality and age) and the sleep quality*depression

interaction term to predict levels of inflammation were analyzed using the PROCESS SPSS macro (Hayes, 2012). This was repeated for all cytokine outcome measures introduced in Specific Aim 1a.

CHAPTER 3: RESULTS

Preliminary Analyses

Sample Description

Ninety-five women comprised the study sample. The average age of this sample was 51.31 years of age (SD = 11.03), and the majority were non-Hispanic White (76.8%). The remaining 23.2% of the sample identified as Hispanic (18.9%), African American (2.1%), Asian (1.1%) and Biracial (1.1%). Participants were generally highly educated, with 23.3% possessing a graduate degree, 33.7% completed college, and 30.5% having completed some college. Most of the women were either married or in an equivalent relationship (43.2%), divorced (18.9%), or involved in a closed, monogamous relationship with a partner (9.5%). Approximately 18.9% were single, 6.3% were widowed, 2.1% identified as separated or other (1.1%). Additionally, 54.7% had children. Many women were on disability (43.2%). The rest were employed full-time (17.9%) or part-time (8.4%), unemployed (12.6%), retired (11.6%), or identified as a volunteer worker (1.1%) or “other” (5.3%).

Descriptive information on sleep quality, fatigue severity and interference, CFS symptom severity and frequency, and circulating cytokines for the sample is provided in Table 1. Mean levels for cytokines were slightly higher than what was previously reported for CFS/ME cases (Fletcher et al., 2009), as shown in Table 3. All pro- and anti-inflammatory cytokines were positively correlated with one another (Table 4).

Fully 95.7% (91/95) of the sample had clinically-significant poor sleep quality as indicated by PSQI sleep quality scores > 5 (Buysse et al., 1989) and 75.8% had clinically-elevated depressive symptoms, as defined by CES-D ≥ 16 . The sample self-reported increased sleep latency (41.4% reported sleep latency > 60 minutes), low sleep efficiency (30.5% endorsed worst score on subscale), and poor sleep quality (34.7%). Self-reported poor sleep duration seemed less of an issue in this sample (15.8% endorsing sleeping less than 5 hours per night), and worse sleep disturbances was also moderately represented in this sample (23.2% reporting the worst score for that subscale). An itemization of the PSQI sleep disturbances subscale score is shown in Table 9. The most predominant reasons that were endorsed for sleep disturbances included not being able to get to sleep within 30 minutes (48.4%), waking up in the middle of the night or early morning (65.3%), feeling too hot (40.0%), and having pain (52.6%). “Other” reasons were also noted (32.6%).

Primary Analyses

Specific Aim 1a

In order to examine the relationship between global poor sleep quality and CFS/ME symptoms, fatigue, and inflammation, multiple regression was conducted adding age and education into the model as covariates. Poor sleep quality was positively related to circulating pro-inflammatory cytokines IL-2 ($\beta=0.202, p=0.043$), IL-6 ($\beta=0.248, p=0.016$), and TNF- α ($\beta=0.294, p=0.005$), which supported the hypothesis that poor sleep quality was positively related to increased circulating pro-inflammatory cytokines in CFS/ME women (Table 4). Pro-inflammatory cytokine IL-1 β and anti-inflammatory cytokines IL-4 and IL-10 were not related to global poor sleep quality (Table 4).

In a model examining the effect of global poor sleep quality on fatigue intensity and frequency (as measured by the Fatigue Symptom Inventory), poor sleep quality was positively related to greater fatigue intensity ($\beta=0.222$, $p=0.032$), and frequency ($\beta=0.290$, $p=0.005$), when controlling for covariates. In another model examining the effect of poor sleep quality on CDC Total Symptom severity and frequency, poorer sleep quality was positively related to greater symptom severity ($\beta=0.446$, $p<0.01$), and frequency ($\beta=0.457$, $p<0.01$), as shown in Table 4.

PSQI subscale analyses. The PSQI sleep quality, sleep latency, sleep disturbances, sleep efficiency, and sleep duration subscales were analyzed in their relation to pro and anti-inflammatory cytokines using multiple regression, with age and education as covariates (Tables 5-9). When the PSQI sleep quality subscale was entered as the independent variable, worse sleep quality was positively related to increased pro-inflammatory cytokine IL-2, IL-6, and TNF- α , and anti-inflammatory cytokine IL-10; but was not associated with pro-inflammatory IL-1 β or anti-inflammatory IL-4 (Table 5).

When the PSQI sleep latency subscale was added as the independent variable, sleep latency was positively related to pro-inflammatory IL-1 β , and TNF- α levels, but not pro-inflammatory IL-2 or IL-6, nor anti-inflammatory IL-4 or IL-10 levels (Table 6).

Using the PSQI subscale sleep disturbances subscale, worse subjective sleep disturbances were positively related to increased IL-6 (Table 7), but not other pro- or anti-inflammatory cytokines (Table 7). When regressing the separate reasons for sleep disturbances on IL-6, only “Cannot get to sleep within 30 minutes” was positive and significant (Table 9). This is interesting given that IL-6 was not positively related to the

PSQI sleep latency subscale score. PSQI subscale sleep efficiency was not related to any pro or anti-inflammatory cytokine (all p 's > 0.05, Table 8). When sleep duration was entered as the independent variable, shorter sleep duration was positively related to greater pro-inflammatory IL-6 and TNF- α , but not pro-inflammatory IL-1 β or IL-2, nor anti-inflammatory IL-4 or IL-10 (Table 9).

Specific Aim 1b

Depression was examined as a moderator of the relationship between global sleep quality (and selected component subscales) and pro- and anti-inflammatory cytokines. The addition of the sleep*depression interaction term accounted for an additional 6.56% of the variance in IL-4 and an additional 4.34% of the variance in IL-6 (Table 10). The main effect of poor sleep quality on circulating IL-4 was not significant (Table 4). When a sleep*depression effect on IL-4 was examined in decomposition analyses, the interaction was significant only at 1 SD above the mean of the modified CES-D score (modified CES-D=36.18, $p=0.0249$), such that the positive relationship between poor sleep quality and IL-4 was stronger for more depressed CFS/ME women, as shown in Figure 1. When a global sleep quality and depression effect on IL-6 was examined in decomposition analyses, the interaction was significant at the mean of the modified CES-D score (CES-D=24.14, $p=0.0036$) at 1 SD above the mean ($p=0.0031$), such that the positive relationship between poor sleep quality and IL-6 was stronger for more depressed CFS/ME women, as shown in Figure 2. A moderating effect of depression on the association of sleep quality and other cytokines was not found (Table 10).

PSQI subscale analyses. Depression did not moderate the effect of sleep quality, sleep latency, sleep disturbances, or sleep efficiency on any cytokine (Tables 11, 12, 14, and 15). Depression had a significant moderating effect for the association of sleep duration and IL-4, explaining an additional 5.43% of the variance in IL-4 (Table 13). The moderating effect of depression was only significant at 1 SD above the mean CES-D modified score (CES-D= 36.18, $p=.0143$). The positive relationship between shorter sleep duration and IL-4 was stronger for more depressed CFS/ME women, as shown in Figure 3. Fatigue severity or interference, CFS symptom intensity and frequency, and depression severity were not significantly related to any cytokine (all p 's > 0.05).

CHAPTER 4: DISCUSSION

I found that poor sleep quality is independently associated with more severe and interfering fatigue, greater severity and frequency of CFS/ME symptoms, and greater inflammatory cytokine levels in women suffering from CFS/ME. These results underscore the importance of subjective poor sleep quality in the experience of CFS/ME on a symptom and biomarker level. This is the first study of women with CFS/ME to show evidence of subjective sleep quality components at the subscale level in relation to cytokine and symptom parameters.

Concordant with previous research, I found that perceived poor sleep quality is highly prevalent in women suffering from CFS/ME (Gotts, Newton, Ellis, & Deary, 2015). In this sample, nearly all cases were clinically defined “poor sleepers,” as defined by global PSQI score > 5. Characterization of sleep difficulties in the context of CFS/ME is virtually as complex and idiosyncratic as the experience of the illness itself (Gotts et al., 2013). Previous research has demonstrated evidence of disrupted sleep by PSG and sleep questionnaires in CFS/ME, albeit not consistently (Aerenhouts et al., 2014; Ball et al., 2004; Fossey et al., 2004; Jackson & Bruck, 2012; Mariman, Vogelaers, Hanouille, Delesie, & Pevernagie, 2012; Neu et al., 2007a; Daniel Neu et al., 2014; Neu, Mairesse, Verbanck, & Le Bon, 2015; Watson, Jacobsen, Goldberg, Kapur, & Buchwald, 2004; Watson et al., 2003). In this sample, women reported more difficulties with sleep latency (41.1%), sleep efficiency (30.5%), and sleep quality (34.7%), than they did about sleep duration (15.8%) and sleep disturbances (23.2%). The clinical relevance of this needs to be determined using

replication studies with larger samples sizes and objective PSG data, especially in light of evidence of possible sleep-specific phenotypes in CFS/ME (Gotts et al., 2013; Kumano-go, Adachi, & Sugita, 2007).

The major reasons that were endorsed for sleep disturbances included not being able to get to sleep within 30 minutes (48.4%), waking up in the middle of the night or early morning (65.3%), feeling too hot (40.0%), and having pain (52.6%). “Other” reasons were also noted (32.6%). The overrepresentation of these symptoms might reflect different subtypes within this patient population and/or our sample. For example, the first reason (not being able to get to sleep within 30 minutes) might indicate a psychologically/cognitively-driven reason for sleep disturbances (i.e. anxiety disorders and/or insomnia). Similarly, waking up in the middle of the night or early morning, might also reflect underlying psychological or cognitive processes such as depression, which is typified by early morning awakening. Alternatively, both reasons could be caused by organic, biological factors idiosyncratic to CFS/ME women and/or behavioral factors that might be especially salient in CFS/ME women (i.e. spending too much time in bed due to fatigue and/or post-exertional malaise). “Feeling too hot” might signify women who are menopausal in our sample, while “having pain” might reflect CFS/ME women who have comorbid fibromyalgia. Each reason, singularly and in myriad combinations with other reasons, might account for different sleep-specific phenotypes in this patient population, and also might affect the etiological or precipitating, predisposing and/or perpetuating factors of their reported poor sleep quality. However, our sample size is relatively small, and menopausal and fibromyalgia status were not coded in this data set. Further research on this subject is warranted in subsequent studies of this heterogeneous patient population,

especially in the context of personalized medicine and precision interventions, where different approaches may be more or less effective for specific patient phenotypes.

Overall, results provide support for the hypothesis that poor sleep predicts greater illness burden in a population of women with CFS/ME (Gotts et al., 2015). Poor sleep quality is positively related to greater fatigue intensity and frequency, and also CDC symptom severity and frequency. This highlights the salience of subjective sleep quality in women's experience of CFS/ME (Gotts et al., 2015). Poor subjective sleep quality has been consistently shown in CFS/ME, and the present study shows that this finding is relevant to the disorder on a biopsychosocial level, including biomarkers of inflammation, fatigue severity and interference in daily living, and the severity and frequency of heterogeneous CFS/ME-related symptoms.

Sleep is commonly disrupted during the course of many chronic medical conditions and sleep dysregulation is a commonly reported complaint among sufferers of chronic diseases in women (Polo-Kantola et al., 2014). This may be due in part from the symptoms of the illness and/or physiological dysfunction due to the illness (Lorton et al., 2006). Disturbed sleep can also play an etiological, precipitating, or maintaining role in many medical conditions (Lorton et al., 2006) and these results suggest that poor subjective sleep quality might maintain and/or account for some of the variance in CFS/ME symptoms in our sample (Gotts et al., 2015). Poor sleep quality also could cause or exacerbate fatigue and the severity and frequency of CFS symptoms over time; however, this remains to be studied using longitudinal research.

The correlation between poor sleep quality and fatigue has been established in CFS/ME (Daniel Neu et al., 2014) and myriad other contexts. Insufficient sleep (i.e. sleep disruption and/or deprivation), as confirmed by PSG is associated with increased mental and physical fatigue. Therefore, we would expect that CFS/ME women who experience insufficient sleep would experience greater fatigue. Notably, CFS/ME women in this study only reported on sleep quality and did not undergo PSG to objectively confirm their subjective account of poor sleep quality. Sleep literature in CFS/ME gives consistent evidence of poor subjective sleep quality, but not of objective sleep parameters (Watson et al., 2004). There are some measurable PSG differences seen in CFS/ME patients (Neu et al., 2009; Neu et al., 2015); however, the relevance and clinical significance of these findings (e.g. increased microarousals) is a matter of debate (Neu et al., 2007b). Nevertheless, if there truly are no objective markers of insufficient sleep in CFS/ME women, their self-report of poor sleep quality is still clinically relevant and can possibly hint at neurological, psychological and/or nociceptive mechanisms behind this sleep quality misperception (Gotts et al., 2015; Harvey & Tang, 2012).

For example, there might be an unknown variable in CFS/ME women (i.e. biomarker) that accounts for the perception of both poor sleep quality and extreme fatigue. Potentially, there exists a nociceptive phenotype that predisposes a CFS/ME patient to be more sensitive to pain and discomfort, which would hypothetically affect the feelings attributed to physical and mental vigor that typically accompany restful sleep (Harvey & Tang, 2012). In this way, poor sleep quality might affect both fatigue severity and interference by decreasing a fatigue perception threshold, making a person more sensitive to the experience of fatigue and also therefore, increase his/her attention biases towards

fatigue. This might lead to the perception of worse fatigue and overestimating its effect on carrying out daily activities. Indeed, in fibromyalgia, but not CFS/ME, allodynia (increased pain sensitivity) is shown and poor sleep quality fully accounted for the direct positive effect between pain and fatigue (Meeus & Nijs, 2006; Nicassio, Moxham, Schuman, & Gevirtz, 2002). Our sample did not exclude patients who presented with comorbid fibromyalgia, therefore our sample may possess a substantial subpopulation who experience increased sensitivity to pain and/or fatigue; however, the exact number of confirmed fibromyalgia cases is unknown in our sample. Larger sample sizes that represent the heterogeneous nature of CFS/ME women (i.e. with and without comorbid fibromyalgia) are needed to fully answer these questions and identify possible psychological or neuroimmune phenotypes in this patient population (Kumano-go et al., 2007).

Possibly, there exists a previously unmeasured sleep variable or construct that either is not detected by PSG or is not conceptualized or discovered in sleep pathology yet which would explain this apparent misperception (Meeus & Nijs, 2006). Potentially, poor subjective sleep is a harbinger of disease progression that might not yet be detected by lab tests or clinical questionnaires. In summary, there remains a good deal of research left to be conducted in this patient population and in this area of sleep medicine in general, possibly also guiding hypotheses about consciousness and perception during sleep and wakefulness that can be used to generalize conclusions that benefit healthier populations as well.

Poor sleep quality has been shown in myriad acute and chronic diseases, including inflammatory illnesses such as inflammatory bowel disorders and rheumatic disorders

(Bellini et al., 2011; Buchanan et al., 2014; Sariyildiz et al., 2013). The present study findings are in line with prior studies of subjective sleep quality in other chronic medical conditions. In ankylosing spondylitis, a severe, painful inflammatory condition, poorer subjective sleep quality is positively associated with pain, disease activity, and increased limitation of mobility, as well as depression and quality of life (Aydin et al., 2015). In fibromyalgia, poor sleep quality (global PSQI score, and subjective sleep quality, habitual sleep efficiency, and sleep disturbances subscale scores) were negatively correlated with pain threshold (Ağargün et al., 1999). Therefore, we would expect a positive association between uncomfortable and painful symptoms as measured by the CDC questionnaire (e.g. sore throat, tender lymph nodes, muscle aches/muscle pain, pain in joints, stomach or abdominal pain, numbness or tingling, light sensitivity) and poor sleep quality in CFS/ME, which can commonly present with comorbid fibromyalgia. In healthy older adults, poor subjective sleep quality as measured by the PSQI is related to differences in cognitive performance (Nebes, Buysse, Halligan, Houck, & Monk, 2009), which is questioned in part of the CDC questionnaire (e.g. “forgetfulness/memory problems”). Severity and frequency of headache symptoms is also measured by the CDC questionnaire, and poor subjective sleep quality has been shown to be related to chronic daily headache patients (Vazquez-Delgado, Schmidt, Carlson, DeLeeuw, & Okeson, 2004). Additionally, poor subjective sleep quality has been implicated in irritable bowel syndrome (IBS) (Bellini et al., 2011; Buchanan et al., 2014), and gastrointestinal symptoms are represented in the CDC questionnaire analyzed in this study. Therefore, our empirical results corroborate evidence from parallel literatures involving similarly chronically ill, distressed patient populations.

Poor Sleep Quality and Cytokines

The present study found that global and subscale subjective sleep quality scores were associated with significantly higher levels of all of the pro-inflammatory cytokines studied, except IL-1 β (Table 4). Cytokine IL-2 levels were associated with only the global and subscale subjective sleep quality scores (out of all the sleep measures), which may in part be explained by the fact that IL-2 is not a widely cited cytokine to be related to sleep, though it was found to have an enhancing effect on slow wave sleep in one study (Kapsimalis et al., 2008). Interestingly, anti-inflammatory IL-10 was positively associated with the poor sleep quality subscale score, along with pro-inflammatory cytokines IL-2, IL-6, and TNF- α . Anti-inflammatory cytokines (such as IL-10) have been known to inhibit slow wave sleep and also inhibit the synthesis of IL-1 and TNF, which are established somnogenic sleep regulatory substances (SRS) (Opp, 2005). It is impossible to derive the cause and effect or exact function of an increase in anti-inflammatory IL-10 along with increased pro-inflammatory cytokines in relation to poor sleep quality in isolation, using cross-sectional data, especially in the context of at least two homeostatic physiological processes (sleep architecture/propensity and immune functioning) (Krueger et al., 2007; ter Wolbeek et al., 2007; K. P. Wright, Jr. et al., 2015). Potentially, IL-10 is increased in response to increased pro-inflammatory cytokines in order to modulate the inflammatory actions of IL-2, IL-6, and TNF- α and/or their somnogenic effects. In a small scale study of non-fatigued, fatigued, and CFS female adolescents, CFS adolescents showing increased somatic complaints and “sickness behaviors” showed increased mitogen-induced anti-inflammatory IL-10 (ter Wolbeek et al., 2007); however, these variables were not correlated with sleep quality. Additionally, in a small study of CFS women with and

without fibromyalgia compared to healthy controls, CFS women without fibromyalgia showed increased circulating IL-10 when measured throughout the night (Nakamura et al., 2010). Further research is needed to confirm this finding and determine its mechanistic and clinical significance.

I also observed that increased sleep latency is highly prevalent in CFS/ME women with more than 40% of the sample reporting difficulty falling asleep (sleep latency > 60 min.). Multivariate analysis showed that greater sleep latencies related to increased circulating IL-1 β and TNF- α levels. This relationship could be explained by the somnogenic effects of IL-1 β and TNF- α as sleep regulatory substances (Krueger et al., 1998). Therefore, increased IL-1 β and TNF- α levels in CFS/ME women with clinically significant sleep latencies might reflect an actively occurring homeostatic process, where chronic sleep deprivation (by primary insomnia) leads to increased sleep propensity via increased TNF- α and IL-1 β . Possibly, the synergistic effects of other cytokines negate sleep initiation, which perpetuate longer sleep latencies and sleep disruption, further contributing to greater inflammation and symptom severity (Kapsimalis et al., 2008; Pandi-Perumal et al., 2007).

Shorter sleep duration was associated with greater IL-6 and TNF- α levels, which may suggest that short, insufficient sleep covaries with more inflammation in this patient population. Additionally, the sleep disturbance subscale was associated with increased IL-6 levels, possibly in accordance with the finding that IL-6 can disturb sleep in mammals, or that IL-6 expression is increased with disturbed, non-refreshing sleep (Clevenger et al., 2012). In contrast, TNF- α and IL-1 β increase slow wave sleep (unlike IL-6, they do not disrupt sleep at increased concentrations); therefore, one would expect that higher TNF- α

and IL-1 β levels would be positively related to deeper and possibly more undisturbed sleep. I did not observe a negative relationship between sleep disturbances and TNF- α and IL-1 β levels; however, the proportion of women who report sleep disturbances and also show increased TNF- α and IL-1 β levels is much higher in our sample and we may not have the power to observe an effect if that subtype is under represented here. Previous studies have shown that persons with CFS/ME exhibit increased slow wave sleep (Neu et al., 2009), and perhaps this finding is capturing a sleep specific phenotype in our sample (Gotts et al., 2013). Relatedly, when the different items within the PSQI sleep disturbance subscale were regressed on to the cytokines analyzed in this study, only the item relating to longer sleep latency was correlated with circulating IL-6, even though the sleep latency subscale was not positively correlated with circulating IL-6. This analysis should be replicated in another sample using validation by PSG and time-lapsed data.

Interestingly, no cytokine was correlated with poor sleep efficiency, even though poor sleep efficiency was relatively high in this sample (>30% score of 3 on subscale). The discrepancy between poor sleep quality and poor sleep efficiency's relationship to inflammation might reflect important perceptual and/or psychobiological differences between healthy insomniac women and CFS/ME women (Harvey & Tang, 2012; Neu et al., 2015). Relatedly, potentially due to cognitive and/or perceptual idiosyncrasies in insomniacs and CFS/ME, the sleep efficiency ratio that is derived from the PSQI retrospective self-report measure may not be reliable.

Moderating Effect of Depression on Poor Sleep Quality and Cytokines

I hypothesized that the relationships between poor sleep quality and inflammation would be stronger for depressed CFS/ME women due to the established links between poor sleep quality and depression on one hand, and poor sleep quality and inflammation on the other in many different healthy and chronically ill populations. Because depression is also associated with dampened sensitivity of immune cell (monocyte) glucocorticoid receptors (Pace et al., 2007), I reasoned that any influence of sleep disruption on increased pro-inflammatory cytokines might be exaggerated in CFS/ME women with comorbid depressive symptom elevations.

When assessing the moderating effect of depression on the relationship between poor sleep quality (overall) and pro- and anti-inflammatory cytokines in this study, the interaction effect was only positive and significant for IL-4 and IL-6. The significant effect on IL-6 is in line with our hypothesis, which is predicated on the evidence that depression is an inflammatory disorder, and may be accompanied by decreased immune cell glucocorticoid receptor sensitivity. Such conditions would be permissive for prolonged elevations of circulating pro-inflammatory levels in the absence of glucocorticoid-mediated regulation of activated immune cells. Accordingly, I hypothesized that the main effect of poor sleep quality on inflammatory indicators would be more pronounced in depressed women. However, this study did not find a significant depression x sleep interactive effect for any of the other pro-inflammatory cytokines.

Why was the sleep x depression interaction effect limited to these cytokines? First, there was evidence that IL-6 concentrations were elevated in the sample (as compared to

another sample of CFS/ME women). Second, depressed individuals show decreased slow wave sleep (Palagini, Baglioni, Ciapparelli, Gemignani, & Riemann, 2013), which might be reflective of IL-6-induced disrupted, insufficient, light sleep. On the other hand, the mitigating effects of TNF- α and IL-1 β on decreased slow wave sleep, might account for why there is no interaction effect for those pro-inflammatory cytokines. Additionally, the somnogenic effects of TNF- α and other pro-inflammatory cytokines might be so robust, that the variance in these cytokines is not affected by depressive status. Indeed, the main effect between poor sleep quality and TNF- α showed the strongest sleep association of all the cytokines observed ($\beta=0.294$). These explanations are clearly little more than speculation and these hypotheses need to be tested using longitudinal research to clarify theoretical mechanisms underlying the specificity of the sleep x depression interactions observed.

In exploratory analyses of anti-inflammatory cytokines IL-4 and IL-10, depression had a significant moderating positive effect on poor sleep quality and anti-inflammatory IL-4, which was not hypothesized. The sleep and cytokine literature does not heavily implicate IL-4 in modulating sleep; however, there is evidence that there is a shift to Th2 cytokines such as IL-4 during late sleep (Dimitrov, Lange, Ticken, Fehm, & Born, 2004). In later stages of sleep, REM duration increases at the expense of NREM sleep. Depression is typified by REM sleep abnormalities (Palagini et al., 2013) and also by Th1 and Th2 cytokine imbalance, including increased IL-4 (Song, Halbreich, Han, Leonard, & Luo, 2009). When analyzing PSQI subscale scores, depression had a significant moderating effect on the relationship between IL-4 and shorter sleep duration, and marginally for increased sleep latencies. Interestingly, the composite poor sleep quality score was

moderated by depression for both IL-6 and IL-4; however, the poor sleep quality subscale score was not moderated by depression in its association with IL-4 or IL-6. While the other components of the composite score (e.g. sleep duration and latency) were moderated by depression status for their association with IL-4, this did not extend to IL-6. These findings may give rise to more evidence that there are sleep specific phenotypes in CFS/ME women and that psychoneuroimmunological factors may play a role in their characterization.

Though not the focus of this study, depressive status was not significantly correlated directly with any cytokine (all p 's > 0.05). This is surprising given the literature implicating depression as an inflammatory illness. In CFS/ME, a hypothesized neuroimmune illness, one might expect that the relationship between depressive status and inflammation would be apparent. However, these results suggest that sleep quality is more salient than depression, in its direct effect on inflammation, though depressive status still can moderate this relationship in CFS/ME, at least for some cytokines. Additionally, most of the sample were depressed, and any interaction effects were usually observed at or above the mean CES-D scores; therefore, our study might not have had enough power to detect interaction effects in less depressed CFS/ME women. Repeating these analyses using a larger, more heterogeneous sample might better capture any direct and/or interactive effects of depression on sleep and cytokines.

Relatedly, fatigue severity or interference and CDC symptom severity or frequency was not correlated with any inflammatory cytokine. This is not in line with a “sickness behavior” hypothesis that increased inflammation is related to fatigue, among other somatic complaints (R. Dantzer, 2009; Robert Dantzer et al., 2008; Kelley et al., 2003). This result also highlights the importance of poor subjective sleep quality's influence on

inflammation, which is not explained by the effects of fatigue severity or interference. If CFS/ME women suffer from a hypothetical over sensitivity to physical discomfort, fatigue, and/or sleep disturbances, which is evidenced in self-reported data from the PSQI, FSI, and CDC questionnaires, one might expect to see a similar pattern of their relationship to inflammatory markers to all three somatic complaints. However, the results show that only poor subjective sleep quality is related to inflammatory cytokines. This is an important distinction that deserves more attention and research, especially in light of hypotheses that CFS/ME is a form of inflammation-mediated sickness behavior, somatization or depression-related disorder (M. Maes, 2011, 2015; M. Maes & Twisk, 2010; M. Maes, F. N. Twisk, M. Kubera, & K. Ringel, 2012) and also that CFS/ME is due in part by undiagnosed sleep disorders (A. Mariman et al., 2013), among other contentiously debated topics related to this syndrome.

At least in CFS/ME women, it seems that the argument is more nuanced than the generalization that pro and anti-inflammatory cytokines exert opposing effects on sleep quality and vice versa, especially because the sleep effect on each anti-inflammatory cytokine was not consistent (i.e. IL-4 vs IL-10). Additionally, all pro and anti-inflammatory cytokines were positively correlated with one another, possibly reflecting that anti-inflammatory cytokines were concurrently upregulated to offset the possibly overshooting actions of the pro-inflammatory cytokines in this sample of CFS/ME women. This is speculative, however, and there are myriad possible reasons for the discrepancies in these analyses.

There remains the possibility that these results are chance findings, or that there is a third unmeasured variable (i.e. BMI) that is accounting for these effects. The sleep data

are self-reported and retrospective, so I might be making inaccurate inferences on actual sleep architecture and quality and their relationship to cytokines and also self-reported fatigue and CFS symptomatology. Despite these shortcomings and discrepancies however, the novel results reported herein necessitate further research that focuses on the mechanistic importance of poor subjective sleep quality and its clinical relevance in the diagnosis and treatment of CFS/ME women, who often present with comorbid depression.

It was surprising that none of the CFS symptom measures or fatigue measures were directly associated with pro-inflammatory cytokine levels. These results suggest that poor sleep may be linked with inflammation, independent of its relationship with CFS symptomatology, but does not rule out the plausibility of a neuroimmune mechanism. The finding does give rise to more questions about the mechanistic relationship between sleep and these symptoms in the context of CFS/ME. As previously noted, this finding is not indicative of the typical “sickness behavior” hypothesis (Robert Dantzer et al., 2008; Lattie et al., 2012), where fatigue severity and interference would be linked to inflammatory cytokine levels. Further research is needed to determine whether a third variable is mediating sleep’s influence on both inflammation and symptomatology. One candidate may be altered HPA axis functioning, which has been associated with CFS symptoms in recent work (Papadopoulos & Cleare, 2012).

Strengths and limitations. This study is the first of its kind to examine the association between global sleep quality and its components and multiple indices of CFS/ME symptomatology and inflammation in a sample of women diagnosed with CFS/ME. The work provides many leads on the role of disturbed sleep in the maintenance of this

poorly understood condition, which may pave the way for ameliorative interventions. However, any interpretations should be tempered by the limitations of our study.

Our study did not include a healthy control group to determine if the effects of poor sleep in women with CFS/ME were significantly different from age- and gender matched poor sleepers who are otherwise healthy. The fact that these results were obtained in a relatively small cross-sectional study warrant caution in assigning temporal relations between the measured variables. Notably, the PQSI, which was administered at the time of the blood draw, asks participants to reflect back on their sleep for the past 30 days, therefore, we suggest that poor sleep may predate inflammatory cytokine levels and the magnitude of CFS symptoms reported. However longitudinal research is needed to confirm these putative temporal associations.

The study is also limited by the lack of polysomnography data, which could have helped identify if there are objectively-defined sleep specific phenotypes in CFS/ME patients that map onto specific symptom patterns and inflammatory indicators (Gotts et al., 2013; Mariman, Vogelaers, Hanouille, Delesie, & Pevernagie, 2012). These phenotypes may be reflected by or due, in part, to different cytokine expression profiles in CFS/ME patients, as different sleep-regulating cytokines (alone or in combination) may contribute to different sleep difficulty profiles (Lorton et al., 2006). Similar analyses conducted with cytokines measured directly before sleep, or throughout the night, might have revealed different patterns (Nakamura et al., 2013; Nakamura et al., 2010).

While the study excluded potential subjects with untreated obstructive sleep apnea (OSA), previous research has shown that primary sleep disorders are underdiagnosed in

primary care settings (Fossey et al., 2004; Qanneta, 2014). In a recent study, a primary sleep disorder, such as OSA, psychophysiologic insomnia or periodic limb movement disorder, was found in 49.8% of the sample of CFS/ME subjects (A. N. Mariman et al., 2013). If there were a large percentage of women in our sample with undiagnosed OSA, our results may be biased, as OSA affects inflammatory cytokine levels, and subjective psychological, sleep and fatigue-related variables (Kapsimalis et al., 2008). Relatedly, excess weight may also affect the variables mentioned. Therefore, future research should substantiate our results with polysomnography data and should use indicators such as Body Mass Index (BMI) as a covariate. Importantly, our subjects were recruited indiscriminate of fibromyalgia diagnosis, which may affect the generalizability of our results, as there are cytokine expression differences between CFS/ME patients with and without fibromyalgia under certain conditions (Nakamura et al., 2013). Finally, the present findings may have been moderated by other psychological states (e.g., stress) or traits (e.g., coping styles) that have been related to many of the variables investigated here (Benhayon et al., 2013b; Robert Dantzer et al., 2008; Papadopoulos & Cleare, 2012).

CHAPTER 5: CONCLUSION

As hypothesized, several sleep parameters were correlated positively with CFS/ME symptom measures in the present study. Poor subjective sleep quality was associated with greater CFS symptom expression (severity and frequency) and fatigue severity and to fatigue-related interference in daily activities. Poor sleep quality and its subscale components were also related to pro and anti-inflammatory cytokines. Finally, depression status positively moderated some but not all of these sleep-inflammation associations. These findings might shed light on precipitating, and perpetuating factors of CFS/ME symptomology and highlight the need for identifying possible neuroimmunological sleep-related phenotypes in this heterogeneous population, who may benefit from interventions aimed to improve sleep quality and psychological factors synergistically.

Our preliminary findings of significant associations between self-reported sleep quality components and inflammatory indicators on the one hand, and CFS/ME symptomology on the other, justify further research in sleep medicine for CFS/ME patients, specifically addressing the mechanism behind these associations. The interpretations of our results are limited by a cross-sectional design; and the lack of a matched healthy control comparison group, nocturnal cytokine measurements, overnight polysomnography data, and important covariates (e.g., BMI). Using these results as a guide, it is important to conduct longitudinal research on this patient population in the future to identify mediating variables for these relationships using neuroendocrine and mood-related indicators. To realize the clinical implications of these associations, future work might identify and

optimize interventions (e.g., Cognitive Behavior Therapy for Insomnia, CBT-I) (Wu, Appleman, Salazar, & Ong, 2015) aimed at improving sleep latency, or other interventions known to improve overall sleep quality in other populations (e.g., Cognitive Behavioral Stress Management, CBSM) (Vargas et al., 2014) in order to modulate neuroimmune processes and CFS/ME-related symptoms.

TABLES

Table 1. Demographic characteristics of the study sample.

		M	SD
Age		51.31	11.03
		N	%
Ethnic Identification			
	Non-Hispanic White	73	76.8
	Hispanic	18	18.9
	African American	2	2.1
	Asian/Asian American	1	1.1
	Biracial	1	1.1
Relationship Status			
	Single	18	18.9
	Married or Equivalent	41	43.2
	Separated	2	2.1
	Divorced	18	18.9
	Widowed	6	6.3
	In a monogamous relationship	9	9.5
	Other	1	1.1
Employment status			
	Full-Time	17	17.9
	Part-Time	8	8.4
	On Disability	41	43.2
	Retired	11	11.6
	Unemployed	12	12.6
	Volunteer worker	1	1.1
	Other	5	5.3
Education level			
	<8 th Grade	1	1.1
	High school or GED	9	9.5
	Trade school	1	1.1
	Some college	29	30.5
	College graduate	32	33.7
	Graduate degree	22	23.2

Table 2. Descriptive statistics of self-report variables.

	Mean	SD	Range
FSI Intensity (0-40)	27.32	15.33	15.0-38.00
FSI Frequency (0-70)	49.2526	12.96	12-70
CDC Total Symptom Severity	2.1036	0.742	0.33-4.06
CDC Total Symptom Frequency	2.14	0.7785	0.22-4.06
CES-D Total Score	25.6947	12.55	2-57
PSQI Total (0-21)	12.263	3.933	3-20
PSQI Subscales (0-3)*	%=3	%=2	%=1 or 0
PSQI sleep duration	15.8	32.6	51.6
PSQI sleep efficiency	30.5	12.6	56.8
PSQI sleep latency	41.1	22.1	36.8
PSQI sleep quality	34.7	34.7	30.5
PSQI sleep disturbances	14.7	52.6	32.6

*Percentage of sample who reported the worst score for that subscale (3), second-worst score (2), and the least severe scores (0 or 1)

Table 3. Descriptive statistics of biological variables.

(pg/mL)	Mean	SD	Range	Expected for CFS/ME Women*	Expected for Female Controls*
IL-1 β^p	29.093	39.753	4.30-152.55	13.4 (4.5-38.3)	6.2 (4.2-38.3)
IL-2 p	6.7193	6.5909	0.50-24.13	2.3 (1.4-5.4)	2.5 (2.1-3.5)
IL-4 a	3.044	5.2569	0.30-22.59	1.7 (0.9-4.3)	0.5 (0.03-1.1)
IL-6 p	7.4699	7.4119	0.20-32.31	6.4 (3.8-14.4)	3.2 (2.1-5.9)
IL-10 a	8.6320	5.38273	1.90-29.85	3.3 (2.1-5.6)	3.6 (2.2-6.4)
TNF- α^p	17.0374	20.221	0.50-71.75	7.3 (3.4-22.6)	6.4 (4.5-38.3)

p .Pro-inflammatory

a Anti-inflammatory

*Expressed as 25th and 75th percentiles (Fletcher et al., 2009)

Table 4. Intercorrelation matrix for biological variables.

	IL-1 β	IL-2	IL-4	IL-6	IL-10	TNF- α
IL-1 β	1	0.269**	0.682**	0.469**	0.434**	0.621**
IL-2		1	0.255*	0.515**	0.248*	0.444**
IL-4			1	0.417**	0.437**	0.538**
IL-6				1	0.534**	0.456**
IL-10					1	0.420**
TNF- α						1

*Significant at the $p < 0.05$ level; ** Significant at the $p < 0.01$ level

Table 5. Regression results for the association of poor sleep quality (total score) and biological and symptom-related variables

Independent variable	Dependent variable	Standardized Beta	<i>p</i> -value
PSQI Total	IL-1 β	0.162	0.127
	IL-2	0.202	0.043*
	IL-4	0.029	0.784
	IL-6	0.248	0.016*
	IL-10	0.151	0.159
	TNF- α	0.294	0.005**
	FSI Intensity	0.222	0.032*
	FSI Frequency	0.290	0.005**
	CDC Total Symptom Severity	0.446	0.000**
	CDC Total Symptom Frequency	0.457	0.000**

Note: analyses controlled for age and educational level.

*Significant at the $p < 0.05$ level; ** Significant at the $p < 0.01$ level

Table 6. Regression results for the association of poor sleep quality sleep quality subscale score and cytokines

Independent variable	Dependent variable	Standardized Beta	<i>p</i> -value
PSQI Sleep Quality	IL-1 β	0.111	0.294
	IL-2	0.200	0.043*
	IL-4	0.129	0.218
	IL-6	0.245	0.016*
	IL-10	0.214	0.043*
	TNF- α	0.216	0.040*

Note: analyses controlled for age and educational level.

*Significant at the $p < 0.05$ level; ** Significant at the $p < 0.01$ level

Table 7. Regression results for the association of poor sleep quality sleep latency subscale score and cytokines

Independent variable	Dependent variable	Standardized Beta	<i>p</i> -value
PSQI Sleep Latency	IL-1 β	0.250	0.017*
	IL-2	0.169	0.087
	IL-4	0.066	0.533
	IL-6	0.163	0.115
	IL-10	0.066	0.534
	TNF- α	0.250	0.017*

Note: analyses controlled for age and educational level.

*Significant at the $p < 0.05$ level; ** Significant at the $p < 0.01$ level

Table 8. Regression results for the association of poor sleep quality sleep disturbances subscale score and cytokines

Independent variable	Dependent variable	Standardized Beta	<i>p</i> -value
PSQI Sleep Disturbances	IL-1 β	0.109	0.304
	IL-2	0.092	0.361
	IL-4	-0.014	0.896
	IL-6	0.265	0.010**
	IL-10	0.089	0.405
	TNF- α	0.082	0.444

Note: analyses controlled for age and educational level.

*Significant at the $p < 0.05$ level; ** Significant at the $p \leq 0.01$ level

Table 9. Analysis of PSQI Sleep Disturbances subscale and its relationship to circulating IL-6.

Reason for Sleep Disturbance	N(%) ≥ 3 times/week	Standardized Beta	P value
Cannot get to sleep within 30 minutes?	46 (48.4)	0.245	0.016*
Wake up in the middle of the night or early morning?	62 (65.3)	0.169	0.103
Have to get up to use the bathroom?	46 (48.4)	0.189	0.072
Cannot breathe comfortably?	14 (14.7)	0.152	0.142
Cough or snore loudly?	18 (18.9)	0.050	0.634
Feel too cold?	19 (20.0)	0.036	0.735
Feel too hot?	38 (40.0)	0.132	0.206
Had bad dreams?	12 (12.6)	0.173	0.104
Have pain?	50 (52.6)	0.085	0.423

Note: analyses controlled for age and educational level.

*Significant at the $p < 0.05$ level

Table 10. Regression results for the association of poor sleep quality sleep efficiency subscale score and cytokines

Independent variable	Dependent variable	Standardized Beta	<i>p</i> -value
PSQI Sleep Efficiency	IL-1 β	0.119	0.260
	IL-2	0.129	0.196
	IL-4	0.069	0.510
	IL-6	0.143	0.167
	IL-10	0.124	0.245
	TNF- α	0.187	0.076

Note: analyses controlled for age and educational level.

Table 11. Regression results for the association of poor sleep quality sleep duration subscale score and cytokines

Independent variable	Dependent variable	Standardized Beta	p-value
PSQI Sleep Duration [‡]	IL-1 β	0.165	0.118
	IL-2	0.171	0.085
	IL-4	0.126	0.229
	IL-6	0.260	0.011*
	IL-10	0.132	0.218
	TNF- α	0.318	0.002**

Note: analyses controlled for age and educational level.

*Significant at the $p < 0.05$ level; ** Significant at the $p < 0.01$ level

[‡] Higher PSQI Sleep Duration Scores Indicate Shorter Sleep Duration

Table 12. Interaction effects of global poor sleep quality and depression on pro- and anti-inflammatory cytokines.

Interaction	Dependent variable	Interaction Beta	<i>SE</i>	<i>p</i> -value	R ² increase
Global PSQI x Depression	IL-1 β	0.0013	0.0032	0.673	0.002
	IL-2	0.0022	0.0019	0.260	0.012
	IL-4	0.0026	0.0010	0.0141*	0.0656
	IL-6	0.0016	0.0008	0.0369*	0.0434
	IL-10	0.0002	0.0005	0.7394	0.0013
	TNF- α	0.000	0.0012	0.9817	0.000

*Significant at the $p < 0.05$ level; ** Significant at the $p < 0.01$ level

Table 13. Interaction effects of sleep quality subscale score and depression on pro- and anti-inflammatory cytokines.

Interaction	Dependent variable	Interaction Beta	<i>se</i>	<i>p</i> -value	R ² increase
Sleep Quality x Depression	IL-1 β	-0.0040	0.0132	0.3168	0.001
	IL-2	0.0034	0.008	0.6725	0.0017
	IL-4	0.0048	0.0045	0.2849	0.0000
	IL-6	0.0016	0.0003	0.9840	0.0434
	IL-10	-0.0024	0.0019	0.9366	0.0155
	TNF- α	-0.0023	0.0051	0.6552	0.0022

Table 14. Interaction effects of sleep latency subscale score and depression on pro and anti-inflammatory cytokines.

Interaction	Dependent variable	Interaction Beta	<i>se</i>	<i>p</i> -value	R ² increase
Sleep Latency x Depression	IL-1 β	-0.0014	0.0102	0.8948	0.0002
	IL-2	0.0053	0.0062	0.4016	0.0068
	IL-4	0.0064	0.0035	0.0679	0.0367
	IL-6	0.0026	0.0026	0.3301	0.0099
	IL-10	0.0006	0.0017	0.7121	0.0016
	TNF- α	-0.0038	0.0040	0.3400	0.0098

Table 15. Interaction effects of sleep duration subscale score and depression on pro and anti-inflammatory cytokines.

Interaction	Dependent variable	Interaction Beta	se	p-value	R ² increase
Sleep Duration x Depression	IL-1 β	0.0164	0.0102	0.1096	0.0282
	IL-2	0.0011	0.0063	0.8636	0.0003
	IL-4	0.0078	0.0034	0.0248*	0.0543
	IL-6	0.0006	0.0025	0.8282	0.0005
	IL-10	0.0000	0.0016	0.9854	0.000
	TNF- α	0.0010	0.0039	0.7956	0.0007

*Significant at the $p < 0.05$ level; ** Significant at the $p < 0.01$ level

Table 16. Interaction effects of sleep efficiency subscale score and depression on pro and anti-inflammatory cytokines.

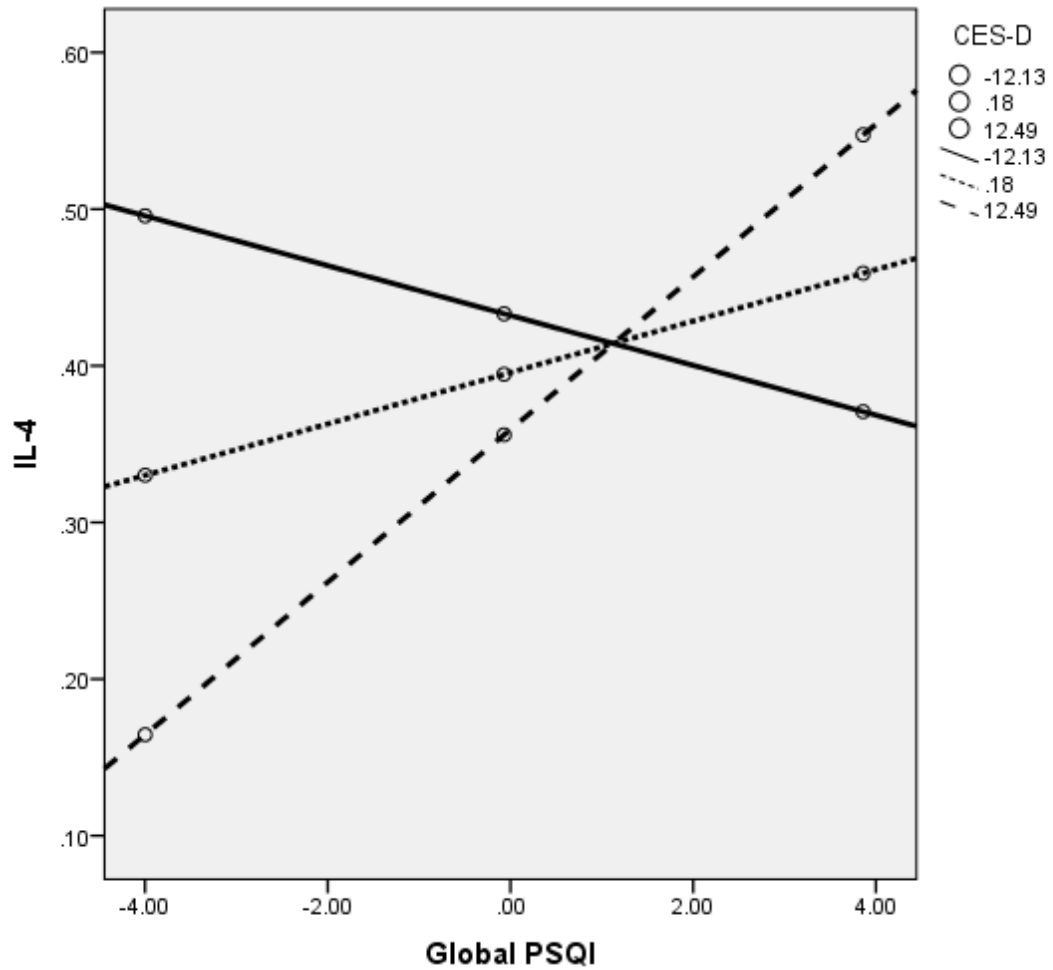
Interaction	Dependent variable	Interaction Beta	<i>se</i>	<i>p</i> -value	R ² increase
Sleep Efficiency x Depression	IL-1 β	0.0034	0.0091	0.7078	0.0016
	IL-2	-0.0066	0.0055	0.2313	0.0139
	IL-4	0.0041	0.0031	0.1828	0.0197
	IL-6	0.0010	0.0023	0.6742	0.0019
	IL-10	0.0008	0.0014	0.6005	0.0032
	TNF- α	-0.0003	0.0035	0.9429	0.0001

Table 17. Interaction effects of sleep disturbances subscale score and depression on pro and anti-inflammatory cytokines.

Interaction	Dependent variable	Interaction Beta	<i>se</i>	<i>p</i> -value	R ² increase
Sleep Disturbances x Depression	IL-1 β	-0.0117	0.0163	0.4738	0.0058
	IL-2	0.0117	0.0099	0.2404	0.0136
	IL-4	0.0047	0.0056	0.4013	0.0079
	IL-6	0.0067	0.0040	0.0940	0.0279
	IL-10	0.0022	0.0026	0.3844	0.0087
	TNF- α	-0.0076	0.0064	0.2370	0.0159

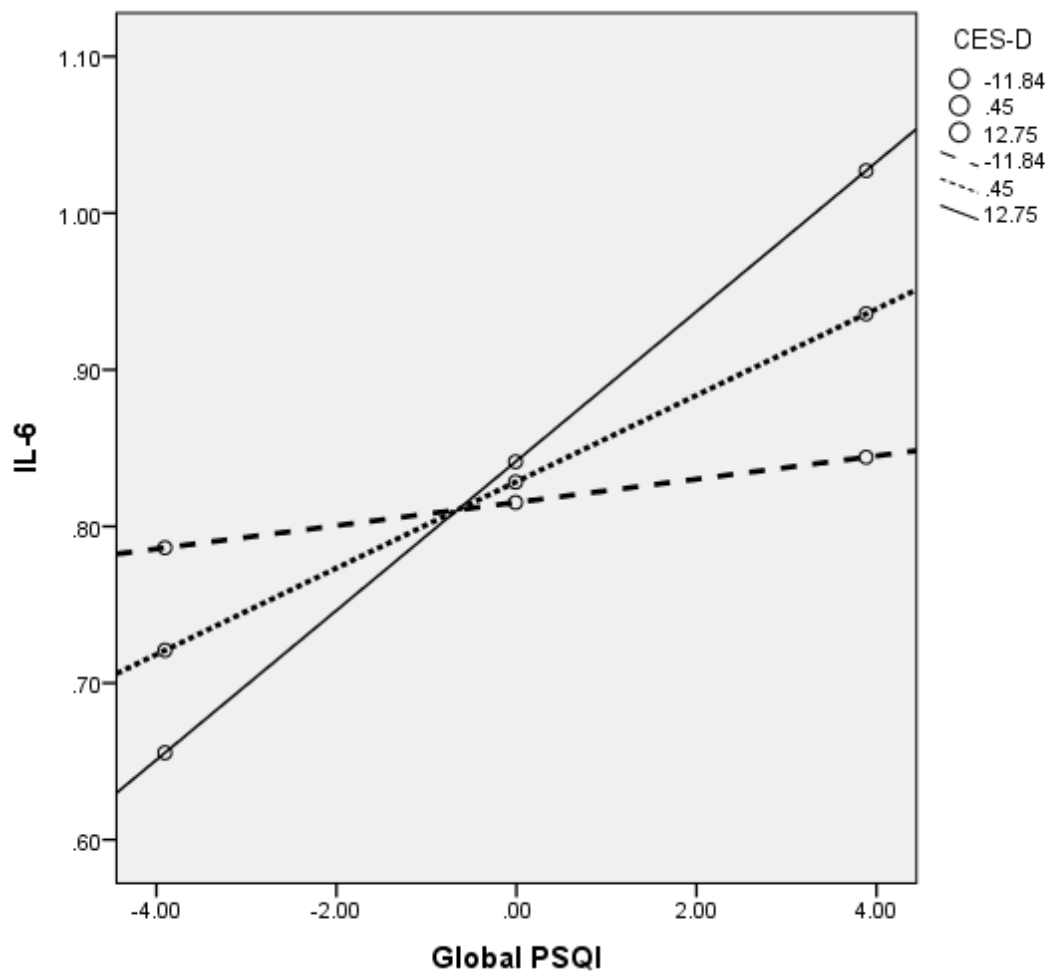
FIGURES

Figure 1. Relationship between global PSQI sleep quality score and circulating IL-4 as moderated by the severity of depressive symptoms



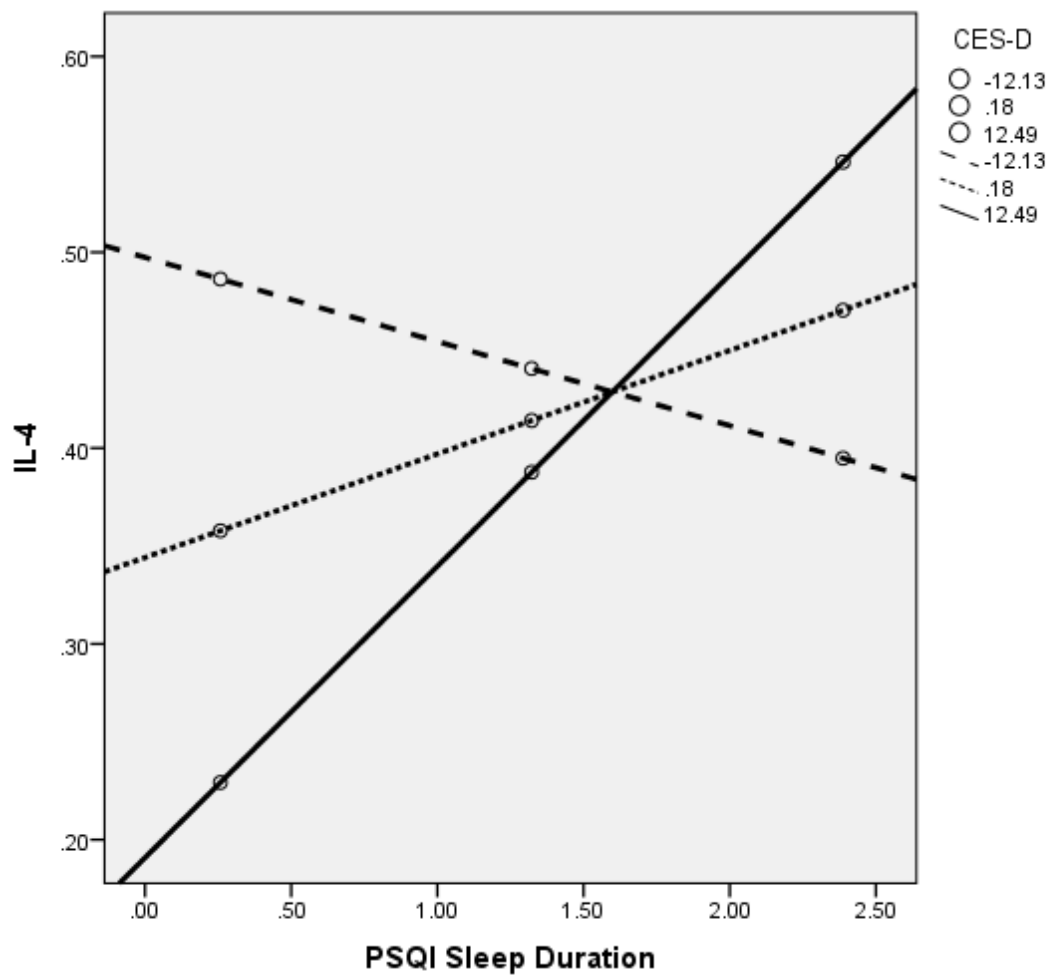
Global PSQI and CES-D scores are centered on the mean. CES-D score is modified by removing the sleep-related items.

Figure 2. Relationship between global PSQI sleep quality score and circulating IL-6 as moderated by the severity of depressive symptoms



Global PSQI and CES-D scores are centered on the mean. CES-D score is modified by removing the sleep-related items.

Figure 3. Relationship between PSQI sleep duration subscale score and circulating IL-4 as moderated by the severity of depressive symptoms



CES-D scores are centered on the mean. CES-D score is modified by removing the sleep-related items.

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