DO SLEEP DIMENSIONS PREDICT PRIMARY AND SECONDARY ANTIBODY RESPONSES TO VACCINATION?

by

Aric Andrew Prather

Bachelor of Arts in Psychology, University of California, Berkeley, 2001

Master of Science in Psychology, University of Pittsburgh, 2006

Submitted to the Graduate Faculty of

Arts and Sciences in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2010

UNIVERSITY OF PITTSBURGH

FACULTY OF ARTS AND SCIENCES

This dissertation was presented

by

Aric Andrew Prather

It was defended on

June 15, 2010

and approved by

Martica Hall, Ph.D., Associate Professor, Department of Psychiatry

Thomas W. Kamarck, Ph.D., Professor, Department of Psychology

Karen A. Matthews, Ph.D., Professor, Department of Psychiatry

Elizabeth Votruba-Drzal, Ph.D., Assistant Professor, Department of Psychology

Dissertation Advisor: Anna L. Marsland, Ph.D., RN, Associate Professor, Department of Psychology

DO SLEEP DIMENSIONS PREDICT PRIMARY AND SECONDARY ANTIBODY RESPONSES TO VACCINATION?

Aric A. Prather, Ph.D.

University of Pittsburgh, 2010

The immune system may contribute to associations between disturbed sleep and increased disease risk. Until recently, however, much of the work examining immune correlates of sleep employed *in vitro* measures of immunity of unknown clinical relevance. To address this limitation, we prospectively examined associations of several sleep parameters (sleep duration, efficiency, and quality) with the magnitude of primary and secondary antibody responses to the hepatitis B vaccination series among a community sample of 125 relatively healthy, older adults. Participants completed electronic sleep diaries for 7 consecutive days (3 days prior, the day of, and 3 days following) at each of the 3 hepatitis B injections. In addition, a subset of participants (n=104) wore an actigraph on the 3 days prior and 3 days following the first injection to provide an objective measure of sleep behavior.

In regard to primary antibody responses following the first dose of the vaccine, poorer sleep efficiency, greater sleep fragmentation, and greater night to night variability in sleep duration were associated with higher antibody responses; however, these associations were reduced after adjustment for sociodemographic covariates, including age, gender, race, and body mass index (BMI). In contrast, shorter sleep duration, measured via actigraphy alone or averaged across all available nights of sleep assessment, was associated with lower secondary antibody levels, assessed 5-months after the second injection, and a poorer likelihood of being a clinically protected at the conclusion of the vaccination series. Participants with low and high

variability in sleep duration also displayed lower secondary antibody levels and decreased likelihood of being clinically protected. These findings remained largely significant after adjustment for sociodemographic covariates.

Taken together, these findings provide preliminary evidence for the influence of sleep on primary and secondary antibody responses to the hepatitis B vaccine. Further exploration of the role of poor sleep in susceptibility to infectious illness is warranted.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

It has been an absolutely amazing journey. The work presented herein would not be possible without the mentorship and guidance of Dr. Anna Marsland. Not only did she provide me with the tools to be a successful clinical-health psychologist, she exemplifies what it takes to be a balanced, caring professional and person. I will always be eternally grateful for the opportunities she extended during my graduate years. I am also indebted to the many faculty members who, in my opinion, went out of their way to ensure my success in the field of health psychology. In particular, I would like to thank Drs. Stephen Manuck, Sheldon Cohen, Matthew Muldoon, Karen Matthews, and Martica Hall. Thank you for continuing to push my thinking and I hope that our collaborations will continue indefinitely. I would also like to thank the remaining members of my dissertation committee who were instrumental in making the present work possible, namely Dr. Elizabeth Votruba-Drzal and Dr. Tom Kamarck.

The research and clinical training I obtained at the University of Pittsburgh was unprecedented. However, beyond the graduate work-load, my experience can be measured by the remarkable friends I made along the way. While there are far too many to single out here, each one of you will always be close to my heart. Whether it was a game of wiffle ball, rooting on the Steelers, or soaking up a far too rare sun-filled afternoon, these friendships have shaped my life in ways that I am only beginning to understand but will always appreciate.

I would like to recognize the influence of my family. I thank my mom for perpetually reminding me that I'm "special" and my dad and step-mom for their continued support. I would also like to thank my mother- and father-in-law who have treated me like a son from the very first day. Furthermore, I would like to honor the memory of my grandparents, particularly Joan, who was a psychologist herself and very excited to see me on this path. Finally, and perhaps most importantly, I would like to thank my wife, Michelle, whose compassion and infectious zeal for life helps me grow as a person each day. Thank you for your support during the ups and downs of this process and for being my best friend. I could not have accomplished half as much without you and I look forward to continuing this journey with you by my side.

1.0 INTRODUCTION

A growing literature demonstrates that sleep is associated with health and disease. Short sleep duration and poor sleep efficiency have been associated with increased incidence and progression of several medical conditions, including type 2 diabetes (Ayas, White, Al-Delaimy et al., 2003; Gangwisch et al., 2007), coronary artery disease (Ayas, White, Manson et al., 2003; Mallon, Broman, & Hetta, 2002; Meisinger, Heier, Lowel, Schneider, & Doring, 2007), metabolic syndrome (Hall et al., 2008), and infectious illness (Cohen, Doyle, Alper, Janicki-Deverts, & Turner, 2009; Cohen, Doyle, Skoner, Rabin, & Gwaltney, 1997) as well as elevated mortality risk (Heslop, Smith, Metcalfe, Macleod, & Hart, 2002; Kripke, Garfinkel, Wingard, Klauber, & Marler, 2002; Patel et al., 2004). In addition, poor subjective sleep quality often covaries with health complaints among healthy, sleep-disordered, and diseased populations (Meisinger et al., 2007; Patil, Schneider, Schwartz, & Smith, 2007; Phillips & Mannino, 2005; Prinz, 1995).

To date, the mechanisms linking sleep and disease remain unclear; however, compelling experimental evidence supports an association between disrupted sleep and alterations in aspects of innate and adaptive immunity (Bryant, Trinder, & Curtis, 2004; Irwin, 2002; Moldofsky, Lue, Davidson, & Gorczynski, 1989; Opp, Born, & Irwin, 2007), raising the possibility that modulation of immune function may be a potential pathway to increased disease risk. Although reliable, the clinical significance of these sleep-related immune changes is unknown. In part, this

is because most studies have relied on *in vitro* markers of immunocompetence that provide a poor estimate of the body's host resistance to disease (Vedhara, Fox, & Wang, 1999). To address this gap in the literature, researchers have turned to an examination of the effects of sleep on immune function in the living organism.

One *in vivo* method that capitalizes on a naturally occurring immune response central to the body's protection against infectious pathogens is measurement of antibody production following vaccination. Using this method, individuals are inoculated with a foreign antigen and their antigen-specific antibodies are then quantified. In general, increases in antigen-specific antibodies are associated with reduced incidence of clinical illness on future exposure to the antigen (Rabin, 1999). Thus, this method is proposed to provide a proximate and clinically-relevant measure of immune function that may inform associations among biobehavioral variables, including sleep and immunity (Cohen, Miller, & Rabin, 2001; Prather & Marsland, 2008; Rabin, 1999).

A growing body of evidence shows that sleep regulates aspects of immunity that facilitate antibody production and maintenance (Opp et al., 2007), with preliminary evidence linking disturbed sleep to poorer antibody responses in humans. To date, much of this work has relied on laboratory based sleep deprivation paradigms to investigate of the effects of sleep loss on vaccination response. Here, acute sleep loss during the days prior to and immediately following vaccination significantly impairs antibody production in response to foreign antigens (Lange, Perras, Fehm, & Born, 2003; Spiegel, Sheridan, & Van Cauter, 2002). Notably, these findings are limited by the use of small samples of largely healthy, young adults and sleep manipulations that may not generalize beyond the laboratory setting. In regard to the latter, one exception is an unpublished study of healthy college freshman that examines the association of

normative variation in sleep with antibody response to vaccination (Pressman, Miller, & Cohen, 2005). Here, shorter sleep duration, averaged over 13 consecutive days of electronic diary assessment, was associated with lower secondary antibody production to the A/Caledonia strain of the trivalent influenza vaccine, as measured 1 and 4 months post-vaccination. Furthermore, relationship, providing preliminary evidence that sleep on the days prior to vaccination may be particularly relevant for subsequent antibody production. While compelling, it remains unclear whether natural variation in *objective* measures of sleep prospectively predict antibody responses to immunization. Furthermore, it remains unknown if these associations generalize to older and thus potentially more immunologically vulnerable populations.

Antibody response to vaccination is directly related to susceptibility to infectious disease (CDC, 1987; Zinkernagel et al., 1996). Accordingly, determining whether modifiable behavioral processes, such as sleep, modulate antibody production is of potential clinical utility. The aim of the proposed study is to examine whether three dimensions of sleep (duration, efficiency, and quality) are significant predictors of primary and secondary antibody responses to a novel antigen (hepatitis B). It is anticipated that findings from this study may inform not only our understanding of the association between sleep and host-resistance in older adults but may also assist in distinguishing relevant time points when sleep interventions may increase vaccination efficacy in vulnerable populations.

2.0 LITERATURE REVIEW

2.1 DIMENSIONS OF SLEEP

Sleep has been defined as a physiological state marked by bouts of reduced consciousness, lessened skeletal movement, and slowed metabolism (Buysse, 2005; Carskadon & Dement, 2000; Zisapel, 2007). Human sleep is regulated by two complementary processes 1) a homeostatic factor and 2) a circadian sleep rhythm (Mongrain, Lavoie, Selmaoui, Paquet, & Dumont, 2004; Zisapel, 2007). Referred to as a sleep "drive", the homeostatic factor is a function of prior wakefulness. As wakefulness accrues during the day, the drive to sleep rises, and decreases precipitously during subsequent sleep (Carskadon & Dement, 2000). The circadian sleep rhythm is an endogenous rhythm generated and controlled by the central nervous system (CNS) (Buysse, 2005; Jones, 2005) and entrained to the external environment by zeitgebers (e.g. daylight, meals) (Carskadon & Dement, 2000; Roenneberg & Merrow, 2007). The pacemaker for this circadian "clock" is localized in the suprachiasmatic nucleus (SCN) of the hypothalamus, with efferent pathways that transmits timing information to other areas of the CNS (Colwell & Michel, 2003). On average, humans have an endogenous circadian sleep rhythm of just over 24 hours (Czeisler & Klerman, 1999). Sleep behavior is also influenced by cognitive arousal and related psychological factors, including stress and anxiety (Morin, Rodrigue, & Ivers, 2003; Ohayon, 2005). Conversely, disrupted sleep can have a negative impact

on psychological states. For example, sleep restriction is associated with increased negative mood, anxiety, and heightened stress sensitivity (Baldwin & Daugherty, 2004; Drake, Richardson, Roehrs, Scofield, & Roth, 2004).

Generally, human adult sleep is consolidated into periods lasting between 7 and 8 hours and, if disturbed, results in problems in wakefulness, including feelings of tiredness, difficulties in concentration, poor motor performance, and poor memory consolidation (Ohayon, 2005; Vgontzas et al., 2004; Walker & Stickgold, 2004). Substantial individual differences exist in both sleep need and circadian rhythm. In regard to the latter, there is mounting evidence for stable differences in an individual's propensity for early morning rising (known as morning larks) versus a tendency to stay up until the late evening hours (known as night owls); such individual differences are known as chronotypes (Mongrain, Carrier, & Dumont, 2006; Mongrain et al., 2004; Paine, Gander, & Travier, 2006).

Sleep consists of two major phases that alternate across the night, rapid eye-movement (REM) sleep and non-REM sleep. Non-REM can be further divided into stages 1, 2, 3, and 4. The stages of non-REM sleep are thought to parallel depth of sleep with stage 4 marking the deepest and stage 1 being the transition from wakefulness to sleep (Buysse, 2005). Sleep stages are defined by physiologic parameters, namely patterns in electrical activity that change in the brain as individuals move in and out of different sleep stages. Assessment of these stages is obtained through polysomnography (PSG), which is considered the "gold standard" for differentiating stages of sleep from wakefulness (Buysse, 2005; Carskadon & Dement, 2000). However, other dimensions of sleep, including duration, efficiency, and quality can be reliably assessed using alternate methods, such as actigraphy and self-report measures.

Actigraphy is an objective method of estimating several sleep parameters, including sleep duration, sleep latency, fragmented sleep, and sleep efficiency (Sadeh, Hauri, Kripke, & Lavie, 1995). Guidelines specified by the American Academy of Sleep Medicine support actigraphy as a reliable and valid method for detecting and quantifying sleep in healthy, normal sleepers (Ancoli-Israel et al., 2003; Littner et al., 2003). Moreover, a review of experimental evidence shows that among healthy subjects the agreement between polysomnography and actigraphy is high (>90%) (Sadeh et al., 1995). When compared with self-report measures, which are subject to recall bias, actigraphy provides a more objective method for estimating sleep latency and number of nighttime awakenings (i.e. sleep fragmentation) (Kushida et al., 2001; Rogers, Caruso, & Aldrich, 1993; Wilson, Watson, & Currie, 1998) with agreement between actigraphy and diary sleep logs for indices of sleep duration ranging between 72%-97% (Usui et al., 1998, 1999).

2.1.1 Sleep Duration

Sleep duration is the amount of time a person is asleep during the night. The average duration of adult sleep has decreased by around 25% over the past 4 decades to around 6.9 hours per night (National Sleep Foundation, 2002),with prospective epidemiologic data showing the average duration across multiple nights to range from 5.5 to 6.5 hours (Knutson, Rathouz, Yan, Liu, & Lauderdale, 2007; Lauderdale et al., 2006; Redline et al., 2004). Experimental manipulation of sleep duration (i.e. sleep deprivation) shows that shortened duration is not only associated with daytime sleepiness (Vgontzas et al., 2004), but also alters biologic processes that may have long term implications for health (Irwin, 2002; Knutson, Spiegel, Penev, & Van Cauter, 2007; Van Cauter et al., 2007; Vgontzas et al., 2004).

2.1.2 Sleep Efficiency

Sleep efficiency generally refers to the proportion of time an individual spends asleep once he or she has attempted to fall asleep. As such, longer sleep latencies (i.e. the time it takes to fall asleep) and the more minutes spent awake after initial sleep onset (i.e. greater sleep fragmentation) contribute to lower sleep efficiency. It has been suggested that sleep efficiency of 85% or greater is indicative of "good sleepers" (Morin, 1993). However, epidemiologic evidence among relatively healthy sleepers indicates appreciable variability. For instance, Lauderdale and colleagues found that among 647 participants (38-50 years old) from the Coronary Artery Risk Development in Young Adults (CARDIA) study the average actigraphy-based sleep efficiency was 80.8% (SD=11.3). Race and gender differences in sleep efficiency have also been documented, with both African American and men showing lower sleep efficiency relative to their Caucasian, female counter parts (Mezick et al., 2008; Lauderdale et al., 2006). Evidence also shows a decline in sleep efficiency across the lifespan. For instance, the Sleep Heart Health Study, a longitudinal study of over 5,000 community volunteers, found an age-related decline in sleep efficiency with the lowest sleep efficiency observed among the elderly (Unruh et al., 2008).

2.1.3 Sleep Quality

Though difficult to define, sleep quality is generally conceptualized as an individual's subjective satisfaction with their sleep (i.e. its restfulness) and is often, but not always, associated with their sleep continuity throughout the night (Grandner & Kripke, 2004; Lichstein, Durrence, Riedel, Taylor, & Bush, 2004). Indeed, laboratory studies show that fragmented sleep (i.e. poor

sleep efficiency) is associated with poorer reported sleep quality the following day (Bonnet & Arand, 2003; Seneviratne & Puvanendran, 2004). Further evidence that disturbed sleep contributes to perceived sleep quality comes from large epidemiologic studies (Ohayon, 2005; Ohayon, Caulet, & Guilleminault, 1997; Ohayon & Zulley, 2001); for instance, in a study of over 25,000 sleepers, those who reported difficulty initiating sleep and frequent night awakenings at least 2 nights/week were more likely to report nonrestorative, unrefreshing sleep than those whose sleep was more efficient (Ohayon, 2005). Psychological factors, including stressful life events and psychiatric conditions (e.g. depression, anxiety) are also associated with poor sleep quality (Jean-Louis, Kripke, & Ancoli-Israel, 2000).

2.2 SLEEP AS A RISK FACTOR FOR DISEASE

2.2.1 Sleep duration and disease

Cross-sectional and prospective epidemiologic evidence demonstrate that short and long sleepers (less than 5 or 6 hours/night or greater than 9 hours/night) are at heightened risk for a number of chronic health conditions, including coronary heart disease (Ayas, White, Manson et al., 2003), diabetes (Ayas, White, Al-Delaimy et al., 2003; Gangwisch et al., 2007; Mallon, Broman, & Hetta, 2005), and the metabolic syndrome (Hall et al., 2008). For instance, in an 8- to 10 year follow-up of the first National Health and Nutritional Examination Survey (NHANES-1), being a short sleeper (5 hours or fewer) or a long sleeper (9 or more hours) was a significant risk factor for developing type 2 diabetes, after controlling for a variety of other sociodemographic and health risk factors, including physical activity, depression, alcohol

consumption, ethnicity, education, marital status, and age (Gangwisch et al., 2007). This finding is consistent with experimental evidence that modest sleep deprivation (i.e. restricting sleep to 4 hours per night) is associated with alterations in metabolic activity, including reduced glucose tolerance, which may contribute to disease risk (Spiegel, Leproult, & Van Cauter, 1999).

Emerging evidence also suggests that shortened sleep duration is associated with decreased host-resistance to infectious disease (Cohen et al., 2009). For instance, Cohen and colleagues (2009) recently reported on 153 healthy volunteers who completed sleep diaries for 14 consecutive days prior to being experimentally inoculated with rhinovirus. Participants were subsequently followed for 5 days to assess subjective reports of an upper respiratory infection (URI), and biologically verified disease. Here, shorter sleep duration (i.e. sleeping less than 7 hours) predicted increased risk of developing a cold by objective and subjective criterion, independently of age, race, gender, BMI, income, education, perceived socioeconomic rank, physical activity, season of exposure, and baseline antibody titers.

2.2.2 Sleep efficiency and disease

Like duration, sleep efficiency has been related to health status in several large epidemiologic investigations, with poorer sleep efficiency being associated with increased frailty (Ensrud et al., 2009), incidence of cardiovascular disease ((Mallon et al., 2002), and type 2 diabetes; (Mallon et al., 2005). Moreover, in a longitudinal study of healthy older adults, individuals displaying a sleep efficiency of <80%, assessed using electroencephalography (EEG), were nearly twice as likely (OR=1.93) to die from all-causes when compared to

participants with a sleep efficiency of ≥80% (Dew et al., 2003). Recent evidence suggests that poor sleep efficiency is also a risk factor for the common cold (Cohen et al., 2009) with sleep efficiency measured prior to viral exposure being inversely associated with risk of developing a biologically-verified cold. Interestingly, this effect was independent of sleep duration, suggesting that they both contribute uniquely to risk for infectious disease.

2.2.3 Sleep quality and disease

Poor sleep quality, assessed as a subjective report of low sleep satisfaction has been associated with number of disease outcomes (Cappuccio, D'Elia, Strazzullo, & Miller, *in press-a;* Jennings, Muldoon, Hall, Buysse, & Manuck, 2007; Leineweber, Kecklund, Janszky, Akerstedt, & Orth-Gomer, 2003; Mallon et al., 2002, 2005), including poorer prognosis for coronary heart disease, fibromyalgia, and chronic fatigue syndrome (Leineweber et al., 2003; Moldofsky, 1993). For example, Leineweber and colleagues (2003) found that women who reported "not feeling well rested" some or most of the time were 2.4 times more likely to experience an incident cardiac event when followed prospectively for 5 years than those reporting rest on a regular basis. These findings were independent of age, BMI, cardiovascular symptoms, smoking status, and education. Poor sleep quality has also been related to risk factors for cardiovascular disease, including hypertension, obesity, insulin resistance, and elevated glucose (Fiorentini, Valente, Perciaccante, & Tubani, 2007; Jennings et al., 2007; Resta et al., 2003; Scheen & Van Cauter, 1998).

Few studies have investigated whether sleep complaints predict decreased host resistance to disease. In part, this may be because diminished sleep quality can be a subclinical symptom of infectious illness. Indeed, growing evidence shows that peripheral immune activation that

accompanies infection communicates with the CNS and results in "sickness symptoms," including increased sleep and daytime fatigue, which are thought to conserve metabolic energy necessary for fighting the infection (Maier & Watkins, 1998). That said, Pressman and colleagues (2005) found that poorer sleep quality, averaged over 13 days of assessment, was associated with a poor antibody response to the A/New Caledonia strain of the influenza vaccine 1-month post-immunization. Furthermore, evidence does show that rotating shift workers, a population marked by poor sleep quality, are at greater risk for infectious illness than day workers. For instance, in a cross-sectional investigation of over 12,000 employees, rotating shift workers were significantly more likely to report developing a cold, experiencing a flu-like illness, or gastroenteritis than stable day workers (Mohren et al., 2002).

Taken together, these findings suggest that shortened sleep duration, poor sleep efficiency, and poor sleep quality are associated with increased risk for disease. To date, however, the mechanisms through which sleep confers increased disease susceptibility remain unclear. It is possible that disturbed sleep contributes to the pathogenesis of health problems; however, the existing sleep literature focuses on chronic health conditions (e.g. diabetes, coronary heart disease) that develop over years, making it difficult to identify specific underlying pathways or critical periods when poor sleep might promote disease. In contrast, it is possible to examine whether sleep is related to susceptibility to acute illnesses, such as infections. Here, compelling experimental evidence shows that shortened sleep and poor sleep efficiency, measured prior to viral exposure, increases susceptibility to URIs (Cohen et al., 2009), raising the possibility that the immune system is a mechanism linking sleep to increased disease risk. In this regard, a growing literature shows an association of sleep with immune function, including experimental evidence that sleep restriction is associated with alterations in immune processes

that play a role in host-resistance to infection. However, before reviewing the literature linking sleep and immunity, a brief overview of the immune system is provided to orient the reader.

2.3 OVERVIEW OF THE IMMUNE SYSTEM

The immune system is comprised of highly complex and integrated network of cells and soluble molecules that work in concert to protect the body (i.e. self) from potentially harmful (i.e. non-self) agents (i.e. antigens). When discussing the components of the immune system, it is useful to distinguish between natural and acquired immunity. Natural immunity is functional at birth and is activated quickly (minutes to hours) to protect the individual from any compounds not recognized as self. Conversely, acquired immunity provides a slower (days to weeks) and more specialized form of protective response that remains inactive unless cells come in contact with their specific (cognate) antigens (Delves & Roitt, 2000a, 2000b; Rabin, 1999).

Natural immune components include physical and anatomical barriers (e.g., skin, mucus membranes) and specialized cells that do not require the specific recognition of an antigen to carry out their functions, such as natural killer (NK) cells and granulocytes (e.g. neutrophils, dendritic cells, and macrophages). NK cells react to and kill malignant cells and cells infected with viruses, while cells like macrophages, in addition to destroying material recognized as non-self, can process antigens and present them to cells of the acquired immune system to initiate a more specific immune response. Macrophages also produce and release cytokines, which are chemical messengers that facilitate cell-to-cell communication and orchestrate immune processes. When activated by pathogens or tissue damage, macrophages release pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α,

which, in turn, recruit lymphocytes to the area of injury, up-regulate the expression of cellular adhesion molecules on the endothelium, and promote the systemic release of acute phase proteins (e.g. C-reactive protein) (Maier & Watkins, 1998).

Acquired immunity is traditionally subdivided into two major components. The first is humoral immunity, which is mediated by soluble factors called antibodies that circulate systemically and defend against bacteria and viruses. The second major component is cell-mediated immunity. Here, defense responses are mediated by specialized cells (lymphocytes) that have evolved to recognize and eliminate foreign antigens. Although there are many types of lymphocytes, each with distinct functions, immune cells are interdependent and respond in a coordinated fashion to achieve immunocompetence.

A cellular immune response is activated when a macrophage or dendritic cell engulfs an antigen and presents it to a T-lymphocyte that is specific to the antigen's surface properties. This initiates a cascade of events beginning with the activation and subsequent proliferation of T lymphocytes. There are two main populations of T cells: cytotoxic T cells and helper T cells. Cytotoxic T (CD8+) cells are able to detect altered self-cells (e.g., virally infected or tumor cells) and enzymatically digest (lyse) them. Activated helper T (CD4+) cells release several cytokines that stimulate the actions of other lymphocytes and macrophages. For example, IL-2, released by a subset of helper T cells called Th1 cells, stimulates B lymphocytes to activate and produce antibodies as well as promotes the proliferation (i.e. cellular division) of T lymphocytes and the activation of monocytes and NK cells. Other cytokines released by Th1 cells include IL-12 and interferon (IFN)-γ. IL-4, IL-5, and IL-10 are produced by another subset of helper T cells, Th2 cells. These cytokines stimulate T-cell division and promote the differentiation of B-cells.

Hence, T-lymphocytes, and the cytokines they produce, regulate the activity of the humoral immune system.

Humoral immune processes are mediated by B-lymphocytes and their antibody products. Here, the immune response begins with the initial recognition of a specific antigen by membrane antibodies on B-cells. Then, with the aid of activating cytokines secreted by helper-T cells and monocytes, B-lymphocytes proliferate and differentiate to form (1) plasma cells which actively secrete antibodies to the invading antigen and (2) memory cells. Antibody molecules bind to the specific antigen, forming antibody-antigen complexes that inactivate viruses and mark them for destruction by phagocytic cells such as macrophages and neutrophils. Some of the progeny of antigen-stimulated B and T lymphocytes become memory cells, which are capable of surviving for long periods (months to years). These cells recognize the specific antigen and enable a faster and more vigorous secondary response to be mounted to repeat infections in the future. This immunologic memory is the basis of protective vaccination against infectious disease. The first time a novel antigen is encountered by the immune system it elicits a *primary immune response*, while any subsequent exposure promotes a *secondary immune response*.

2.3.1 Measurement of immunity

Measures of the human immune system are largely limited to enumerative and functional parameters that can be assessed in peripheral circulation. Enumerative measures include quantifying the absolute numbers or percentages of specific populations of immune cells and their biochemical mediators (e.g. cytokines). Changes in the relative distribution of cell subtypes are often assessed as an indication of immune activation. For example, acute infectious disease is associated with an increase in circulating numbers of lymphocytes. Moreover, it is possible that

changes in the absolute numbers of various cell subsets in circulation reflect the redistribution of immune cells between the peripheral blood and lymphoid organs. These changes may influence immunocompetence by determining whether immune cells are likely to encounter a foreign antigen at a particular location.

Beyond enumerative measures, several *in vitro* assays allow for the assessment of functional aspects of immunity. For instance, lymphocyte division (proliferation) can be assessed by stimulating cell subsets in the laboratory (e.g. T cells) with non-specific mitogens (e.g. phytohemagglutinin (PHA)). Greater proliferation is thought to be associated with a more effective immune response (Vedhara, Fox et al., 1999). Cytotoxicity (i.e. a cell's ability to kill) can also be assessed *in vitro*. NK cell cytotoxicity is routinely assayed as a measure of innate immunity, and provides information about NK cells' cytotoxic potential. Finally, researchers routinely quantify the magnitude of cellular production of cytokines following *in vitro* stimulation. For instance, levels of pro-inflammatory cytokines (e.g. IL-6) are measured after monocytes/macrophages are stimulated with endotoxin. The magnitude of the pro-inflammatory cytokine response to immune activation is critical; insufficient response may leave the organism vulnerable to infection, whereas excessive response can increase risk for inflammatory diseases (Nathan, 2002; Pavlov & Tracey, 2004).

In vitro assays provide valuable information about the functional capacity of specific cell populations. However, in order to accurately quantify such immune changes, immune cells are removed from the host environment and thus provide a poor estimation of *in vivo* host resistance. In contrast, *in vivo* measures assess integrated immune responses within the organism. For instance, measuring the number of antigen-specific antibodies produced following vaccination offers a functional estimate of the coordinated innate and acquired immune response.

The immune system is a dynamic network that acts quickly to meet the demands of an ever-changing environment, with the goal of protecting the organism from pathogens and the onset of disease. It is well documented that aspects of immunity are modulated by several psychological and behavioral processes, including sleep (Rabin, 1999; Segerstrom & Miller, 2004). In this regard, a growing body of literature demonstrates that disrupted sleep can have deleterious effects on immune function (Benca & Quintas, 1997; Opp et al., 2007).

2.4 SLEEP AND IMMUNITY

2.4.1 Normal sleep and the immune system

To date, a number of studies have examined diurnal changes in immune parameters across 24-hour cycles that include a period of regular, undisturbed nocturnal sleep (for reviews, Benca & Quintas, 1997; Irwin, 2002; Moldofsky, 1995; Opp et al., 2007). Findings show that cell subtypes in peripheral circulation vary markedly across the sleep-wake cycle. For instance, circulating numbers of granulocytes, monocytes, and lymphocyte subsets, including T-helper cells, cytotoxic T-cells, and B-cells, peak in the evening or early night and gradually decline to a nadir in the morning hours (Born, Lange, Hansen, Molle, & Fehm, 1997; Born et al., 1995; Irwin et al., 1996). Conversely, peripheral NK cell numbers reach their highest level in the mid-afternoon, with a decrease in number and function by around midnight (Born et al., 1997). It is likely that diurnal changes in peripheral cell numbers reflect migration of cells to and from lymphoid tissue and immune organs (e.g., spleen), and changes in the marginalization (i.e. stickiness) of cells to walls of blood vessels, rather than absolute changes in cell number.

Concomitant with diurnal changes in circulating cell numbers, the production of T-cell derived cytokines also varies across the sleep-wake cycle. For example, levels of IFN-y produced by Th1 cells are elevated during the early part of the night (Dimitrov, Lange, Tieken, Fehm, & Born, 2004) as is the production of IL-12 by monocytes and dendritic cell precursors, the latter of which is integral to the antigen presentation that initiates the adaptive immune response (Dimitrov, Lange, Nohroudi, & Born, 2007). IL-2 activity is also markedly increased during sleep as compared with levels during nocturnal wakefulness (Born et al., 1997; Irwin, Thompson, Miller, Gillin, & Ziegler, 1999; Lissoni, Rovelli, Brivio, Brivio, & Fumagalli, 1998).

2.4.2 Sleep deprivation and immune function

The hypothesis that sleep loss might impair host immune defenses in clinically significant ways is supported by an association between decreased sleep and increased morbidity in humans (e.g. Ayas, White, Al-Delaimy et al., 2003; Ayas, White, Manson et al., 2003) and by the finding that prolonged sleep deprivation results in death in an animal model (Rechtschaffen & Bergmann, 2002; Rechtschaffen, Bergmann, Everson, Kushida, & Gilliland, 1989). In regards to the latter, biopsies of chronically sleep deprived rats reveal elevated rates of bacteremia, suggesting that breakdown in immune function and subsequent infection may have contributed to death (Benca & Quintas, 1997; Everson, 1993).

In humans, researchers have relied on *in vitro* measures of immunity to investigate the impact of sleep loss on host resistance. Here, total sleep deprivation ranging from 24 to 48 hours has been associated with elevated numbers of lymphocytes, decreased numbers of NK cells, and increased systemic levels of pro-inflammatory cytokines, such as IL-6, IL-1β, and TNF-α (Born et al., 1997; Heiser et al., 2000; Moldofsky et al., 1989; Vgontzas et al., 1999). Furthermore,

sleep deprivation results in the down-regulation of a number of measures of immune function, including reduced T-cell proliferation following stimulation with a nonspecific mitogen (e.g. phytohemagglutinin) (Palmblad, Petrini, Wasserman, & Akerstedt, 1979), and diminished NK cell cytotoxicity (Moldofsky et al., 1989). Restricting sleep to only a couple hours per night (i.e. partial sleep deprivation) results in similar immune changes, including reduced NK cell activity and IL-2 production (Irwin et al., 1994; Irwin et al., 1996; Uthgenannt, Schoolmann, Pietrowsky, Fehm, & Born, 1995) and increased systemic and monocyte-derived production of IL-6 and TNF-α (Irwin, Wang, Campomayor, Collado-Hidalgo, & Cole, 2006; Uthgenannt et al., 1995; Vgontzas et al., 2004). Notably, these enumerative and functional immune changes are short-lived, returning to baseline levels quickly when sleep returns to normal.

In sum, growing evidence supports an association between sleep and immunity. Sleep deprivation experiments show the down-regulation of several immune processes integral to the development of host resistance to infectious pathogens (e.g. T-cell division, cytokine production). To date, however, it remains to be determined whether these sleep-related changes in immune function translate to greater susceptibility to disease. A first step in examining this possibility is to employ an *in vivo* model of immune response to a novel pathogen, a measure that is more proximally related to host resistance to infectious disease. Here, researchers have begun to investigate whether sleep disturbances are associated with antibody response to vaccination. Prophylactic vaccination is designed to simulate infection and induce the formation of memory T and B lymphocytes and the production of antibodies specific to the targeted pathogen. As antibody levels are directly related to protection against infectious illness, the magnitude of antibody response to vaccination provides a clinically-relevant measure of host resistance to disease. Importantly, individuals vary substantially in their ability to mount and maintain

antibody responses to vaccination. This variability is of health interest as poor responders are likely to be at risk of clinical illness on exposure to the specific pathogen (CDC, 1987; Francis et al., 1982; Hadler et al., 1986). Before describing the preliminary literature supporting a relationship between sleep and vaccination response, a brief discussion of several other factors known to influence vaccination response are presented.

2.5 FACTORS THAT INFLUENCE VACCINATION

2.5.1 Sociodemographic factors and vaccination response

Several sociodemographic factors are associated with variability in antibody response to vaccination, including age, gender, and body mass. It is well documented that aging is associated with a decline in immune function, known as immunosenescence, which includes deterioration of innate and adaptive components of immunity (Weinberger, Herndler-Brandstetter, Schwanninger, Weiskopf, & Grubeck-Loebenstein, 2008). In regard to vaccination response, randomized clinical trials demonstrate that influenza vaccinations are 70-90% effective among young healthy adults, but only 17-53% effective among the elderly (Goodwin, Viboud, & Simonsen, 2006). Similar age-effects have been observed in antibody response to hepatitis B vaccination (Averhoff et al., 1998; Bock et al., 1996; Roome, Walsh, Cartter, & Hadler, 1993; Wood et al., 1993). For example, in a study of over 1,700 health care workers administered the hepatitis B vaccine, those over 40 years of age were 2.2 times more likely to be non-responders to the vaccine relative to those 40 years old or under (Averhoff et al., 1998).

Men and overweight individuals also tend to display poorer antibody responses to vaccination than females and leaner individuals (Averhoff et al., 1998; Bock et al., 1996; Hui et al., 2006; Zuckerman, 2006). For instance, Wood and colleagues (1993) found that, among nearly 600 hospital workers receiving the hepatitis B vaccination series, being male ($p<.03$) or having a BMI of over 29 ($p<.01$) was significantly associated with being a non-responder, as measured 6 months post-vaccination. Moreover, BMI and gender remained independent predictors after controlling for other sociodemographic and behavioral factors (e.g. age, smoking status).

2.5.2 Psychological factors and vaccination response

In addition to sociodemographic factors, it is well documented that psychosocial factors modulate antibody responses to vaccination. Consistent with a large body of evidence showing that psychological stress modulates aspects of innate and adaptive immunity (for review, see Segerstrom & Miller, 2004), a number of studies have demonstrated an inverse association between psychological stress and antibody titers (for review, see Burns, Carroll, Ring, & Drayson, 2003; Cohen et al., 2001; Marsland, Bachen, Cohen, & Manuck, 2001; Pedersen, Zachariae, & Bovbjerg, 2009). To date, these studies have focused primarily on the magnitude of secondary antibody responses in response to vaccination (e.g. influenza vaccination) and have found that chronic stress (e.g. caregiving for a chronically ill family member)(Glaser, Kiecolt-Glaser, Malarkey, & Sheridan, 1998; Kiecolt-Glaser, Glaser, Gravenstein, Malarkey, & Sheridan, 1996; Phillips et al., 2006; Vedhara, Cox et al., 1999), reporting higher perceived stress (Burns, Carroll, Drayson, Whitham, & Ring, 2003; Jabaaij et al., 1993; Miller et al., 2004), and more stressful life events (Burns, Carroll, Ring, Harrison, & Drayson, 2002) are associated

with lower secondary antibody levels. However, not all studies are consistent (Glaser et al., 1992; Jabaaij et al., 1996; Petry, Weems, & Livingstone, 1991) and interpretations of findings are complicated by variability in the timing of stress relative to the immune outcome and in the nature and duration of the stressors (Miller et al., 2004). The importance of timing is exemplified by the fact that both acute stress and eccentric exercise experienced immediately prior to vaccination enhance secondary antibody responses (Edwards, Burns, Allen et al., 2007; Edwards et al., 2006) suggesting that psychological and behavioral factors may influence immune processes early in antibody production.

While there is accumulating evidence that psychosocial factors influence secondary antibody responses, research on primary antibody production is limited. In this regard, the hepatitis B vaccination model provides an opportunity to explore these associations because it is possible to identify individuals who are naïve to the antigen. Petrie and colleagues (1995) reported no differences in primary antibody responses among participants assigned to a "disclosure" intervention designed to reduce stress and controls (Petrie, Booth, Pennebaker, Davison, & Thomas, 1995). In contrast, Glaser and colleagues (1992) found that medical students who reported higher levels of stress during examinations were less likely to produce antibodies in response to the first vaccination. Unfortunately this association was based on composite of stress reported across the six month vaccination period, making it unclear whether stress around the time of the first vaccination was related to primary antibody response.

Although evidence demonstrates that sociodemographic and psychosocial factors contribute to individual differences in magnitude of antibody responses to vaccination, a large portion of the variance remains unexplained. Given evidence that disturbed sleep modulates aspects of immune function that are involved in antibody production as well as covaries with

psychological stress and sociodemographic factors shown to impact humoral immunity, it is plausible that sleep contributes to variation in antibody response. In this regard, a small, but compelling, literature has investigated the relationship between sleep and vaccination response.

2.5.3 Sleep and vaccination response

Preliminary animal and human literature supports an association between sleep and antibody response. In a provocative study of mice challenged with influenza, animals deprived of sleep for 7 hours prior to immunization displayed reduced viral-specific antibodies, increased viral titers, and failure to clear the virus relative to non-sleep-deprived mice (Brown, Pang, Husband, & King, 1989). Subsequent studies, however, have failed to replicate these findings (Renegar, Crouse, Floyd, & Krueger, 2000; Renegar, Floyd, & Krueger, 1998*a*; Renegar, Floyd, & Krueger, 1998*b*).

To date, two human studies have investigated the effects of acute sleep deprivation on antibody response. In one study, Lange and colleagues (2003) randomly assigned nine healthy, young participants to 36 hours of sleep deprivation following vaccination against hepatitis A. All participants were naïve to hepatitis A prior to vaccination and antibody responses were assessed 14 and 28 days later. Participants subjected to sleep deprivation showed a reduction in antibody production 2 weeks post vaccination when compared with 10 non-sleep deprived controls. Moreover, at 28 days post-vaccine, maximum antibody levels were nearly 100% greater in the non-sleep deprived control group. This finding is compelling for two reasons 1) it suggests that deviations from one's normal sleep pattern may affect the primary immune response and 2) it indicates that modulation of this response may occur early in antibody development.

Similar results were obtained in another study that employed a more generalizable sleep deprivation protocol. Here, Spiegel, Sheridan, and Van Cauter (2002) assigned 11 participants to 6 nights of partial sleep deprivation (4 hours/night) followed by 7 nights of extended sleep (12 hours/night) to recover from sleep loss. All participants were seropositive for anti-influenza antibody titers at baseline; hence, this study focused on secondary immune responses. Participants were vaccinated against influenza on the morning of the fifth day and followed for approximately 30 days. Ten days post-vaccination, antibody titers from sleep deprived participants were significantly lower than a normal sleep control group. However, group differences disappeared by the 30-day follow-up.

Finally, one unpublished study examined the relationship between sleep and secondary antibody responses to influenza vaccination in the natural environment (Pressman et al., 2005). Here, Pressman and colleagues examined whether dimensions of sleep (duration, quality, and efficiency) collected using sleep diaries for 13 days (2 days prior, the day of, and 10 days following vaccination) predicted antibody titers to the influenza vaccine 1 and 4 months later among 83 healthy freshman undergraduates. Prospective analyses showed that lower average sleep duration across the 13 days of monitoring predicted lower antibody response at both follow-up times. Poor average sleep quality predicted lower antibody levels 1 month post-immunization. When analyzed day by day, however, shorter sleep duration and poor sleep quality the night before vaccination predicted lower antibody responses at 1 month and 4 months follow-up. Notably, this relationship was only observed for one of the three viral strains (A/New Caledonia) in the influenza vaccine and relied solely on self-reported sleep. Nevertheless, these findings provide initial evidence that short sleep duration and poor sleep are associated with diminished secondary antibody responses to vaccination.

3.0 SUMMARY AND STATEMENT OF PURPOSE

It has become clear that sleep plays an important role in the maintenance of health. Short sleep duration, poor sleep efficiency, and poor sleep quality are associated with increased risk for chronic disease and susceptibility to acute infectious illness. Growing evidence suggests that the immune system may be one pathway linking sleep disturbance to health risk, with acute sleep loss modulating immune processes responsible for maintaining host-resistance to disease. However, until recently, studies demonstrating an association between sleep and immunity have relied upon *in vitro* markers of immunocompetence that are of unknown clinical significance and provide a poor overall estimate of the body's ability to resist disease. For this reason, recent attention has turned to examining the impact of sleep on immune function in the living organism. One naturally occurring immune response relevant to protection from infectious pathogens is antibody production in response to vaccination. Use of *in vivo* vaccination models, provides a unique opportunity to examine sleep-immune associations that are directly related to host resistance to infectious disease.

Preliminary evidence suggests that modest sleep disruption on the days surrounding vaccination is associated with diminished primary and secondary antibody responses; the literature is in its infancy and it remains to be determined whether initial findings are reliable and generalize beyond young, healthy adults. In this regard, it might be expected that the association between sleep and antibody response would be stronger, and perhaps more clinically meaningful,

among older populations who show greater sleep disruption and immunosenescence. Furthermore, prior evidence for an association between sleep and antibody levels has relied on either experimental manipulation or self-reported sleep behavior, which may not generalize to the natural environment or accurately estimate sleep parameters. No study, to date, has examined whether natural variation in sleep behavior, measured objectively (e.g. via actigraphy), is associated with magnitude of primary and secondary antibody production to vaccination.

To address these gaps in the extant literature, the primary aims of the current study are to investigate whether sleep parameters, including duration, efficiency, and quality assessed through *subjective* and *objective* means, predict primary and secondary antibody responses to the hepatitis B vaccination series among a sample of relatively healthy, older community volunteers.

4.0 STUDY QUESTIONS AND HYPOTHESES

Question 1: Do sleep parameters, measured prior to the first vaccination, predict primary antibody responses to hepatitis B immunization?

Growing epidemiologic and experimental evidence suggests that short sleep duration, poor sleep efficiency, and poor sleep quality are associated with onset and progression of chronic disease and infectious illness (Ayas, White, Al-Delaimy et al., 2003; Ayas, White, Manson et al., 2003; Cohen et al., 2009; Gangwisch et al., 2006; Hall et al., 2008; Leineweber et al., 2003; Mallon et al., 2002; Meisinger et al., 2007) . To date, no study has investigated whether natural variation in sleep duration, efficiency, or quality prior to vaccination is associated with magnitude of primary antibody response. Based on the existing evidence, we make the following hypotheses:

Hypothesis 1*:* Shorter sleep duration, as measured using actigraphy over the 3 days prior to the first immunization, will be associated with lower primary antibody responses to the hepatitis B vaccination.

Hypothesis 2*:* Poorer sleep efficiency, as measured using actigraphy over the 3 days prior to the first immunization, will be associated with lower primary antibody responses to the hepatitis B vaccination.

Hypothesis 3*:* Poorer subjective sleep quality, as measure using electronic sleep diaries over the 4 days prior to the first immunization, will be associated with lower primary antibody responses to the hepatitis B vaccination.

Question 2: Do sleep parameters, measured prior to second vaccination, predict secondary antibody responses to hepatitis B immunization?

In contrast to the limited literature examining psychological and behavioral correlates of primary antibody responses, a larger number of studies have examined secondary antibody responses. Consistent evidence shows that prolonged stress is associated with lower secondary antibody responses to a number of vaccinations, including influenza and hepatitis B (Glaser et al., 1998; Kiecolt-Glaser et al., 1996; Miller et al., 2004; Phillips et al., 2006; Vedhara, Cox et al., 1999). In regard to sleep, initial evidence suggests that poor sleep in the laboratory and the field is associated with lower secondary antibody responses measured months later (Pressman et al., 2005; Spiegel et al., 2002). Nevertheless, no study has examined the influence of sleep, measured objectively in the natural environment, on secondary antibody response to the hepatitis B vaccine. Based on the existing literature we make the following hypotheses:

Hypothesis 4: Shorter sleep duration, as measured using both electronic sleep diaries over the 4 days prior to the second vaccination and actigraphy over the 3 days prior to the first immunization, will be associated with lower secondary antibody responses to hepatitis B vaccination.

Hypothesis 5: Poorer sleep efficiency, as measured using actigraphy over the 3 days prior to the first vaccination, will be associated with lower secondary antibody responses to hepatitis B immunization.

Hypothesis 6: Poorer sleep quality, as measured across 4 days prior to the second vaccination, will be associated with lower secondary antibody responses to hepatitis B immunization.

Question 3: Do sleep parameters, measured prior to the first vaccination, predict secondary antibody responses to hepatitis B immunization independent of sleep parameters measured prior to the second vaccination period?

Psychological and behavioral factors experienced immediately prior to vaccination appear to have a substantial impact on secondary antibody response, with preliminary evidence supporting the impact of acute stress, partial sleep deprivation, and exercise in the laboratory on secondary antibody responses (Edwards, Burns, Allen et al., 2007; Edwards et al., 2006; Pressman et al., 2005). It is possible that variation in sleep on the days prior to initiating the hepatitis B vaccination series may significantly influence secondary antibody responses independent of sleep that occurs prior to the second vaccination. Accordingly, we hypothesize the following:

Hypothesis 7: Shorter sleep duration, as measured using actigraphy over the 3 days prior to the first vaccination, will be associated with lower secondary antibody responses to hepatitis B immunization after adjusting for sleep duration measured prior to the second vaccination.

Hypothesis 8: Poor sleep efficiency, as measured using actigraphy over the 3 days prior to the first vaccination, will be associated with lower secondary antibody responses to hepatitis B immunization after adjusting for sleep efficiency measured prior to the second vaccination.

Hypothesis 9: Poorer sleep quality, as measured using sleep diaries over the 4 days prior to the first vaccination, will be associated with lower secondary antibody responses to hepatitis B immunization after adjusting for sleep quality measured prior to the second vaccination.

In addition to addressing these primary hypotheses, this study will also investigate in secondary (i.e. exploratory) analyses whether sleep fragmentation and intra-individual variability in sleep behavior are associated with primary and secondary antibody responses. Moreover, we will explore, using all available measurement days, whether individual differences in sleep duration, efficiency, or quality predict primary and secondary antibody levels. Finally, in instances where sleep is related to secondary antibody responses, an additional set of analyses will be computed to test whether these sleep parameters predict the likelihood of clinical protection (i.e. mounting anti-HBa ≥10 mIU/ml), thus providing prospective evidence that sleep impacts susceptibility to infectious illness.

5.0 METHODS

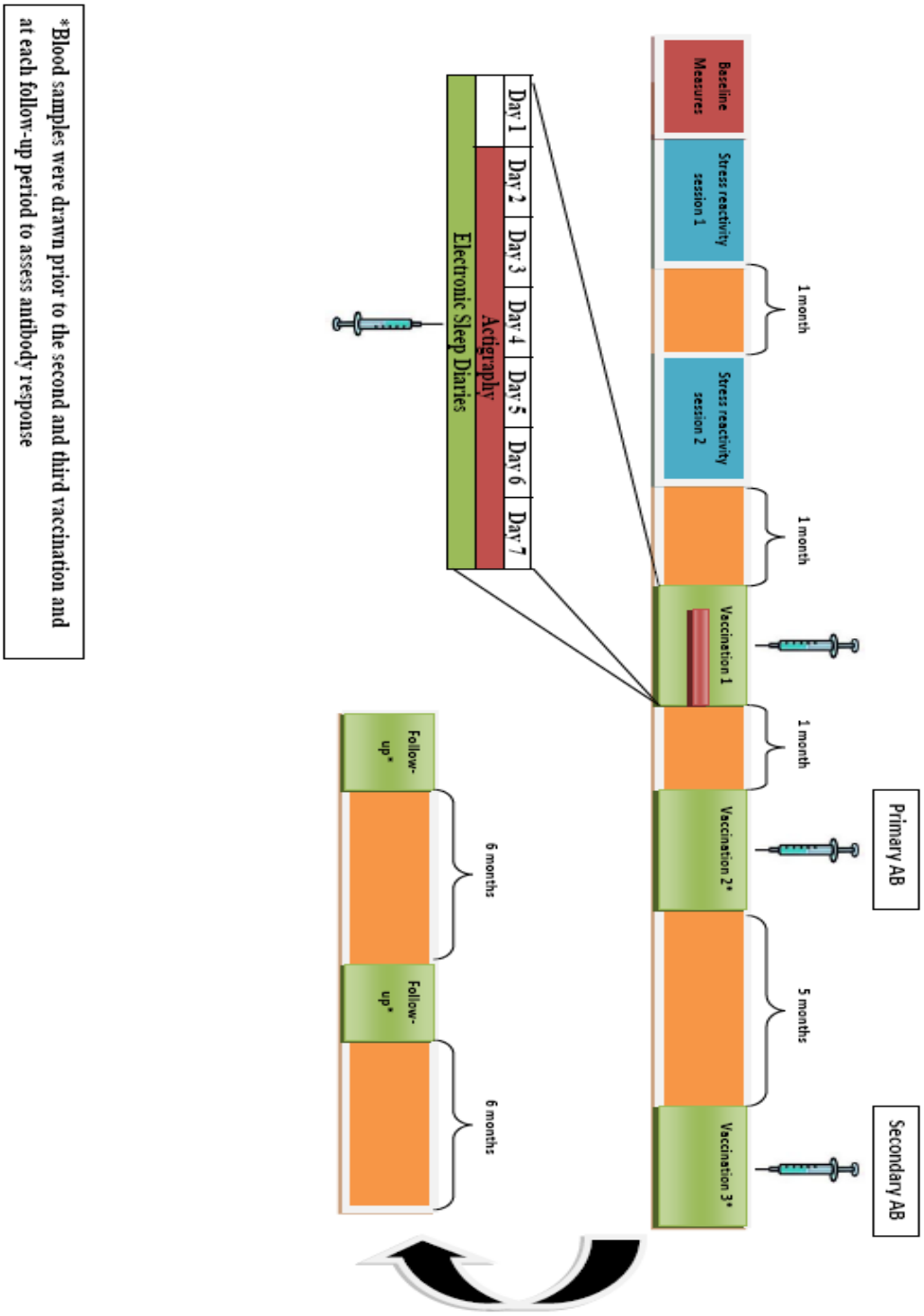
5.1 PARTICIPANTS

Participants were 70 women and 55 men (91.3% Caucasian), aged between 40 and 60 years old (Mean=50.1 ± 5.4) who were recruited via mass mail solicitation in Western Pennsylvania (primarily Allegheny County). Eligible participants were non-smokers, reported being in good general health (including no history or symptoms of myocardial infarction, asthma, cancer treatment in the past year, current or past psychiatric illness, or other systemic disease known to affect the immune system), and free from medications known to affect the nervous, endocrine, or immune systems in the past 3 months (not including oral contraceptives). Women who were pregnant or lactating were ineligible. In addition, participants more than 30% overweight, as estimated by BMI, were excluded. Prior to full enrollment, otherwise eligible participants underwent a blood draw to assess levels of hepatitis B core (HBc), surface antigens (HBsA), and surface antibodies (anti-HBa), indicating current or past exposure or prior vaccination. Individuals who showed any serological evidence of prior exposure to the antigen were excluded.

5.2 PROCEDURES

The VIP project was a prospective study that consisted of three separate phases 1) a laboratory based reactivity phase, 2) a vaccination phase, and 3) a follow-up period, as displayed in Figure 1. In the reactivity phase, participants underwent an acute laboratory challenge (an evaluative speech task) on two occasions, scheduled one month apart, during which time immune, cardiovascular, and endocrine measures were obtained. This was followed by the vaccination phase of the study when all participants received the standard 3 dose hepatitis B vaccination sequence. The first dose of the hepatitis B vaccine was administered approximately 1 month after the reactivity phase followed by the second and third dose administered 1 month and 6 months after the first immunization, respectively. Each vaccination period included daily assessment of health behaviors using electronic diaries that were completed for 7 days surrounding each of the vaccinations (see "Ecological Momentary Assessment" below). A subgroup of participants also wore actigraph watches over the same 7 day assessment period surrounding the first immunization providing 6 nights of behavioral sleep data (see "Actigraphy" below). Blood draws were obtained moments before administration of the second and third dose of the vaccine to assess antibody levels (i.e. primary and secondary responses to vaccination) as well as 6 and 12 months following completion of the vaccination series (i.e. follow-up phase). Subjects were paid $230 for completing this study in its entirety. Data included in the present analysis was largely restricted to the vaccination phase of the VIP project and focuses on sleep data and primary and secondary antibody responses to vaccination; however, secondary analyses investigating the relation between sleep and clinical protection status, which was assessed at the conclusion of the vaccination series (i.e. 6 months following the third injection), will also be presented.

Figure 1: Diagram of the study design for Vaccination Immunity Project



5.2.1 Hepatitis B Vaccination

Participants received three 20 microgram doses of recombinant hepatitis B vaccine (Engerix-B, Glaxo SmithKline), administered intramuscularly into the deltoid muscle. The first two immunizations were spaced 1 month apart, and the third booster dose was administered 6 months after the first. The first injection stimulated a primary immune response with subsequent doses activating secondary processes. To assess primary and secondary antibody responses to the hepatitis B vaccination, 10 ml blood samples were drawn on visits when participants received the second and third immunizations but prior to these injections. Antibody levels were also measured 6 and 12 months after the third immunization to assess clinical protection (i.e. anti-HBa ≥10mIU/ml) and antibody maintenance.

5.2.2 Ecological Momentary Assessment

In order to assess the influence of sleep on antibody responses, participants completed electronic diaries for 7 consecutive days (3 days prior to, the day of, and 3 days after the immunization) at all three vaccination time points. To facilitate data collection, participants were trained to use an electronic palm-pilot type computer (Palm Zire21). Four times per day (1, 4, 9, and 11 hours after scheduled awakening) participants were signaled to complete questions about a variety of daily experiences, including affect, levels of stress, and health behaviors. Assessment of sleep duration, latency, and quality was included in the first assessment of each day regarding the previous night. This method of data collection, also known as ecological momentary assessment (EMA), has been demonstrated to be a more valid method of capturing day-to-day variability in psychosocial processes than retrospective reports, which are subject to

recall bias (Stone et al., 1998). In order to limit data lost to diary malfunction, data was uploaded to the study database when participants came in for their vaccination visit (day 4 of EMA data collection) and immediately following the 7-day collection period.

5.2.3 Actigraphy

A subset of participants (n=104) wore an actigraph watch (Actiwatch-64,Respironics, Inc.) on their non-dominant wrists continuously for 7 days around the first hepatitis B inoculation, providing behavioral sleep data for 6 consecutive nights (3 nights before and 3 nights after the first immunization). Actigraphy measures movement as a proxy for wakefulness and, when used in sleep research, capitalizes on the fact that sleep is marked by prolonged inactivity. In this study, actigraphy was used as a more objective assessment of sleep duration, efficiency, and sleep fragmentation. Experimental evidence supports actigraphy as a reliable and valid measure of sleep behavior in healthy, community samples (Ancoli-Israel et al., 2003; Littner et al., 2003) that does not suffer the methodological weaknesses associated with retrospective reporting.

5.3 MEASURES

5.3.1 Background Variables

Demographic information, including age, gender, race, and BMI (kg/m^2), was obtained by the study nurse at the time of the first study visit.

5.3.2 Predictor Variables (Primary)

5.3.2.1 Sleep Diary Measures

The electronic daily diary included questions aimed at assessing sleep duration, sleep onset latency, and sleep quality. Each morning participants were prompted to respond to the following questions: "What time did you go to bed last night?" and "What time did you wake up this morning?" In addition, they recorded how long, in minutes, "after the lights went out" it took until they fell asleep (i.e. sleep onset latency) and rated the quality of that night's sleep (1=very poor thru 4=very good).

Sleep Duration

Diary-based sleep duration was calculated for each diary entry as follows: [(Time Went to Bed - Time Woke Up) - Time Until Fell Asleep]. This calculation considers the amount of time an individual reports sleeping once able to fall asleep, but does not account for awakenings during the night. Pre-vaccination averages of diary-based sleep duration were calculated using data collected on Day 1 thru Day 4 of the diary assessment at each immunization period, yielding measures of diary-based sleep duration prior to the first, second, and third vaccination. However, the primary hypotheses of this study focus on sleep measures collected prior to the first and second hepatitis B immunizations.

Sleep Efficiency

Diary-based sleep efficiency was calculated for each diary entry as follows: [(Sleep duration)/(Time Went to Bed- Time Woke Up)*100]. This calculation estimates the proportion of time an individual is asleep after attempting going to bed. Unfortunately, the electronic diary

did not include a question regarding perceived minutes awake after sleep onset. Therefore, variation in the diary-based sleep efficiency is completely accounted for by sleep onset latency. Pre-vaccination averages of diary-based sleep efficiency were calculated using data collected on Day 1 thru Day 4 of the diary assessment at each immunization period.

Sleep Quality

Diary-based measures of sleep quality were calculated by averaging sleep quality scores during the pre-vaccination days for each immunization period (Day 1 thru Day 4). Again, primary hypotheses of this study focus on sleep measures collected prior to the first and second hepatitis B immunizations.

5.3.2.2 Actigraphy Sleep Measures

Actigraphy provides an objective measure of activity level and employs a software algorithm to determine sleep based on inactivity. While not a direct measure of sleep behavior, per se, it has been shown to be reliable when compared to polysomnography (Ancoli-Israel et al., 2003; de Souza et al., 2003). Analysis of actigraphy data was conducted using the manufacture's supplied software (Actiware 5.02, Minimitter, Inc). Data was stored in 1 minute epochs. The software scores each 1 minute epoch as either sleep or wake based on the activity counts within that epoch as well as the counts registered in the epochs 2 minutes before and 2 minutes after. The selected threshold for scoring an epoch as wake was set at 40 activity counts (medium threshold). The sleep/wake algorithm per 1 minute epoch is as follows:

$$\mathbf{D = A_{-2}*(1/25) + A_{-1}*(1/5) + A_0*(1) + A_{+1}*(1/5) + A_{+2}*(1/25)}$$

where A_X= accelerometer activity count for that minute. If D>40, participants were scored as awake. Sleep onset was defined as 10 consecutive minutes of D<40.

The algorithm requires that "rest" intervals are set to calculate sleep parameters. Bedtimes and wake times were set by researchers based primarily on electronic diary responses. However, when diary data were unavailable (46 data points), rest intervals were set based on careful visual inspection of actigraphy data. In this regard, actigraphy rest intervals were set based on the longest period of inactivity during nighttime hours. Complete actigraphy data from 5 participants was independently scored by an experienced technician (r=.84). Moreover, files that were particularly difficult to interpret were discussed with experienced editors and consensus was reached regarding rest interval periods. Once rest intervals were set, sleep onset was automatically calculated by the software. Sleep onset is defined as 10 consecutive minutes in which the maximum of one epoch has an activity count of 40 (medium threshold). Sleep end is identified as the last 1 minute epoch in the last 10 minute period with no more than one epoch that has an activity count of greater than 40. The primary actigraphy-based sleep measures of interest in this study were sleep duration and sleep efficiency. However, sleep fragmentation was also examined in secondary analyses.

Sleep Duration

Assumed sleep duration was calculated as the length of time between the actigraph-estimated sleep start and end times and does not subtract actigraph-identified awakenings throughout the night. Therefore, it is an estimate of general sleep-wake patterns and less highly associated with sleep efficiency (r=.29) and sleep fragmentation (r= -.12) than total sleep time (TST) (r=.61 and r= -.45, respectively). In the present study this measure of sleep duration was preferred over TST because it was operationally more similar to the diary-based measure of sleep duration collected at the second vaccination period, thus providing consistency in analyses where actigraphy-derived sleep duration at the first vaccination period was treated as a predictor of

secondary antibody responses, *after adjusting* for diary-based sleep duration at the second vaccination period (i.e. hypothesis 7). However, because TST is commonly reported in the literature as a measure of actigraphy-based sleep duration, analyses were re-run using TST as a predictor of primary and secondary antibody responses and are presented in Appendix A. Pre-vaccination averages of actigraphy-based sleep duration used in the analyses were calculated using data from the first three days of measurement (Day 2 through Day 4) prior to the first immunization.

Sleep Efficiency

Assumed sleep efficiency was calculated based on the following equation:

Sleep efficiency= (Total Sleep Time/Rest interval)*100

Sleep efficiency was calculated by the software algorithm to estimate the proportion of time scored asleep given the specified rest interval. Unlike actigraphy-based sleep duration, efficiency accounts for sleep lost during the night/early morning prior to the final awakening. Measures of pre-vaccination sleep efficiency were calculated using the three consecutive days of actigraphy data (Day 2 thru Day 4) collected prior to the first immunization.

5.3.3 Predictor Variables (Secondary)

Sleep Fragmentation

Though not a primary sleep variable of interest, emerging evidence suggests that fragmented sleep may be related to physiologic (e.g. catecholamines) and psychological factors (i.e. psychological stress) known to impact immunity (Irwin, Clark, Kennedy, Christian Gillin, & Ziegler, 2003; Mezick et al., 2009; Sadeh, Keinan, & Daon, 2004). Therefore, pre-vaccination

averages of sleep fragmentation were calculated to determine their effect on primary and secondary vaccination responses in secondary analyses. This measure of nocturnal awakening is derived from indices of nocturnal movement and is calculated as follows:

(% of 1-minute intervals of movement during sleep+ % of 1-minute intervals of immobility)/total 1-minute immobility intervals

This calculation provides an estimation of the proportion of awakening after sleep onset, with a higher score indicating more fragmented sleep. Measures of average sleep fragmentation were calculated using the three consecutive days of actigraphy data (Day 2 thru Day 4) collected prior to the first immunization.

Intra-Individual Variability in Sleep Parameters

There is growing evidence that night to night variability in sleep is substantial, often eclipsing the variability observed between individuals (Buysse et al., in press; Knutson, Rathouz et al., 2007; Mezick et al., 2009; van Hilten et al., 1993). To examine whether intra-individual variability in sleep parameters predict vaccination response, standard deviations (SD) for each sleep parameter were computed for each participant. For instance, using all 6 consecutive days of actigraphy-based measures of sleep duration (i.e. measurements that occurred 3 days prior to and 3 days following the primary vaccination), a SD score was calculated for each individual. This score reflects the degree to which that person deviates from his or her own average sleep, with a higher SDs indicating greater variability in sleep duration. Similar SDs were calculated for the other actigraphy-based measures (i.e. sleep efficiency and sleep fragmentation). In contrast, SDs based on electronic diary sleep measures (i.e. diary-based sleep duration, efficiency, and quality) were computed using all available measurements across the three

vaccination time points. Therefore, diary-based SDs were computed using as many as 21 days of measurement for each participant.

Individual Differences in Sleep

Epidemiologic evidence suggests that individual differences in sleep parameters (i.e. habitual sleep), particularly shortened sleep duration, are associated with increased morbidity and mortality (Cappuccio, D'Elia, Strazzullo, & Miller, *in press-b;* Hall et al., 2008; Mallon et al., 2002). To examine whether dispositional differences in sleep duration, efficiency, and quality are associated with magnitude of primary and secondary antibody responses to the hepatitis B vaccination, we computed averages of sleep duration, efficiency, and quality for each participant using all available actigraphy and electronic diary measures. For instance, to calculate individual differences in sleep efficiency, we averaged the 6 days of actigraphy collected around the first vaccination period, the 7 days of electronic sleep diary measures obtained around the both second and third vaccination periods. As sleep quality was obtained by electronic sleep diary at each vaccination period, we averaged diary-based sleep quality over the 21days of assessment for each study participant.

5.3.4 Outcome Variables

5.3.4.1 Hepatitis B Antibody Response

At the second and third vaccination, 10 mls of blood were collected for quantifying levels of antigen-specific antibodies to hepatitis B (anti-HBa). Fresh blood samples were sent to Central Laboratory Services (University of Pittsburgh Medical Center) for antibody assessment. When antibody levels exceeded levels of detection by Central Laboratory Services (>1000

mIU/ml), frozen serum samples were sent to a commercial laboratory (Arup Laboratories; Salt Lake City, UT) for further analysis. Preliminary testing showed good reliability between both laboratories (r=.998).

Primary Antibody Response

To determine the primary antibody response to the initial vaccination, 10 mls of blood was drawn immediately prior to receiving the second vaccination (i.e. 1-month following the initial immunization), and anti-HBa levels were quantified. In healthy populations, it is expected that only 25% of participants produce quantifiable levels of antibodies to this initial vaccination. Consequently, this variable was dichotomized with those producing quantifiable levels identified as "responders" and those without antibodies following the initial immunization as "non-responders."

Secondary Antibody Response

The secondary antibody response was determined by obtaining 10 mls of blood immediately prior to the third immunization, which occurred approximately 5 months after the second vaccination. This outcome variable was treated as a continuous variable.

Clinical Protection

Though not a primary outcome of the present study, we assessed clinical protection against the hepatitis B virus using a 10 ml blood sample drawn 6 months following the third vaccination. A circulating anti-HBa antibody level of ≥10 mIU/ml is the clinical threshold for protection (CDC, 1987). In this sample, 18 participants (14.6%) failed to mount ≥10 mIU/ml of anti-HBa. This clinical protection variable was dichotomized, categorizing participants as "protected" or "not protected".

5.4 DATA ANALYSIS

Preliminary analyses were conducted to examine distribution of all continuous variables. Inter-correlations between covariates (gender, age, race, BMI), pre-vaccination sleep averages, and antibody levels were calculated using Pearson's r (continuous) and point biserial (continuous and dichotomous) and phi-correlation (dichotomous) coefficients All statistical analyses were performed using SPSS 17.0.

5.4.1 Primary Hypotheses

Hypotheses 1-3 examined whether sleep duration, efficiency, and quality, measured prior to the first immunization, predicted the likelihood of mounting a detectable antibody response to the first hepatitis B vaccination. To this end, separate unadjusted logistic regressions were computed, followed by hierarchical logistic regressions, entering sociodemographic variables (age, gender, race, BMI) in the first step of the model, followed by the sleep parameter of interest in the second step.

Hypotheses 4-6 examined whether sleep duration, efficiency and quality, measured prior to the second vaccination, predicted antibody responses to the second immunization. In general, each hypothesis was tested using an unadjusted linear regression followed by a hierarchical linear regression; however, in addition to adjusting for sociodemographic variables in the each model, we also controlled for responder status to the initial vaccination (i.e. whether an individual mounted a detectable primary antibody response). Indeed, individuals who displayed detectable antibodies in response to the first immunization also showed higher antibody levels in response to the second vaccination ($r=.22$, $p<.05$).

To test hypothesis 4, we utilized two analytic strategies. First, to capitalize on the objective nature of actigraphy, it was initially proposed to treat actigraphy-based sleep duration, measured prior to the first immunization, as a stable construct and use it as a predictor of secondary antibody responses. However, test-retest reliability over the pre-vaccination period suggested poor stability (*ICC=.49; see* Psychometric Data). Accordingly, we relied on diary-based measures of sleep duration assessed prior to the second vaccination period. Using unadjusted linear regression followed by hierarchical linear regression, where sociodemographic variables (age, gender, race, and BMI) and responder status were entered in the first step and pre-vaccination averages of diary-based sleep duration in the second step.

While psychometric data failed to support stability in actigraphy-based sleep duration over the 3 days of assessment prior to the first vaccination, test-retest reliability appeared sufficiently high over the 6 days of measurement (3 days prior to and 3 days following the first immunization; *ICC=.68; see* Psychometric Data). Therefore, in a second analytic strategy, we computed another unadjusted linear regression followed by a hierarchical linear regression entering sociodemographic variables and responder status in the first step and actigraphy-based sleep duration, averaged over 6 days of assessment, in the second step.

Hypothesis 5 examined whether sleep efficiency predicted secondary antibody levels in response to the second hepatitis B vaccination. Sleep efficiency displayed adequate reliability (*ICC*=.75). Accordingly, actigraphy-based sleep efficiency was treated as a stable construct and used as a predictor of secondary antibody responses. First an unadjusted linear regression was computed followed by a hierarchical linear regression, again entering sociodemographic characteristics and responder status in the first step and then actigraphy-derived pre-vaccination sleep efficiency in the second step.

Hypothesis 6 investigated the influence of subjective sleep quality, assessed prior to the second vaccination, on secondary antibody responses. Here, we relied on electronic sleep diaries completed on the four consecutive days prior to the second immunization. An unadjusted linear regression was computed followed by a hierarchical linear regression, entering sociodemographic variables and responder status in the first step, followed by diary-based pre-vaccination sleep quality in the second step.

Hypotheses 7-9 tested the independent contribution of sleep duration, efficiency, and quality measured prior to the first vaccination on secondary antibody responses. To this end, we computed separate hierarchical linear regression analyses first adjusted for sleep measures that occurred immediately prior to the second vaccination followed by the sleep measures prior to second vaccination. We then computed another hierarchical regression, entering sociodemographic characteristics and responder status in the first step, the pre-vaccination sleep measure prior to the second vaccination in the second step, followed by the pre-vaccination sleep measure from the first immunization in the final step.

5.4.2 Secondary Analyses

In secondary analyses, we sought to investigate the influence of sleep fragmentation and intra-individual variability of sleep measures and individual differences in sleep on antibody responses to hepatitis B vaccination. To this end, we employed unadjusted and adjusted logistic regression to examine whether pre-vaccination averages in sleep fragmentation, intra-individual variability in sleep parameters, and averages of sleep duration, efficiency, and quality predicted primary antibody responses (i.e. responder status). To address these sleep effects on secondary

antibody responses, we employed unadjusted and hierarchical linear regression analyses. All analyses were adjusted for sociodemographic variables (i.e. age, gender, race, and BMI).

We also investigated whether sleep parameters predicted the likelihood of being clinically protected from the hepatitis B virus at the conclusion of the vaccination series. Clinical protection was defined by displaying ≥10mIU/ml anti-HBa in peripheral circulation 6 months following the third immunization. In this study, clinical protection was significantly related to higher antibody levels following the second immunization (r=.54) but not to responder status following the initial vaccination (r=.09). Accordingly, analyses of clinical protection were limited to sleep parameters found to be statistically significant predictors of secondary antibody responses. We employed hierarchical logistic regressions to examine the effects of sleep on likelihood of being clinically protected. All analyses were adjusted for sociodemographic variables.

5.4.3 Non-linear Relationships

It is plausible that several of the relations between sleep parameters and antibody production may be non-linear in nature. This was determined post-hoc based upon graphical representation of the data. If a non-linear relationship was indicated, regression models were recomputed using the appropriate non-linear function (e.g. quadratic term); however, to correct for the multi-collinearity that may result by entering the linear and non-linear effect in the same regression model, non-linear variables were first regressed onto their related linear effects and the standardized residuals were saved. Regression models were computed, entering any relevant covariates in the first step followed by the linear term in the second step. In the final step, the saved standardized residual was entered, which represents the non-linear effect.

5.5 DATA TRANSFORMATIONS

5.5.1 Background Variables

Measures of age, gender, and body mass index (BMI) were normally distributed. With respect to racial make-up, the sample was comprised of 114 Caucasians, 9 African-Americans, 1 Hispanic-American, and 1 Asian-American. As such, race was dichotomized as "Caucasian" and "non-Caucasian." Complete background variable data was available for all 125 participants in this study.

5.5.2 Electronic Sleep Diaries

Each of the 125 participants completed electronic diaries to assess sleep behavior four days prior to and three days after the first, second, and third hepatitis B vaccination. At the end of the first vaccination period, 66.4% (n=83) of participants provided data on all 7 days of collection and 90% provided data on 5 of 7 days (n=112). With regards to the pre-vaccination period (Day 1 thru Day 4), data was complete on 80% (n=100) of participants, with 113 (90.4%) participants providing data for at least 2 of the 4 days of measurement. Similar results were observed during the second vaccination period 75.8% of participants (n=94) provided sleep data for all 7 collection days and 94% (n=117) providing diary data on 5 of 7 days. Complete pre-vaccination data was available for 78.4% (n=96) of participants, with 118 (94.4%) completing sleep diary measures on 2 of the 4 days prior to the second hepatitis B injection. Finally, 62.4% (n=78) provided data on all 7 days of diary collection during the third vaccination and 91.2% (n=114) provided data on at least 5 of the 7 days of collection. Pre-vaccination data (Day 1 thru

Day 4) was complete on 76% (n=96) of participants, with 119 (95.2%) of participants providing sleep data for 2 of the 4 nights prior to the final injections. In all instances, missing data points were due to hardware/software malfunction.

Because the primary hypotheses of this study focus exclusively on sleep measures collected prior to the first and second hepatitis B immunization (i.e. pre-vaccination periods), averages of diary-based sleep duration and sleep quality were calculated using data collected on Day 1 thru Day4. Pre-vaccination averages were only calculated if a participant completed at least 2 diary entries. As a result, data on 12 participants were lost at the first vaccination and data on 7 participants were lost at the second vaccination, yielding a full sample of 113 and 118 participants for later hypothesis testing.

5.5.3 Actigraphy

Actigraphy collection was initiated after beginning the VIP study. As such, data were collected continuously on 104 participants for 7 consecutive days around the first vaccination, providing behavioral sleep data for 6 consecutive nights (Day 2 thru Day 7; 3 days prior to and 3 days following the initial vaccination). Data were lost on 9 participants due to hardware/software malfunction and on 2 participants due to removal of the actigraph watch during sleep periods on all collection nights. As such, data on 93 participants were available for editing.

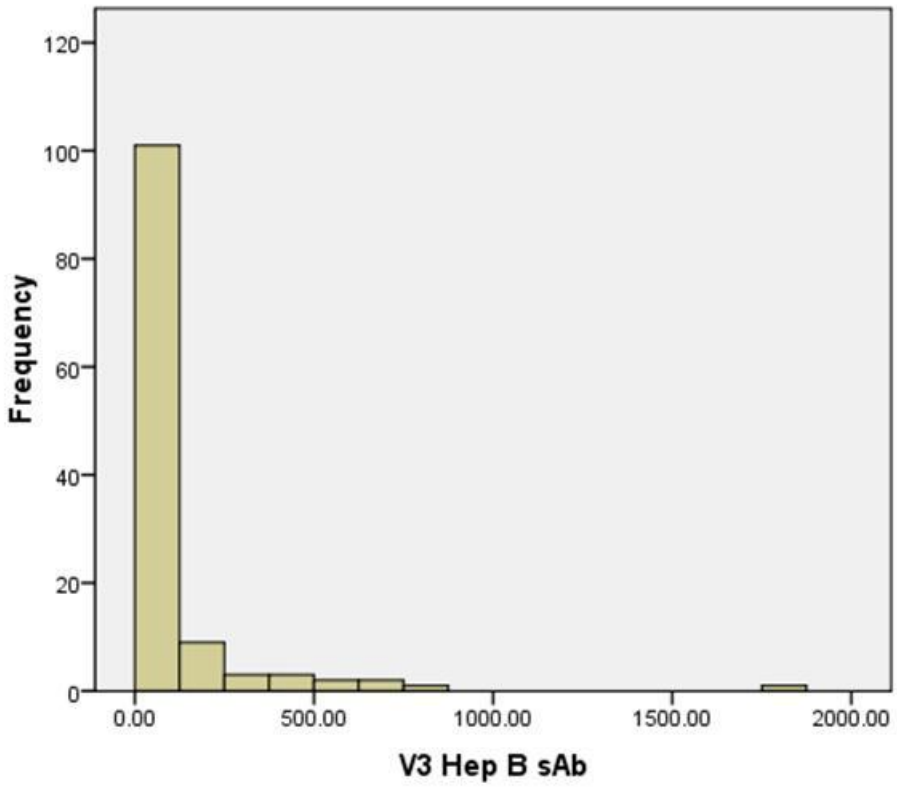
As mentioned, diary-based bedtimes and wake-up times were used to set the rest intervals for actigraphy data. In the event that bedtime or wake time estimates differed by more than 2 hours between the electronic diary and actigraphy, they were considered to be outliers and treated as missing (Mezick et al., 2009). This resulted in 18 missing data points (3.2%) across 14

participants. Actigraphy-based averages of pre-vaccination sleep duration, efficiency, and fragmentation were calculated for participants who had at least 2 available nights of data. This resulted in the loss of 4 participants and yielded a final sample of 89 participants with whom averages in actigraphy sleep data were available for hypothesis testing.

5.5.4 Antibody Levels

Raw antibody levels were obtained prior to the second and third vaccination, representing primary and secondary antibody responses to the first and second vaccination, respectively. Only 31 of 125 participants (24.8%) responded to the first vaccination with detectable antibody levels. Therefore, a dichotomized variable (responder vs. non-responder) was created to examine associations between sleep measures and primary immune responses to immunization. Antibody levels assessed five months after the second vaccination were available on 124 participants; however, one sample was lost to laboratory error and one sample was deemed an outlier (11, 600 mIU/ml; >9 standard deviations above the mean) and was subsequently dropped from the analyses, leaving 122 participants with available secondary antibody data. Secondary antibody levels were positively skewed, and were normalized using a natural log transformation. The raw distribution of secondary antibody levels is displayed in Figure 2. Finally, antibody levels measured 6 months after the final vaccination were used to assess clinical protection to the hepatitis B virus (n=123). In this regard, 14.6% of participants (n=18) failed to meet the clinical threshold for protection (anti-HBa $\geq$ 10 mIU/ml). Consequently, a dichotomous variable was created (protected vs. non-protected) to examine associations between sleep measures and protection status.

Figure 2: Raw distribution of secondary antibody levels.



5.6 DATA IMPUTATION

Quantitative researchers have advocated for the use of imputation techniques over conventional listwise deletion (Babyak, 2005; Schafer & Olsen, 1998). Therefore, two sets of analyses were conducted when testing the primary study hypotheses. The first set of analyses was based on sleep averages for participants who had at least 2 days of pre-vaccination sleep measurements. The second set of analyses employed imputed data to create pre-vaccination averages, generally resulting in an increased sample size. In this second set of analyses, missing sleep parameters were imputed using the expectation maximization (EM) approach (Little &

Rubin, 1987). This approach is characterized by a two-step iterative procedure marked first by an expectation step where an expected value of the completed data set is computed. Next, in the maximization step, the expected values are substituted in for missing values and a maximum likelihood function is estimated until convergence is achieved. Imputed data were used only if this approach resulted in an increase in sample size. Moreover, imputed values were only substituted for participants who had at least one day of data to contribute to the imputed average (i.e. averages comprised of entirely imputed data were not included in these analyses). Notably, while use of imputed data modestly increased our sample size in these analyses, it did not appreciably influence the study findings. As such, imputed analyses are provided in Appendix B.

6.0 RESULTS

6.1 SAMPLE DESCRIPTION

The present sample included 125 medically healthy participants (56% female, 91.2% Caucasian) aged between 40 and 60 years old (M=50.1 ± 5.4) derived from the Vaccination Immunity Project (N=208); 83 participants were excluded from the present analysis because they did not receive the hepatitis B vaccination series or were deemed ineligible once enrolled due to prior hepatitis B exposure. Sociodemographic characteristics for the included and excluded samples are provided in Table 1. Participants included in the present analyses were similar in age, gender composition, and BMI to those excluded, but differed in racial composition. Specifically, participants included in this study were less racially diverse (91.2% Caucasian) when compared to those excluded (68% Caucasian; $X^2(1)=17.31$, $p<.001$).

Table 1: Sociodemographic characteristics among those included and excluded in the present analyses. Displayed as means and (standard deviations) or percentages.

	Included (n=125)	Excluded (n=83)
Gender (% Female)	56%	63.9%
Age (years)	50.1 (5.4)	50.1 (5.5)
Race (% Caucasian)	91.2%*	68.7%
Body Mass Index (kg/m2)	25.2 (3.3)	25.5 (3.2)
Education: Some college (%)	92.5%	
Employment Status (% Full time)	64.8%	
Family Income (%)		
<$50,000	31.2%	
$50,000-$74,999	20.0%	
$75,000-$99,999	13.6%	
≥ $100,000	24.8%	
No response	10.4%	

*p<.05

Table 2 displays pre-vaccination sleep averages captured by both actigraphy and electronic diary during this study as well as antibody responses to the first and second immunization. Prior to testing the primary and secondary study hypotheses, we investigated the reliability/stability and relative validity of the sleep measures employed in this study.

Table 2: Pre-vaccination sleep averages and antibody responses among the study participants.

	Mean (SD) or %	[Range]
Vaccination 1		
Actigraphy-based measures	n=89	
Total sleep time (mins)	344.3 (53.5)	[184.5-456]
Sleep onset latency (mins)	24.8 (22.0)	[0-105]
Sleep duration (hrs)	6.5 (1.0)	[3.9-8.6]
Sleep efficiency (%)	80.0 (8.5)	[47-94.6]
Sleep fragmentation (%)	30.2 (11.8)	[8-76.2]
Diary-based measures	n=113	
Sleep onset latency (mins)	13.1 (9.6)	[1-53.8]
Sleep duration (hrs)	7.0 (0.8)	[4.5-8.7]
Sleep efficiency (%)	96.9 (2.3)	[86.8-99.7]
Sleep quality (very poor [0-4] very good)	3.1 (0.5)	[1.3-4.0]
Vaccination 2		
Diary-based measures	n=118	
Sleep onset latency (mins)	12.2 (8.6)	[1-53.3]
Sleep duration (hrs)	7.1 (0.9)	[4.5-9.7]
Sleep efficiency (%)	97.1 (2.1)	[87.2-99.8]
Sleep quality (very poor [0-4] very good)	3.3 (0.5)	[1.8-4.0]
Vaccination 3		
Diary-based measures	n=119	
Sleep onset latency (mins)	11.8 (7.8)	[1-58.3]
Sleep duration (hrs)	7.0 (0.9)	[4.4-9.6]
Sleep efficiency (%)	97.3 (1.7)	[87.8-99.8]
Sleep quality (very poor [0-4] very good)	3.2 (0.5)	[1.8-4.0]
Antibody response		
Primary antibody levels (mIU/ml)[a]	4.76 (26.5) median=0.0	[0-252]
Primary antibody responder status (% with detectable antibodies)	24.8%	
Secondary antibody levels (mIU/ml)[b]	96.0 (217.4) median= 23.1	[0-1780]
% Clinically protected (≥10 mIU/ml)[c]	84.8%	

[a]=125, [b]=122, [c]=123

6.2 PSYCHOMETRIC DATA

6.2.1 Reliability of Sleep Parameters

Test-retest reliability analyses were conducted on all primary sleep measures to assess the stability of sleep from night to night. These analyses yielded aggregated intraclass correlation (*ICC*) coefficients for sleep measures assessed prior to each vaccination (Day 1 thru Day 4), post-vaccination (Day 5 thru Day 7), and across all nights of measurement (Day 1 thru Day 7). An $ICC \geq .70$ is generally considered the threshold for adequate test-retest reliability in sleep research (Acebo et al., 1999; Sadeh, Sharkey, & Carskadon, 1994). When *ICC* values were calculated using all available days of measurement, all of the sleep variables were considered reliable (Table 3), with the exception of actigraphy-based sleep duration across the first vaccination period (*ICC*=.68) and diary-based duration and efficiency across the third vaccination period (*ICC*=.66 and .68), all of which approached adequate reliability.

When reliability was calculated using only measurements that occurred prior to immunization, only actigraphy-based sleep efficiency and fragmentation were adequately reliable (*ICC*=.75 and .84, respectively). These findings are consistent with previous research showing that at least 6 days of consecutive measurement in necessary to reliably assess most sleep parameters, including sleep duration (Knutson, Rathouz et al., 2007; van Hilten et al., 1993).

Table 3: Test-retest reliability: Intraclass Correlation Coefficients (*ICC*) estimates from aggregated mean values over successive numbers of recording nights (pre-vaccination Day 1(or 2) thru 4; post vaccination Day 5 thru Day 7; across pre and post vaccination Day 1(or 2) thru Day 7). *ICC*≥.70 indicates adequate reliability.

	Pre-vaccination	Post-vaccination	Across pre and post vaccination
Sleep Duration ICC			
V1 Act. sleep duration	.49	.58	.68
V1 Diary sleep duration	.59	.66	**.75**
V2 Diary sleep duration	.64	.63	**.76**
V3 Diary sleep duration	.63	.55	.66
Sleep Efficiency ICC			
V1 Act. sleep efficiency	**.75**	**.71**	**.83**
V1 Diary sleep efficiency	.62	**.79**	**.77**
V2 Diary sleep efficiency	.68	.69	**.78**
V3 Diary sleep efficiency	.58	**.70**	.68
Sleep Quality ICC			
V1 Diary sleep quality	.66	.60	**.74**
V2 Diary sleep quality	.63	.59	**.79**
V3 Diary sleep quality	.69	.69	**.78**
Sleep Fragmentation ICC			
V1 Act. fragmentation	**.84**	**.74**	**.90**

6.2.2 Validity of Sleep Parameters

While this study did not employ polysomnography, considered as the "gold standard" in sleep assessment, high correlations between sleep diaries and actigraphy within each day of assessment would provide some evidence that these tools are measuring a similar construct. Here, sleep duration and efficiency were assessed using both actigraphy and electronic diary across the first vaccination period. Actigraphy-based sleep duration differed from diary-based assessment by an average of 38.6 minutes (S.D. =30.0 minutes). Day to day correlations between actigraphy and diary-based sleep duration were very high (r's= .86-.93) and are provided in Table 4. This was not surprising, however, given that the rest intervals used to calculate actigraphy derived sleep measures were based on the bedtimes and wake times obtained via electronic sleep diaries.

Day to day correlations between actigraphy and diary-based sleep efficiency are provided in Table 5. Unlike sleep duration, daily sleep efficiency measured using electronic diaries was not highly correlated with concomitant actigraphy-based measures of sleep efficiency (r's= -.03-.33). In part, this may be due to the fact that sleep efficiency calculated using actigraphy accounts for awakenings during the nighttime. The electronic sleep diary did not include a question regarding the amount of awakening a participant experienced after sleep onset, thus likely overestimating his or her sleep efficiency on any given night.

Table 4: Day to day correlations between actigraphy and diary-based measures of sleep duration assessed during the first vaccination period.

Diary-based sleep efficiency	Actigraphy-based sleep efficiency							
		Day1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	Day 1	--	.08	-.14	.06	.15	.21	-.08
	Day 2	--	**.33**	.08	.31	-.03	.20	-.02
	Day 3	--	.13	**.11**	.13	.05	.09	-.06
	Day 4	--	.05	-.09	**.31**	-.10	.06	-.16
	Day 5	--	.09	-.05	.27	**.09**	.21	-.15
	Day 6	--	.16	-.05	.18	.09	**.28**	-.07
	Day 7	--	.15	.21	.51	.04	.21	**-.03**

Table 5: Day to day correlations between actigraphy and diary-based sleep efficiency measured during the first vaccination period.

Diary-based sleep efficiency	Actigraphy-based sleep efficiency							
		Day1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	Day 1	--	.08	-.14	.06	.15	.21	-.08
	Day 2	--	**.33**	.08	.31	-.03	.20	-.02
	Day 3	--	.13	**.11**	.13	.05	.09	-.06
	Day 4	--	.05	-.09	**.31**	-.10	.06	-.16
	Day 5	--	.09	-.05	.27	**.09**	.21	-.15
	Day 6	--	.16	-.05	.18	.09	**.28**	-.07
	Day 7	--	.15	.21	.51	.04	.21	**-.03**

6.2.3 Stability of Sleep Averages Over Time

Diary-based measures of sleep duration, efficiency, and quality were assessed over 7 days at each of the three immunization periods. Accordingly, we were able to assess the stability of sleep averages over 1-month (i.e. test-retest reliability between V1 and V2), 5-month (i.e. test-retest reliability between V2 and V3), and 6-month (i.e. test-retest reliability between V1 and V3) periods. Aggregate intra-class correlation (*ICC*) coefficients for diary-based averages of sleep duration, efficiency, and quality are provided in Table 6. In general, diary-based sleep duration was relatively stable over time when averaged across the pre-vaccination, post-vaccination, and all days of measurement (*ICC's .60-.82*). In contrast, diary-based sleep efficiency appeared to be stable over a 1-month period (i.e. from V1 to V2; *ICC=.59-.78*), but not over longer periods of time. Finally, diary-based sleep quality displayed moderate stability over time (*ICC's .68-.80*).

Table 6: Intraclass Correlation Coefficients estimates from aggregated mean values of pre-vaccination, post-vaccination, and consecutive 7 days of assessment to assess stability in averages of diary-based sleep duration, efficiency and quality over one month (V1 and V2), five months (V2 and V3), and six months (V1 and V3).

	Pre-vaccination	**Post-vaccination**	**Across Pre and Post**
Sleep Duration ICC			
V1 and V2	.69	**.74**	**.81**
V2 and V3	**.73**	.66	**.82**
V1 and V3	**.72**	.60	**.78**
Sleep Efficiency ICC			
V1 and V2	.59	**.78**	**.74**
V2 and V3	.49	.54	.62
V1 and V3	.49	.67	.54
Sleep Quality ICC			
V1 and V2	.63	**.71**	**.80**
V2 and V3	**.75**	.61	**.77**
V1 and V3	.53	.64	.68

6.2.4 Impact of Vaccination on Sleep Parameters

While the primary aim of this study was to examine the influence of sleep on antibody responses, experimental animal and human evidence suggest that vaccination-related immune activation can also affect sleep (Bryant et al., 2004; Imeri & Opp, 2009). To address this possibility, paired t-tests were employed to test differences in sleep parameters assessed pre and post the first, second, and third vaccination. Results showed an increase in sleep duration from before to after the first vaccination (measured via actigraphy: $t(86)= -2.81$, $p<.01$; via electronic diary: $t(106)= -2.74$, $p<.01$). In addition, subjective sleep quality and diary-based sleep efficiency improved across the same period (sleep quality: $t(110)= -2.05$, $p<.05$; sleep efficiency: $t(106)=-2.26$, $p<.05$, respectively). As displayed in Table 7, no other significant differences in sleep parameters pre and post vaccination were observed. Post hoc examination of day-to-day differences in sleep across first vaccination period revealed that shorter sleep duration, poorer efficiency and poorer sleep quality on the night before the immunization (i.e. Day 4) accounted for the apparent improvements in sleep observed following the vaccination, possibly as a consequence of anxiety and waking up early for the vaccination appointment. Indeed, removal of Day 4 data from the pre-vaccination averages resulted in non-significant pre/post differences in sleep duration (measured via actigraphy: $t(79)= -1.91$, $p=.06$; via electronic diary; $t(102)=-1.40$, $p=.16$), efficiency ($t(106)=-1.29$, $p=.20$), and quality ($t(106)=-1.06$, $p=.29$). Nevertheless, Day 4 data remained in our calculations of pre-vaccination sleep averages as removal of this data led to additional reductions in reliability and sample size.

Table 7: Pre and post-vaccination means (sd) for actigraphy and diary-based sleep measures. P-values based on paired t-tests.

	Pre-vaccination	Post-vaccination	p-value
Sleep Duration			
V1 actigraphy sleep duration (hrs) (n=87)	6.5 (0.9)	6.8 (0.9)	**<.01**
V1 diary sleep duration (hrs) (n=107)	7.0 (0.8)	7.2 (0.9)	**<.01**
V2 diary sleep duration (hrs) (n=115)	7.0 (0.9)	7.1 (1.0)	.20
V3 diary sleep duration (hrs) (n=107)	7.0 (0.9)	7.1 (0.9)	.28
Sleep Efficiency			
V1 actigraphy sleep efficiency (%) (n=87)	80.4 (7.8)	80.8 (7.8)	.54
V1 diary sleep efficiency (%) (n=107)	96.9 (2.3)	97.3 (1.0)	**.03**
V2 diary sleep efficiency (%) (n=115)	97.1 (2.1)	97.2 (2.2)	.50
V3 diary sleep efficiency (%) (n=107)	97.2 (1.8)	97.3 (1.8)	.33
Sleep Quality			
V1 diary sleep quality (n=111)	3.1 (0.5)	3.2 (0.5)	**.04**
V2 diary sleep quality (n=115)	3.2 (0.5)	3.2 (0.5)	.20
V3 diary sleep quality (n=107)	3.2 (0.5)	3.2 (0.5)	.58
Sleep Fragmentation			
V1 actigraphy fragmentation (%) (n=87)	29.9 (11.7)	29.1 (12.5)	.37

6.3 PRELIMINARY RESULTS

6.3.1 Associations among Sleep Parameters and Background Variables

Pearson-product moment and point-biserial correlations of the primary pre-vaccination sleep measures and background variables (i.e. gender, age, race, and BMI) are displayed in Table 8. In general, men showed more sleep fragmentation prior to the initial vaccination ($r=.33$, $p<.01$) and poorer sleep efficiency ($r=-.19$, $p<.05$) prior to the third immunization than women.

Age was inversely associated with sleep duration prior to the third vaccination ($r=-.18$, $p<.05$). Finally, poorer actigraphy-based sleep efficiency prior to the first immunization and longer diary-based sleep duration prior to the second vaccination were observed among non-Caucasian participants when compared with Caucasian participants ($r=-.26$, $p<.05$; $r=.30$, $p<.05$, respectively). There were no significant relations between BMI and sleep measures.

Table 9 displays correlations among pre-vaccination sleep measures. As expected, poorer sleep efficiency was associated with shorter sleep duration ($r=.32$, $p<.01$) and greater sleep fragmentation ($r=-.66$, $p<.001$). Moreover, actigraphy-based sleep duration and efficiency were significantly associated with diary-based measures of duration and efficiency obtained prior to the first immunization ($r=.75$ and $r=.25$, respectively). In addition, diary-based measures of duration, efficiency, and quality were correlated significantly across all three vaccination periods (p's $<.05$).

Table 8: Bivariate correlations among sociodemographic characteristics and actigraphy and diary-based pre-vaccination sleep measures.

	Age	Gender(female=1, male=2)	Race(Caucasian=1, non-Caucasian=2)	BMI
Vaccination 1				
Actigraphy				
Sleep duration	-.05	-.15	-.16	-.06
Sleep efficiency	.15	-.11	-.26*	-.10
Sleep fragmentation	-.17	.33*	.16	-.01
Diary				
Sleep duration	-.02	-.17	-.04	-.14
Sleep efficiency	.15	-.03	-.11	-.16
Sleep quality	.14	-.06	.02	.00
Vaccination 2				
Diary				
Sleep duration	-.14	-.01	.30*	-.09
Sleep efficiency	.07	-.02	.06	-.16
Sleep quality	.18	-.10	.03	.01
Vaccination 3				
Diary				
Sleep duration	-.18*	-.10	.02	-.12
Sleep efficiency	.13	-.19*	-.04	-.17
Sleep quality	.07	-.07	-.16	-.08

*p<.05

6.3.2 Associations among Background Variables and Vaccination Response

Pearson, point biserial, and phi-correlations were computed to examine associations of background variables (age, gender, race, and BMI) and primary and secondary antibody response to hepatitis B vaccination. Here, females produced higher levels of secondary antibodies in response to the second hepatitis B immunization than males ($r=-.33$, $p<.001$). Moreover, a greater proportion of females was clinically protected (i.e. displaying anti-HBa $\geq$ 10 mIU/ml) 6

months after the third immunization relative to males (r=-.25, p<.05). There were no other significant associations.

Table 9: Bivariate correlations among pre-vaccination sleep averages at each vaccination time point.

	1	2	3	4	5	6	7	8	9	10	11	12
Vaccination 1												
1. Act. Sleep duration	--											
2. Act. Sleep efficiency	.32*	--										
3. Act. Sleep fragmentation	-.04	-.66*	--									
4. Diary Sleep duration	.75*	-.10	.10	--								
5. Diary Sleep efficiency	.10	.25*	-.20	.29*	--							
6. Diary Sleep quality	-.09	.20	-.19	.11	.39*	--						
Vaccination 2												
7. Diary Sleep duration	.29*	-.19	.15	.54*	-.07	-.01	--					
8. Diary Sleep efficiency	-.06	.05	-.09	.08	.42*	.10	.29*	--				
9. Diary Sleep quality	.00	.04	-.12	.09	.24*	.51*	.14	.29*	--			
Vaccination 3												
10. Diary Sleep duration	.49*	-.06	.15	.57*	.02	.05	.57*	.04	-.03	--		
11. Diary Sleep efficiency	-.03	.05	-.15	.04	.36	.10	-.05	.33*	.20*	.10	--	
12. Diary Sleep quality	-.05	.08	-.10	-.09	.08	.38*	-.11	.08	.60*	-.05	.22*	--

*p<.05

6.4 PRIMARY HYPOTHESIS TESTING

Question 1: Do sleep parameters, measured prior to the first vaccination, predict primary antibody responses to hepatitis B immunization?

H1: Shorter sleep duration, as measured using actigraphy over the 3 days prior to the first immunization, will be associated with lower primary antibody responses to the hepatitis B vaccination.

Experimental evidence suggests that acute sleep loss can negatively influence primary antibody response to vaccination (Lange et al., 2003). However, as displayed in Table 10, logistic regression analyses revealed no association of sleep duration with likelihood of

responding (i.e. displaying detectable antibody levels) to the first dose of vaccine (OR, 0.82; 95% CI, 0.46-1.46, p=.50). This relationship remained non-significant after adjustment for the standard set of covariates (age, gender, race, BMI).

H2: Poorer sleep efficiency, as measured using actigraphy over the 3 days prior to the first immunization, will be associated with lower primary antibody responses to the hepatitis B vaccination.

Growing evidence suggests that poor sleep efficiency negatively impacts host resistance to infectious illness (Cohen et al., 2009). On the contrary, in the present study, logistic regression analyses revealed that individuals showing poorer actigraphy-based sleep efficiency were more likely to respond to the first vaccination (OR, 0.93; 95% CI, .88-.99, p=.02; Table 10). Based on this logit model, a participant with a pre-vaccination sleep efficiency of 85% would have a 12% probability of responding with detectable primary antibody levels, while a participant with a sleep efficiency of 75% would have a 22% probability of being a responder, nearly a doubling in likelihood. After adjusting for age, gender, race, and BMI, however, this prospective association fell below statistical significance (OR, 0.94; 95% CI, .88-1.00, p=.06).

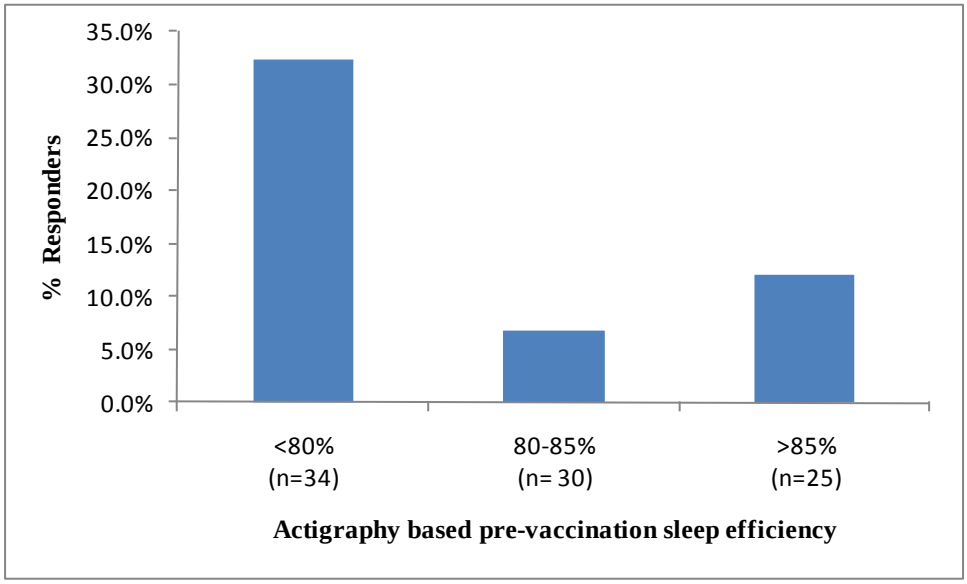
To illustrate the unadjusted association between sleep efficiency and response status, sleep efficiency was categorized into approximate tertiles (low efficiency <80%, n=34; medium efficiency= 80-85%, n=30; high efficiency>85% n= 25). As displayed in Figure 3, the relationship appears non-linear. Accordingly, this relationship was re-analyzed, including sleep efficiency as a quadratic term in addition to modeling the linear effect. As displayed in Table 10, the addition of this quadratic term did not improve the model fit of the association between sleep efficiency and responder status.

Table 10: Unadjusted and adjusted logistic regression analyses examining whether pre-vaccination sleep duration, efficiency, and quality predicts likelihood of mounting detectable antibodies (i.e. being a responder) in response to the first hepatitis B injection. Analyses employed listwise deletion.

	B	SE	Wald	p-value	Odds Ratio	95% C.I.
DV: Responder Status						
Sleep Duration (n=89)						
1. V1 actigraphy pre-vac. sleep duration	-.20	.29	.46	.50	.82	.49-1.46
After covariate adjustment						
1. Covariates[a]						
2. V1 actigraphy pre-vac. sleep duration	-.15	.31	.25	.62	.86	.47-1.57
Sleep Efficiency (n=89)						
1. V1 actigraphy pre-vac. sleep efficiency	-.07	.03	5.98	.02	.93	.88-.99
After covariate adjustment						
1. Covariates[a]						
2. V1 actigraphy pre-vac. sleep efficiency	-.06	.03	3.64	.06	.94	.88-1.00
3. V1 act. pre-vac. sleep efficiency (quadratic effect)	.18	.30	.38	.54	1.20	.67-2.16
Sleep Quality (n=113)						
1. V1 diary-based sleep quality	-.36	.42	.72	.40	.70	.31-1.59
After covariate adjustment						
1. Covariates[a]						
2. V1 diary-based sleep quality	-.25	.43	.32	.57	.78	.33-1.83

[a] age, gender, race, BMI

Figure 3: Percentage of participants mounting detectable antibodies 1-month after receiving the first hepatitis B vaccination as categorized by actigraphy-based pre-vaccination sleep efficiency.



H3: Poorer subjective sleep quality, as measure using electronic sleep diaries over the 4 days prior to the first immunization, will be associated with lower primary antibody responses to the hepatitis B vaccination.

Subjective sleep quality averaged over the 4 days prior to the first vaccination did not predict responder status to the first vaccination with or without covariates in the model (Table 10).

Question 2: Do sleep parameters, measured prior to the second vaccination, predict secondary antibody responses to hepatitis B immunization?

H4: Shorter sleep duration, as measured using both sleep diaries over the 4 days prior to the second vaccination and actigraphy over the 3 days prior to the first immunization, will be associated with lower secondary antibody responses to hepatitis B vaccination.

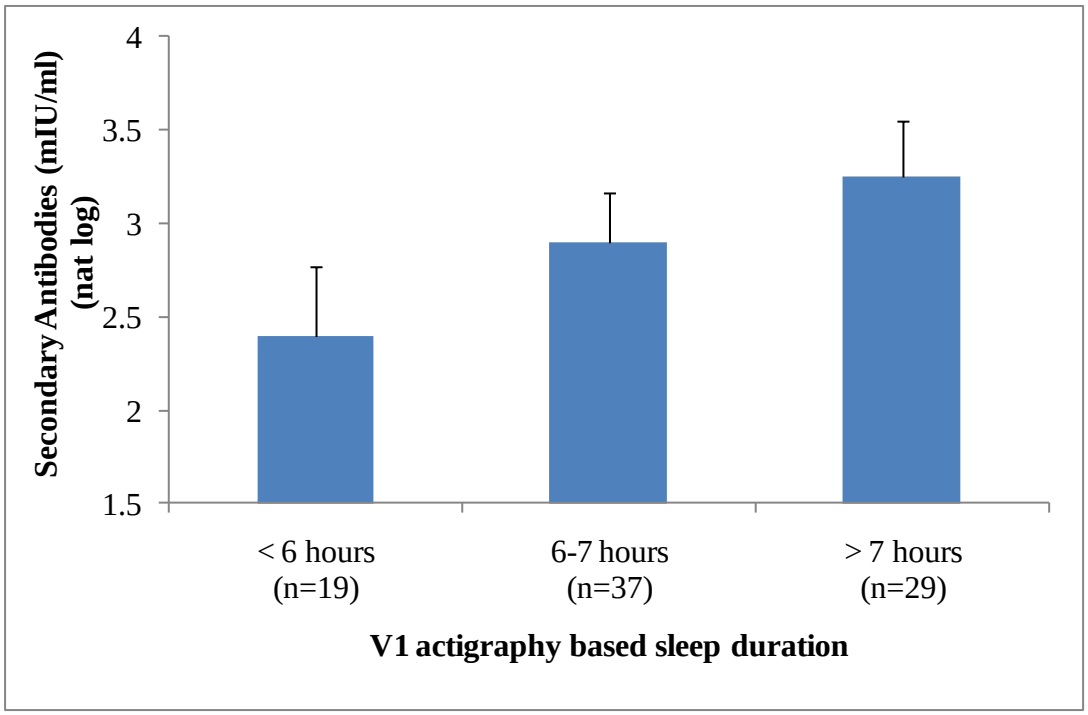
Two analytic strategies were employed to test whether sleep duration was associated with secondary antibody responses to the hepatitis B vaccination. First, because actigraphy data was not collected on the days immediately prior to the second vaccination, we relied on diary measures of sleep duration averaged over the 4 days prior to the second immunization. As displayed in Table 11, linear regression analyses revealed no significant association of diary-based sleep duration with secondary antibody levels, either without or with adjustment for covariates (i.e. gender, age, race, BMI, and primary responder status).

Psychometric data suggested poor reliability in our actigraphy-based measure of sleep duration averaged over 3 days (pre-vaccination; *ICC*=.49); however, we observed modest reliability when averaged over 6 days of measurement (*ICC*=.68; Table 3). Accordingly, we also used this measure as predictor of secondary antibody responses to vaccination. Linear regression analyses showed that shorter average sleep duration, as assessed by actigraphy, was associated with lower secondary antibody levels ($F(1, 84)=4.50$, p=.04; b=.50, SE=.23, p=.04). This association remained significant after adjustment for age, gender, race, BMI, and responder status ($F(6, 78)=4.50$, $p<.001$; $\Delta R^2=.03$; b=.43, SE=.22, p=.05; Table 11).

To better illustrate this relationship, sleep duration was categorized into approximate tertiles (< 6 hours per night n=19; 6-7 hours per night, n=37; >7 hours per night n= 29). As displayed in Figure 4, the association was largely linear, suggesting that the more hours of sleep individuals obtained on the days prior to and following the initial vaccination resulted in higher antibody levels in response to the second vaccination. Because secondary antibody levels were natural log transformed, we calculated predicted geometric means to evaluate the magnitude of this effect. Accordingly, it was predicted that a participant sleeping 6 hours would mount 85.4 mIU/ml antibodies compared to 131.3 mIU/ml antibodies for a participant sleeping 7 hours per

night on average. Based on this model[1], it can be inferred that for each additional hour of sleep, we can expect a 54% increase in secondary antibody response.

Figure 4: Actigraphy-based sleep duration, averaged over 6 consecutive days (3 days prior to and 3 days following the initial vaccination), predicts secondary antibody levels after adjustment for age, gender, race, BMI, and responder status.



H5: Poorer sleep efficiency, as measured using actigraphy over the 3 days prior to the first vaccination, will be associated with lower secondary antibody responses to hepatitis B immunization.

Actigraphy-based sleep efficiency displayed adequate reliability in as few as 3 days of measurement (*ICC*=.78), providing evidence that sleep efficiency may be relatively stable over time. Accordingly, we examined whether actigraphy-based sleep efficiency predicted antibody

[1] Model adjusted for age (50.4 years old), gender (female), race (Caucasian), BMI (25.3 kg/m^2), responder status (non-responder)

levels in response to the second immunization. Here, linear regression analyses revealed no association between sleep efficiency and secondary antibody levels with and without covariates in the model (Table 11).

H6: Poorer sleep quality, as measured across 4 days prior to the second vaccination, will be associated with lower secondary antibody responses to hepatitis B immunization.

There were no significant associations of pre-vaccination sleep quality with secondary antibody levels in unadjusted and covariate adjusted models (Table 11).

Table 11: Unadjusted and adjusted linear regression models examining whether sleep measures predict secondary antibody levels following the second vaccination. Analyses employed listwise deletion.

	B	SE	p-value	R^2	ΔR^2
DV: Secondary Antibody Levels (nat. log)					
Sleep duration (diary based; n=115)					
1. V2 diary pre-vac. sleep duration	.02	.20	.94	.00	---
After covariate adjustment					
1. Covariates[a]				.22	---
2. V2 diary pre-vac. sleep duration	-.08	.19	.67	.21	-.01
Sleep duration (actigraphy based; n=85)					
1. V1 actigraphy sleep duration	.50	.23	.04	.04	---
After covariate adjustment					
1. Covariates[a]				.17	---
2. V1 actigraphy sleep duration	.43	.22	.05	.20	.03
Sleep efficiency (n=86)					
1. V1 actigraphy pre.-vac. sleep efficiency	.02	.02	.35	.00	---
After covariate adjustment					
1. Covariates[a]				.16	---
2. V1 actigraphy pre.-vac. sleep efficiency	.03	.02	.15	.18	.02
Sleep quality (n=115)					
1. V2 diary pre.-vac. sleep quality	.18	.37	.64	.00	---
After covariate adjustment					
1. Covariates[a]				.22	---
2. V2 diary pre.-vac. sleep quality	.04	.34	.90	.21	-.01

[a]age, gender, race, BMI, and responder status

Question 3: Do sleep parameters, measured prior to the first vaccination, predict secondary antibody responses to hepatitis B immunization independent of sleep parameters measured prior to the second vaccination period?

Emerging evidence suggests that perturbation around the time of the initial exposure to antigen can impact the magnitude of subsequent secondary antibody response. For example, acute sleep loss, psychological stress, and eccentric exercise on the day prior to vaccination influence the magnitude of antibody responses captured months later (Edwards, Burns, Allen et al., 2007; Edwards et al., 2006; Lange et al., 2003; Spiegel et al., 2002). Accordingly, we investigated whether natural variation in sleep duration, efficiency, and quality assessed prior to the initial vaccination predicted secondary antibody responses independent of the influence of sleep measured on the days immediately prior to the second vaccination.

H7: Shorter sleep duration, as measured using actigraphy over the 3 days prior to the first vaccination, will be associated with lower secondary antibody responses to hepatitis B immunization after adjusting for sleep duration measured prior to the second vaccination.

Analyses revealed that actigraphy-derived sleep duration measured prior to the first vaccination did not predict antibody levels to the second vaccination after adjustment for sleep duration measured on the 4 days prior to the second vaccination ($F(2, 80)= 1.92$, p=.154; ΔR^2=.02; b=.40, SE=.21, p=.07). Further adjustment for demographic variables and responder status did not alter this finding (Table 12).

H8: Poor sleep efficiency, as measured using actigraphy over the 3 days prior to the first vaccination, will be associated with poorer secondary antibody responses after adjusting for sleep efficiency measured prior to the second vaccination.

Next, we explored the possibility that poor sleep efficiency on the days prior to the initial vaccination would influence magnitude of secondary antibody responses independently of levels of sleep efficiency prior to the second immunization. As shown in Table 12, actigraphy-based sleep efficiency was unrelated to the magnitude of secondary antibody production. This finding remained the same in analyses that adjusted for covariates, including gender, age, race, BMI, and responder status.

H9: Poorer sleep quality, as measured using sleep diaries over the 4 days prior to the first vaccination, will be associated with lower secondary antibody responses to hepatitis B immunization after adjusting for sleep quality measured prior to the second vaccination.

We investigated whether poor sleep quality prior to the initial vaccination was associated with lower secondary antibody levels independently of sleep quality observed on the days preceding the second immunization. Again, hierarchical linear regressions revealed no significant relationships in the initial models when adjusting for covariates (Table 12).

Table 12: Unadjusted and adjusted linear regression analyses examining whether actigraphy and diary-based measures of sleep, assessed prior to the first vaccination, predict secondary antibody levels after controlling for the effects of sleep occurring prior to the second vaccination . Analyses employed listwise deletion.

	B	SE	p-value	R^2	ΔR^2
DV: Secondary Antibody Levels (nat. log)					
Sleep duration (n=83)					
1. V2 diary pre-vac. sleep duration				.00	---
2. V1 actigraphy pre-vac. sleep duration	.39	.21	.07	.02	.02
After covariate adjustment					
1. Covariates[a]				.18	---
2. V2 diary pre-vac. sleep duration				.18	.00
3. V1 actigraphy pre-vac. sleep duration	.33	.20	.11	.20	.02
Sleep efficiency (n=83)					
1. V2 diary pre-vac. sleep efficiency				.00	---
2. V1 actigraphy pre-vac. sleep efficiency	.02	.02	.36	.00	.00
After covariate adjustment					
1. Covariates[a]				.18	---
2. V2 diary pre-vac. sleep efficiency				.18	.00
3. V1 actigraphy pre-vac. sleep efficiency	.03	.02	.14	.19	.01
Sleep quality (n=105)					
1. V2 diary pre-vac. sleep quality				.00	---
2. V1 diary pre-vac. sleep quality	.08	.42	.85	.00	.00
After covariate adjustment					
1. Covariates[a]				.27	---
2. V2 diary pre-vac. sleep quality				.27	.00
3. V1 diary pre-vac. sleep quality	.22	.37	.56	.27	.00

[a] age, gender, race, BMI, and responder status

6.5 SECONDARY ANALYSES

Secondary analyses were conducted to explore the association between less well understood sleep parameters and antibody response to vaccination. Table 13 provides descriptive statistics for actigraphy-based sleep fragmentation, intra-individual variability in sleep parameters, and individual differences in sleep measures (averaged across all three immunization time points).

6.5.1 Sleep Fragmentation

One advantage of using actigraphy is the opportunity to obtain an objective measure of sleep fragmentation. To date, no study has examined whether sleep fragmentation impacts immune function, including antibody responses to vaccination. While sleep fragmentation was not assessed prior the second vaccination period, it was measured before the initial immunization providing an opportunity to determine whether fragmented sleep predicts responder status. Moreover, the aggregated *ICC* value for pre-vaccination sleep fragmentation was .84 (see Table 3), suggesting adequate stability over 3 days of measurement. Accordingly, we treated fragmentation as a stable individual difference and explored whether fragmentation predicted antibody responses.

First, logistic regression was employed to determine whether actigraphy-based fragmentation predicted responder status. Here, greater sleep fragmentation was associated with increased likelihood of mounting detectable antibodies in response to the initial vaccination (OR,

1.05; CI 95% 1.00-1.10, p=.03). However, after for covariates this association was no longer statistically significant. Next, we investigated whether sleep fragmentation predicted secondary antibody levels. Here, an unadjusted linear regression model showed no significant association before ($F(1, 84)=0.73$, p=.40; $\Delta R^2=.00$; b=-.01, SE=.02, p=.40) or after adjustment for covariates ($F(6, 79)=3.56$, p=.004; $\Delta R^2=.00$; b=.00, SE=.02, p=.86).

6.5.2 Intra-individual Variability in Sleep Parameters

There is growing interest in the impact of night to night variability in sleep on health. Indeed, individuals with marked variability in sleep patterns, such as rotating shift workers, are at increased risk of several medical conditions (e.g. cardiovascular disease) as well as report greater incidence of infectious illness, including the common cold (Fujino et al., 2006; Mohren et al., 2002). There is widespread within person variability in sleep, often eclipsing the variability observed between individuals. Mixed models were utilized to determine the proportion of variance in sleep parameters attributable to within and between participants' factors. For this purpose, we computed single measure intra class correlation (ICC_s) coefficients. This type of *ICC* (denoted here on as ICC_s) differs from the aggregated *ICC* discussed earlier as it describes the reliability at the level of the individual (i.e. each participant). In contrast, the aggregated *ICC* provides an *average* measure of reliability at the group level (i.e. study sample).

ICC_s values indicate that proportion of variance that can be attributed to between person differences; therefore, [1-ICC_s] is the proportion of the total variance attributable to within person differences. As displayed in Table 13, ICC_s values ranged from .19 to .61, suggesting that 39%-81% of the variability in our sleep measures were due to within person differences. This is consistent with a small but compelling set of studies that support high intra-individual

variability in presumed healthy sleepers (Knutson, Rathouz et al., 2007; Mezick et al., 2009; van Hilten et al., 1993). Estimates of intra-individual variability for each sleep parameter were obtained by calculating standard deviations (SD) over the 6 days of actigraphy measurement and as many as 21 days of electronic sleep diary measurement for each participant.

Logistic and linear regression models were computed to quantify the effect of intra-individual variability in sleep parameters on primary and secondary antibody responses to vaccination. Sociodemographic variables (age, gender, race, and BMI) were included as covariates in all models; responder status was also included in models predicting secondary antibody responses. In addition, in separate models, we adjusted for mean sleep parameters, averaged over all measurement time points, in the final step of each respective regression model to test whether the relationship between intra-individual sleep variability and antibody response was independent of average sleep.

Table 13: Descriptive statistics for secondary sleep variables

Sleep Variable	**Mean (SD)**	***ICC*$_s$**
V1 Pre-vac. sleep fragmentation (%)	30.2 (11.8)	---
Intra-individual variability		
1. Actigraphy-based measures		
Sleep duration (mins)	60.2 (29.1)	.26
Sleep efficiency (%)	6.0 (3.6)	.46
Sleep fragmentation (%)	9.1 (4.9)	.61
2. Diary-based measures		
Sleep duration (mins)	57.7 (18.2)	.23
Sleep efficiency (%)	1.8 (1.4)	.19
Sleep quality (very poor [0-4] very good)	0.56 (0.20)	.32
Individual differences in sleep		
Sleep duration (hrs)	6.9 (0.7)	---
Sleep efficiency (%)	88.8 (3.9)	---
Sleep quality (very poor [0-4] very good)	3.2 (0.4)	---

6.5.2.1 Intra-individual Variability in Sleep Duration

To investigate whether intra-individual variability in sleep duration predicted primary and secondary antibody responses, we relied on measures derived from both actigraphy and electronic sleep diaries. With respect to actigraphy, an unadjusted logistic regression model suggested that greater night to night variability in sleep duration, measured over 6 nights of actigraphy assessment, was associated with a greater likelihood of displaying detectable antibody titers one month following the first vaccination (OR, 3.07; 95% CI, 1.07-8.84, p=.04; Table 14). However, this association fell below statistical significance after adjusting for covariates and average sleep duration.

With respect to whether intra-individual variability in sleep duration influences secondary antibody response, hierarchical linear regression analyses did not support an effect of variability in actigraphy-based sleep duration (Table 15); on the contrary, variability in electronic diary-based duration did significantly predict secondary antibody levels. Here, hierarchical linear regression analyses revealed that, controlling for the effects of age, gender, race, BMI, and responder status, greater variability was associated with lower secondary antibody responses ($F(6, 115)=6.11$, $p<.001$; $\Delta R^2=.04$; b=-1.35, SE=.55, p=.02). Furthermore, this relationship was independent of average sleep duration assessed across all three vaccination periods (Table 15).

To further illustrate this association, we categorized participants into tertiles based on variability in diary-based sleep duration (< 45 minutes, n=37; 45 to 65 minutes, n=44; >65 minutes, n=41). As displayed in Figure 5, the association between variability in diary-based sleep duration and natural log transformed secondary antibody levels appeared non-linear. Accordingly, an additional hierarchical linear regression was computed including a negative

quadratic term in the final step of a regression to model this curvilinear relationship. The negative quadratic term was a significant predictor of secondary antibody production (Table 15).

Table 14: Unadjusted and adjusted logistic regression analyses examining whether intra-individual variability (IIV) in sleep duration, efficiency, quality, and fragmentation predicts likelihood of mounting detectable antibodies (i.e. being a responder) in response to the first hepatitis B injection.

	B	SE	Wald	p-value	Odds Ratio	95% C.I.
DV: Responder Status						
IIV actigraphy-based sleep duration (n=90)						
1. IIV actigraphy sleep duration	1.21	.54	4.31	.04	3.07	1.07-8.84
After covariate adjustment						
1. Covariates[a] & V1 act. sleep duration						
2. IIV actigraphy sleep duration	.97	.60	2.63	.11	1.02	.82-8.54
IIV diary-based sleep duration (n=125)						
1. IIV diary sleep duration	.01	.01	1.53	.22	1.01	.99-1.04
After covariate adjustment						
1. Covariates[a] & average sleep duration						
2. IIV diary sleep duration	.02	.01	1.72	.19	1.02	.99-1.04
IIV actigraphy-based sleep efficiency (n=90)						
1. IIV actigraphy sleep efficiency	.10	.07	2.13	.14	1.11	.97-1.27
After covariate adjustment						
1. Covariates[a] & V1 act. sleep efficiency						
2. IIV actigraphy sleep efficiency	.07	.10	.47	.49	1.07	.87-1.29
IIV diary-based sleep efficiency (n=125)						
1. IIV diary sleep efficiency	-.03	.15	.05	.83	.97	.73-1.29
After covariate adjustment						
1. Covariates[a] & average sleep efficiency						
2. IIV diary sleep efficiency	-.07	.20	.13	.72	.93	.62-1.39
IIV diary-based sleep quality (n=125)						
1. IIV sleep quality	.01	1.05	.00	.99	1.01	.13-7.88
After covariate adjustment						
1. Covariates[a] & average sleep quality						
2. IIV diary sleep quality	-.35	1.17	.09	.76	.70	.07-6.90
IIV actigraphy-based sleep fragmentation (n=90)						
1. IIV actigraphy sleep fragmentation	.06	.05	1.42	.23	1.06	.96-1.17
After covariate adjustment						
1. Covariates[a] & average sleep fragmentation						
2. IIV actigraphy sleep fragmentation	.02	.06	.07	.79	1.02	.90-1.15

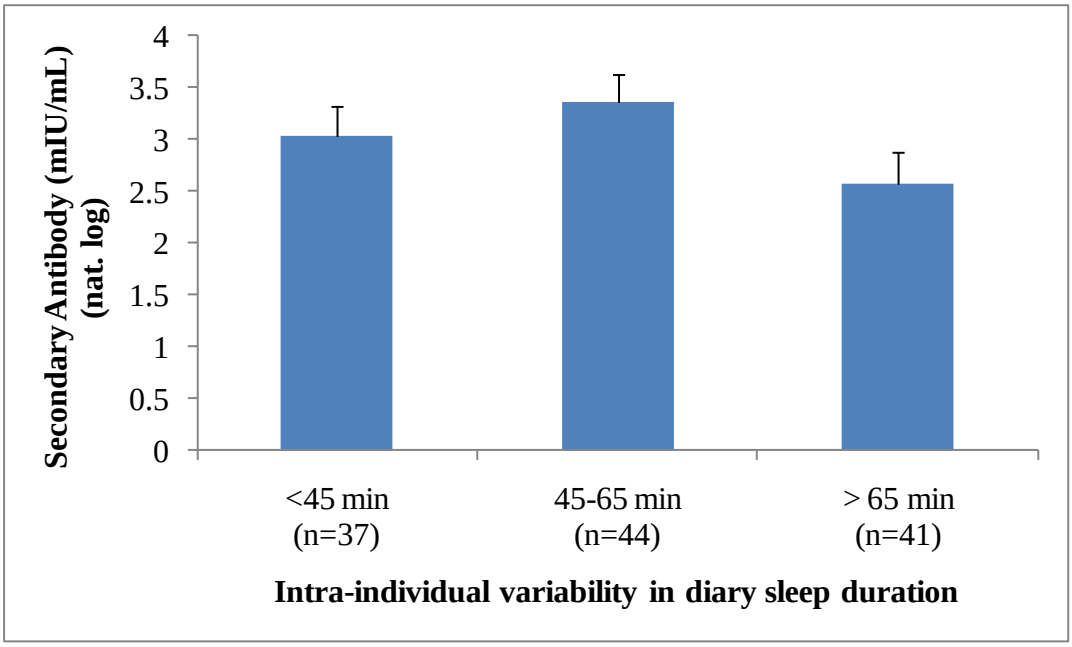
[a] age, gender, race, BMI

Table 15: Unadjusted and adjusted linear regression analyses examining whether intra-individual variability (IIV) in sleep predicts secondary antibody responses to the second vaccination.

	B	SE	p-value	R^2	ΔR^2
DV: Secondary antibody levels (nat. log)					
IIV actigraphy-based sleep duration (n=87)					
1. IIV actigraphy sleep duration	-.19	.40	.63	.00	---
After covariate adjustment					
1. Covariates[a] & V1 act. sleep duration				.27	---
2. IIV actigraphy sleep duration	-.55	.38	.15	.29	.02
IIV diary-based sleep duration (n=122)					
1. IIV diary sleep duration	-.53	.57	.35	.00	---
After covariate adjustment					
1. Covariates[a] & average sleep duration				.16	---
2. IIV diary sleep duration	-1.35	.55	.02	.20	.04
3. IIV diary sleep duration (quadratic effect)	.37	.16	.02	.23	.03
IIV actigraphy-based sleep efficiency (n=87)					
1. IIV actigraphy sleep efficiency	-.06	.05	.28	.00	---
After covariate adjustment					
1. Covariates[a] & V1 act. sleep efficiency				.23	---
2. IIV actigraphy sleep efficiency	-.10	.06	.10	.26	.03
IIV diary-based sleep efficiency (n=122)					
1. IIV diary sleep efficiency	-.08	.12	.50	.00	---
After covariate adjustment					
1. Covariates[a] & average sleep efficiency				.16	---
2. IIV diary sleep efficiency	-.26	.15	.08	.18	.02
IIV diary-based sleep quality (n=122)					
1. IIV sleep quality	-.65	.88	.46	.00	---
After covariate adjustment					
1. Covariates[a] & average sleep quality				.16	---
2. IIV diary sleep quality	-.84	.85	.32	.16	.00
IIV actigraphy-based sleep fragmentation (n=87)					
1. IIV actigraphy sleep fragmentation	.00	.04	.92	.00	---
After covariate adjustment					
1. Covariates[a] & average sleep fragmentation				.16	---
2. IIV actigraphy sleep fragmentation	-.01	.04	.91	.15	-.01

[a] age, gender, race, BMI, and responder status to initial vaccination

Figure 5: Intra-individual variability in diary-based sleep duration predicts the magnitude of secondary antibody responses to the second immunization. This association is adjusted age, gender, race, BMI, responder status, and diary-based sleep duration averaged over the three vaccination periods.



6.5.2.2 Intra-individual Variability in Sleep Efficiency

Night-to-night variability in sleep efficiency was assessed using actigraphy over 6 days at the time of the first immunization and via electronic diaries for 7 days at each of the 3 vaccination periods. Logistic regression analyses showed no significant associations of actigraphy or diary derived measures of variability in sleep efficiency and probability of responding to the first vaccination, including after controlling for sociodemographic characteristics and average sleep efficiency across the same period (Table 14).

Similar analyses examining the associations of variability in sleep efficiency with secondary antibody levels revealed an inverse relationship between actigraphy-based measures and antibody response that withstood adjustment for covariates ($F(6, 80)=4.65$, $p<.001$; $\Delta R^2=.04$; b=-.10, SE=.05, p=.05); however, this association was not independent of average actigraphy-

based sleep efficiency measured over this same time period. Intra-individual variability in diary-based sleep efficiency was unrelated to secondary antibody responses, with and without covariate adjustment (Table 15).

6.5.2.3 Intra-individual Variability in Sleep Quality

Variability in subjective sleep quality was assessed across 7 consecutive days at each of the three vaccination periods. However, there were no associations between this measure and either probability of mounting a primary response or magnitude of secondary antibody response (Tables 14 and 15).

6.5.2.4 Intra-individual Variability in Sleep Fragmentation

Finally, variability in sleep fragmentation was assessed across the first vaccination period using the actigraphy data. Regression analyses revealed no significant associations of this measure with probability of mounting a detectable antibody response to the first vaccine or magnitude of secondary response. These associations remained non-significant in models that controlled for sociodemographic characteristics and average sleep fragmentation over the first vaccination period (Tables 14 and 15).

6.5.3 Individual Differences in Sleep

Much of the work linking sleep and health comes from large epidemiologic samples that focus on dispositional differences in sleep parameters. For instance, “short” sleepers (e.g. sleeping less than 6 hours per night) and, to some extent, “long” sleepers (i.e. sleeping longer the 8 or 9 hours per night) are at increased risk for several chronic medical conditions, including

hypertension, diabetes, and the metabolic syndrome (Gangwisch et al., 2006, 2007; Hall et al., 2008). While the present study was designed to examine prospectively whether variation in sleep prior to either the first or second vaccination period influenced the magnitude of primary and secondary antibody responses, it is also plausible that dispositional differences in sleep affect antibody production.

6.5.3.1 Individual Differences in Sleep Duration

Average sleep duration was calculated by averaging actigraphy-based measures of sleep duration obtained over the first vaccination period and electronic diary-based data obtained over the second and third vaccination period. With respect to the primary antibody response, logistic regression revealed no significant relationship between average sleep duration and responder status before and after covariate adjustment (Table 16). In contrast, linear models revealed a significant positive association of dispositional sleep duration and magnitude of secondary antibody response ($F(1, 85)=4.84$, p=.03; $R^2=.04$; b= .62, SE=.28, p=.03). However, this association was only marginally significant after adjusting for sociodemographic characteristics and responder status (p=.06; Table 17).

Table 16: Unadjusted and adjusted logistic regression analyses examining whether individual differences in sleep duration, efficiency, and quality predicts likelihood of mounting detectable antibodies (i.e. being a responder) in response to the first hepatitis B injection.

	B	SE	Wald	p-value	Odds Ratio	95% C.I.
DV: Responder Status						
Average sleep duration (n=90)						
1. Average sleep duration	.21	.42	.26	.61	1.23	.55-2.79
After covariate adjustment						
1. Covariates[a]						
2. Average sleep duration	.10	.43	.05	.82	1.10	.48-2.54
Average sleep efficiency (n=90)						
1. Average sleep efficiency	-.18	.07	7.09	.01	.84	.73-.95
After covariate adjustment						
1. Covariates[a]						
2. Average sleep efficiency	-.14	.08	3.48	.06	.87	.75-1.01
Average sleep quality (n=125)						
1. Average sleep quality	-.81	.56	2.10	.15	.44	.15-1.33
After covariate adjustment						
1. Covariates[a]						
2. Average sleep quality	-.66	.57	1.34	.25	.52	.17-1.58

[a] age, gender, race, BMI

Table 17: Unadjusted and adjusted linear regression analyses examining whether sleep duration, efficiency, and quality, averaged across all three vaccination periods, predicts secondary antibody levels following the second vaccination.

	B	SE	p-value	R^2	ΔR^2
DV: Secondary Antibody Levels (nat. log)					
Average sleep duration (n=87)					
1. Average sleep duration	.62	.28	.03	.04	---
After covariate adjustment					
1. Covariates[a]				.17	---
2. Average sleep duration	.50	.26	.06	.20	.03
Average sleep efficiency (n=87)					
1. Average sleep efficiency	.05	.05	.29	.00	---
After covariate adjustment					
1. Covariates[a]				.17	---
2. Average sleep efficiency	.06	.05	.25	.18	.01
Average Sleep quality (n=122)					
1. Average sleep quality	-.06	.45	.90	.00	---
After covariate adjustment					
1. Covariates[a]				.17	---
2. Average sleep quality	-.13	.42	.75	.17	.00

[a] age, gender, race, BMI, responder status to initial vaccination

6.5.3.2 Individual Differences in Sleep Efficiency

Average sleep efficiency was calculated by combining actigraphy measures across the first vaccination period with diary measures assessed during the second and third vaccination periods. Logistic regression analysis revealed an inverse association of average sleep efficiency with likelihood of mounting a detectable primary antibody response (OR, 0.83; 95% CI, .73-.95, p=.01). However, this relationship remained only marginal after adjustment for age, gender, race, and BMI (p=.06; Table 16). There were no associations between average sleep efficiency and magnitude of secondary antibody response before or after covariate adjustment.

6.5.3.3 Individual Differences in Sleep Quality

Regression analyses revealed no significant associations of average sleep quality with probability of mounting a response to the first immunization or with magnitude of secondary antibody response either before or after controlling for covariates (Tables 16 and 17).

6.5.4 Clinical Protection

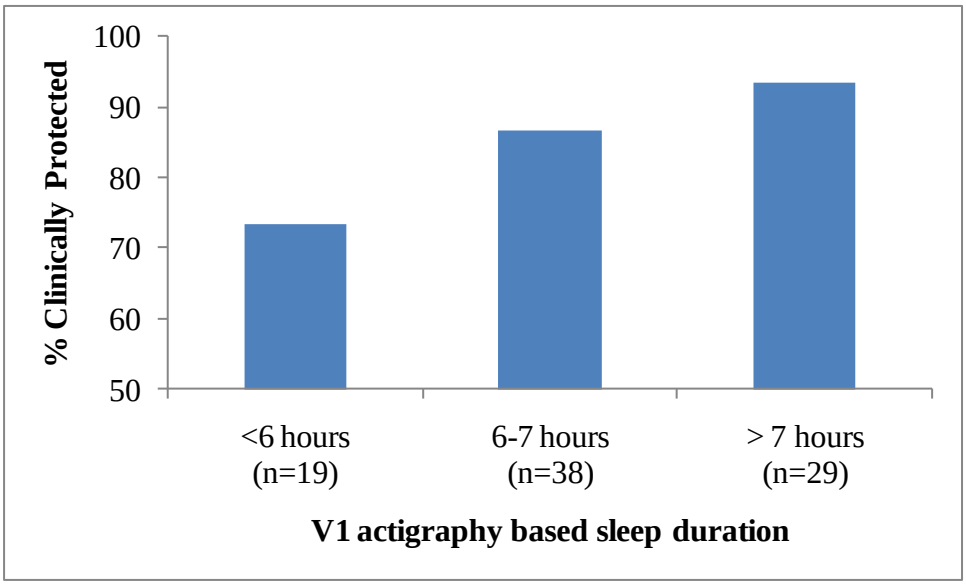
Finally, beyond establishing prospective associations between sleep parameters and magnitude of primary and secondary antibody production, this study was able to test whether sleep parameters are associated with the probability of mounting a clinically protective response to the hepatitis B sequence. In this regard, the established criterion for clinical protection is a circulating antibody level of ≥10 mIU/ml (CDC, 1987). The present study indicated that shorter sleep duration, measured via actigraphy only or when averaged across all available vaccination time points, predicted lower secondary antibody responses. In addition, intra-individual variability in sleep duration and efficiency were related to secondary antibody production. Therefore, in secondary analyses, we examined whether these parameters were also associated with the likelihood of clinical protection 6-months after the conclusion of the vaccination series.

6.5.4.1 Clinical Protection: Sleep Duration

Consistent with primary analyses revealing that shorter sleep duration at the time of the first immunization predicted lower secondary antibody responses, logistic regression revealed that shorter sleep duration was also associated with a decreased likelihood of being clinically protected 6-months after the final dose of vaccine (OR, 3.35; 95% CI, 1.34-8.37, p=.01). As displayed in Figure 6, 73.7% of participants who slept less than 6 hours per night were protected

at the end of the vaccination series. In comparison, 86.8% participants who slept between 6 and 7 hours were protected with participants who slept greater than 7 hours showing an even higher proportion of clinical protection (93.5%). With respect to the effect size, this logit model indicates that a participant who slept 6 hours on average over the days surrounding the first vaccination period would have a 79.9% probability of displaying anti-HBa ≥10 mIU/ml while an individual sleeping 7 hours would have a 91.6% probability of being protected. This association remained significant after adjustment for age, gender, race, and BMI (Table 18).

Figure 6: Actigraphy-based sleep duration, averaged over 6 consecutive days (3 days prior to and 3 days following) the initial vaccination, is associated with likelihood of being clinically protected (i.e. anti-HBa ≥ 10 mIU/ml) 6-months following the third immunization. This association is adjusted for age, gender, race, and BMI.



6.5.4.2 Clinical Protection: Individual Differences in Sleep Duration

We assessed whether sleep duration averaged over all three vaccination time points predicted likelihood of clinical protection. Logistic regression revealed that shorter average sleep duration was associated with decreased likelihood of clinical protection from the hepatitis

B virus 6 months after the vaccination series (OR, 3.57; 95% CI, 1.26-10.11, p=.02; Table 18). However, this association was only marginally significant when adjusting for covariates.

Table 18: Unadjusted and adjusted logistic regression analyses examining whether sleep measures predict the likelihood of being clinically protected (i.e. anti-HBa ≥ 10mIU/ml) 6-months following the final hepatitis B vaccination.

	B	SE	Wald	p-value	Odds Ratio	95% C.I.
DV: Protection Status						
V1 actigraphy-based sleep duration (n=86)						
1. V1 actigraphy sleep duration	1.21	.47	6.71	.01	3.35	1.34-8.37
After covariate adjustment						
1. Covariates[a]						
2. V1 actigraphy sleep duration	1.21	.55	4.77	.03	3.34	1.13-9.88
Average sleep duration (n=88)						
1. Average sleep duration	1.27	.53	5.74	.02	3.57	1.26-10.11
After covariate adjustment						
1. Covariates[a]						
2. Average sleep duration	1.23	.65	3.52	.06	3.42	.95-12.31
IIV diary-based sleep duration (n=123)						
1. IIV diary sleep duration	-.67	.81	.68	.41	.51	.11-2.50
After covariate adjustment						
1. Covariates[a] & average sleep duration						
2. IIV diary sleep duration (linear effect)	-.02	.02	1.39	.24	.98	.95-1.01
3. IIV diary sleep duration (quadratic effect)	.88	.39	5.27	.02	2.42	1.14-5.14
IIV diary-based sleep efficiency (n=123)						
1. IIV diary sleep efficiency	-.19	.16	1.54	.22	.83	.61-1.12
After covariate adjustment						
1. Covariates[a] & average sleep efficiency						
2. IIV diary sleep efficiency	-.44	.26	2.97	.09	.65	.39-1.06

[a]age, gender, race, BMI

6.5.4.3 Clinical Protection: Intra-individual Variability in Sleep Duration

Next, we examined whether intra-individual variability in diary-based sleep duration was associated with the likelihood of being clinically protected 6-months after the completion of the hepatitis B vaccination series. Regression analyses revealed that high and low sleep duration variability were not only associated with lower secondary antibody responses, but also with a decreased likelihood of mounting a clinical protective response. In this regard, logistic

regressions were used to explore this relationship including both linear and non-linear effects as described earlier. Here, we found that a non-linear relationship remained statistically significant after controlling for age, gender, race, BMI, the linear effect, and sleep duration averaged over all three vaccination periods (OR, 2.42; 95% CI, 1.14-5.14, p=.02; Table 18), with those with high and low variability less likely to be clinically protected than those with medium variability.

6.5.4.4 Clinical Protection: Intra-individual Differences in Sleep Efficiency

Finally, prior analyses indicated that greater stability in sleep efficiency across nights was associated with more robust secondary antibody responses. However, regression analyses did not show a similar association between variability in sleep efficiency and probability of mounting a clinically protective response (Table 18).

6.5.5 Influence of Covariates on Dependent Variables

As expected, several of the study covariates were significantly related to the dependent outcomes. As seen in Tables 10-12, the covariates accounted for 17-25% of the variance in antibody responses. With respect to the influence of specific covariates, younger individuals were more likely to mount detectable antibodies to the first vaccination (OR, 0.87; 95% CI, .77-.98, p=.02). Conversely, secondary antibody responses were higher among women, those with a higher BMI, and participants who mounted detectable antibodies to the initial vaccination (p's<.05).

7.0 DISCUSSION

Despite a large literature linking sleep and health, the mechanisms of these associations remain unclear. Laboratory evidence in animals and humans supports the immune system as a plausible biological pathway; however, the majority of this work has been limited to *in vitro* immune measures where the clinical relevance is unclear. Accordingly, we have turned to a more integrated measure of immunity, namely antibody response to vaccination.

The goal of the present study was to examine whether natural variation in three dimensions of sleep (duration, efficiency, and quality) predicted the magnitude of primary and secondary antibody responses to the hepatitis B vaccination series among a sample of relatively healthy older adults. In addition, this study explored the impact of several less well researched sleep parameters (e.g. sleep fragmentation and intra-individual variability in sleep) on vaccination response. Findings uncovered several intriguing relationships, adding to our growing understanding of the influence of sleep on *in vivo* immune function. Before turning to the potential implications of this work, however, a brief discussion of these findings and how they fit within the context of the existing literature is provided.

7.1 SLEEP AND PRIMARY ANTIBODY RESPONSE

It was hypothesized that shorter sleep duration, poorer sleep efficiency, and poorer sleep quality prior to the initial vaccination would be associated with fewer primary antibodies as assessed 1-month later. Contrary to expectations, neither duration nor quality predicted probability of mounting a detectable primary antibody response. Interestingly, individuals who mounted a response to the first dose of the vaccine showed poorer sleep efficiency, measured objectively using actigraphy alone (see Figure 3) and when averaged across all three vaccination periods; however, neither of these associations withstood adjustment for sociodemographic covariates. Secondary unadjusted analyses also revealed that greater sleep fragmentation and greater intra-individual variability in sleep duration around the days of the first immunization were associated with probability of mounting a response to the first dose of vaccine, suggesting that poorer sleep continuity within and between nights promoted greater antibody production to the first immunization.

To date, only one study has examined the association of sleep parameters with primary antibody response (Lange et al., 2003). Findings showed that when compared with normal sleep, a 36 hour period of sleep deprivation was associated with lower primary antibody responses to the hepatitis A vaccination (Lange et al., 2003). Our failure to observe a similar effect of sleep duration in the current study may be attributable to several methodological differences. For instance, 36 hours of sleep restriction likely confers substantially different effects on the body when compared to the natural variation in sleep quantified in our study. Total and partial sleep deprivation have been associated with changes in enumerative and functional measures of immunity that precipitously return to baseline levels upon nights of unrestricted sleep (for review, Opp et al., 2007). In contrast, the physiologic cost of naturally occurring sleep loss is

less clear, though alterations in immune and autonomic functioning have been reported (Irwin et al., 2003). It is also possible that discrepant findings result from differences in timing and outcome measures across studies. In the current study, only 24.8% of participants mounted detectable antibody levels in response to the initial hepatitis B vaccination, leading us to dichotomize our dependent outcome (i.e. responders vs. non-responders). Accordingly, the amount of variability that could be explained by sleep duration was limited. Furthermore, we focused on sleep duration prior to administering the first immunization. Conversely, Lange and colleagues (2003) restricted sleep immediately post-vaccination, potentially leading to alterations in disparate immune mechanisms.

Contrary to hypotheses, poorer sleep efficiency, greater sleep fragmentation, and greater intra-individual variability in duration predicted a greater likelihood of mounting a detectable primary antibody response in the current study. These interesting findings are somewhat consistent with an emerging literature suggesting that enhanced physiologic activity can prime immune pathways to respond more vigorously to an antigenic challenge (Edwards, Burns, Carroll, Drayson, & Ring, 2007; Matzinger, 2002). In this regard, experimental human and animal evidence shows that acute bouts of psychological stress and exercise promote short-lived, rapid changes in enumerative and functional measures of innate and adaptive immunity, including parameters that facilitate antigen recognition and antibody production (Dhabhar, 2002; Marsland, Cohen, Rabin, & Manuck, 2001; Marsland et al., 1997; Matthews et al., 1995; Segerstrom & Miller, 2004). Of particular relevance, recent experimental evidence demonstrates that acute mental stress and exercise also enhance antibody responses to vaccination (Edwards, Burns, Allen et al., 2007; Edwards et al., 2006). For example, Edwards and colleagues (2006) found that participants randomized to either a 45 minute mental stressor

(i.e. serial addition task with social evaluation) or dynamic exercise (i.e. ergonomic cycling) displayed higher antibody titers to the A/Panama strain of the influenza vaccine 4 and 20 weeks post immunization when compared to a resting control condition.

To our knowledge, the current findings provide the first prospective evidence for an association between poor sleep continuity, measured in the field, and primary antibody production. Fragmented sleep, poor sleep efficiency, and greater intra-individual variability in sleep duration have been associated with elevated catecholamine release and alterations in sympathovagal balance across the night span (Irwin et al., 2003; Stamatakis & Punjabi, in press; Tiemeier, Pelzer, Jonck, Moller, & Rao, 2002), which, in turn, have been associated with the modulation of immune parameters that play a role in antibody production (Kin & Sanders, 2006). Similarly, patients with obstructive sleep apnea (OSA), who by definition experience fragmented sleep, show elevations in inflammatory activity (e.g. higher circulating IL-6) (Arnardottir, Mackiewicz, Gislason, Teff, & Pack, 2009; Shamsuzzaman, Gersh, & Somers, 2003) and lymphocyte activation (Dyugovskaya, Lavie, & Lavie, 2005) when compared to non-OSA sleepers. Taken together, these findings suggest that poor sleep continuity may contribute to a more robust primary immune response to antigen challenge.

7.2 SLEEP AND SECONDARY ANTIBODY RESPONSE

While analyses did not reveal prospective associations of sleep efficiency or quality with secondary antibody response, shorter actigraphy-based sleep duration was associated with lower secondary antibody production. Furthermore, this finding remained statistically significant after adjusting for several relevant covariates, including age, gender, race, BMI, and responder status

to the initial vaccination (see Figure 4). This finding was further supported by secondary analyses utilizing all available sleep duration data and is consistent with existing laboratory and field evidence in humans. Indeed, partial sleep deprivation (i.e. restricting participants' sleep from 8 to 4 hours/night) for 4 consecutive nights prior to vaccination resulted in a 57% reduction in secondary antibody production 10 days post immunization with influenza relative to non-deprived sleepers (Spiegel et al., 2002). Similarly, Pressman and colleagues (2005) found that among college freshman shorter sleep duration, measured in the field, was associated with lower secondary antibodies to the influenza vaccine 1-month and 4-months later. The current findings are also consistent with experimental evidence that short sleep duration is associated with increased susceptibility to upper respiratory infection (Cohen et al., 2009). Taken together, growing evidence, including our own, suggests that short sleep duration may place people at elevated risk for infectious disease.

The prospective design of the current study allowed for investigation of temporally unique relationships between sleep dimensions and secondary antibody responses (Hypotheses 7 through 9). In this regard, shorter actigraphy-based sleep duration prior to the first dose of the vaccine was modestly associated with fewer secondary antibody responses, independently of sleep occurring on the days prior to the second vaccination ($p=.07$; Table 12), raising the possibility that the days prior to beginning the vaccination series may be a critical period when sleep confers unique influence on vaccination. Adjustment for sociodemographic covariates and responder status further diminished the magnitude of this effect. However, further examination of this possibility in a bettered powered sample is warranted. Furthermore, actigraphy measurement at all vaccination time points will aid in the elucidation of temporally vulnerable periods when sleep may affect antibody production.

Unlike much of the sleep-health literature that has relied on single nights of assessment or retrospective report to derive habitual sleep averages, this study capitalized on objective and subjective sleep data captured serially over multiple nights. Consistent with prior studies (Buysse et al., in press; Knutson, Rathouz et al., 2007; Mezick et al., 2009; van Hilten et al., 1993), we found that within person variation in sleep from night to night was substantial (Table 13), eclipsing the between person variation. Here, intra-individual variability in sleep efficiency, measured via actigraphy, and sleep duration (see Figure 5), measured across all three vaccination time points, predicted magnitude of secondary antibody responses. Variability in sleep duration remained a significant predictor after adjusting for sociodemographic covariates and average sleep duration; however, the association with antibody response was non-linear, with lower secondary antibody responses among those with low and high night-to-night variability. Factors distinguishing participants with either low or high variability in sleep duration remain to be determined. Differential influences of dispositional characteristics shown to affect antibody production (e.g. trait negative and positive affect; Marsland, Cohen et al., 2001; Marsland, Cohen, Rabin, & Manuck, 2006; Phillips, Carroll, Burns, & Drayson, 2005) may partially account for this non-linear finding. Nevertheless, this is the first study to document effects of intra-individual variability in sleep on *in vivo* immune function and may provide insight into the health risks observed among rotating shift workers, including increased prevalence of infectious illness (Mohren et al., 2002).

Contrary to our hypotheses, subjective sleep quality was unrelated to both primary and secondary antibody responses in this study. This is in contrast to an earlier study that found poorer sleep quality prior to the influenza vaccination predicted lower secondary antibodies 1-month later (Pressman et al., 2005). Moreover, cross-sectional findings support an association

between poor sleep quality and risk factors for disease, including hypertension, obesity, insulin resistance, and elevated glucose (Fiorentini et al., 2007; Jennings et al., 2007; Resta et al., 2003; Scheen & Van Cauter, 1998). Although it is plausible that subjective sleep quality is unrelated to antibody production, our null finding may be partially accounted for by the restricted range of sleep quality responses. Indeed, the majority of participants described their sleep as either "good" or "very good" over the days of the first and second vaccination periods (71.4% and 72.6% of participants, respectively). Accordingly, future studies may benefit from employing a more sensitive measurement of sleep quality and inclusion of individuals with greater variability in their subjective sleep quality.

7.3 SLEEP AND CLINICAL PROTECTION

Perhaps the most striking finding from this study was the association of actigraphy-based sleep duration with probability of mounting a clinically protected response to the vaccine, as assessed 6-months after the final injection (Figure 6). Indeed, participants sleeping less than 6 hours per night over the actigraphy measurement period had a 25% lower probability of being clinically protected when compared to participants sleeping more than 7 hours per night. This finding remained statistically significant after adjustment for age, gender, race, and BMI. Furthermore, shorter sleep duration, averaged across all three vaccination periods, was associated with a lower probability of protection, providing additional evidence that individual differences in duration may confer risk for infectious illness. Consistent with the association of greater sleep variability with magnitude of secondary antibody response, participants with low and high night-

to-night variability in sleep duration were also less likely to mount a protective response to the vaccine.

Several sociodemographic factors are related to probability of mounting a protective response to hepatitis B vaccination. In the current study, the relationship between sleep duration and probability of being protected at the conclusion of the vaccination was independent of factors known to confer risk, including age, gender, and BMI. Moreover, the magnitude of the sleep effects observed in this study is comparable to risk ratios derived from epidemiologic studies that report associations of demographic and health factors with vaccination response. For instance, Averhoff and colleagues found that individuals older than 40 years, obese, or current smokers had relative risks of 2.2, 1.6, and 1.9, for being unprotected following the vaccination series compared to individuals under the age of 40, non-obese, or non-smokers (Averhoff et al., 1998). Comparable risk ratios have been presented in other studies (Zeeshan et al., 2007; Zuckerman, 2006). In the current study, participants whose sleep averaged less than 6.5 hours per night had a risk ratio of 2.3 for not mounting a protective response to the vaccination compared to those sleeping 6.5 hours of more per night.

Taken together, these findings provide intriguing evidence that sleep parameters, occurring prior to vaccination, and when averaged over time, influence the magnitude of primary and secondary antibody responses to the hepatitis B vaccine. Moreover, this study provides preliminary evidence that night-to-night variability in sleep duration as a previously undocumented correlate of magnitude of antibody response. Beyond accounting for variability in antibody production, the current findings suggest that short sleep duration may aid in identifying individuals at increased risk of not mounting a clinically-protective response to hepatitis B vaccination series.

7.4 POTENTIAL MECHANISMS LINKING SLEEP AND ANTIBODY PRODUCTION

7.4.1 Immune/Endocrine Mechanisms

Antibody production requires a complex, integrated set of immune processes, marked by antigen uptake, processing and presentation, and proliferation of antigen-specific memory T and B cells that facilitate the production and release of antigen-specific antibody into systemic circulation (Rabin, 1999). While the specific underlying pathways by which sleep affects antibody production have yet to be determined, several immune processes that contribute to antibody production appear sensitive to variability in sleep (Lange, Dimitrov, & Born, in press).

The immune response to foreign antigens is initiated by macrophages and dendritic cells that ingest the antigen and present it to other aspects of the immune system, including T cells. Recent human evidence suggests that sleep modulates antigen presentation. For example, Dimitrov and colleagues (2007) found that 24-hours of wakefulness was associated with a significant decline in number of myeloid precursor dendritic cells and their capacity to produce IL-12, a cytokine critical in orchestrating T-helper cell maturation. Sleep deprivation has also been associated with shifts in the number of T- and B-cells in peripheral circulation, which may impact that probability of antigen-presenting cells being able to access lymphocytes in secondary lymphoid tissue.

Diurnal fluctuations in immune parameters are primarily regulated by the neuroendocrine axis and autonomic nervous system (Lange et al., in press). Cortisol and catecholamines (e.g. epinephrine and norepinephrine) reach their relative nadirs in the early and late evening during normal sleep. Variation in these hormonal systems is intimately related to the distribution of

immune cells in the periphery. Indeed, administration of exogenous epinephrine or cortisol at physiologic levels has been shown to modulate several leukocyte subsets, including NK cells, naïve and memory helper and cytotoxic T cells (Dimitrov et al., 2009). Similar changes have been observed in response to acute sleep deprivation (Opp et al., 2007). Furthermore, it has been speculated that low levels of cortisol and epinephrine observed during normal sleep enable naïve T helper cells to travel from the bone marrow to lymphatic tissue (Lange et al., in press). Thus, it is possible that disrupted sleep may modulate adaptive immune function as a result of disruptions in the circadian regulation of hormones.

Interleukin (IL)-6 is thought to play an important role in switching T helper cells from Type 1 phenotype to Type 2 cells that promote humoral immune activity, including antibody production (Diehl & Rincon, 2002). Interestingly, elevations in inflammatory activity, including IL-6, have been observed the morning following partial sleep deprivation (Irwin et al., 2006). Furthermore, stress-related elevations in circulating IL-6 were found to statistically mediate the effect of acute stress and enhanced antibody production to the influenza vaccine (Edwards et al., 2006). Epidemiologic evidence also supports an association between shorter sleep duration and elevations in systemic levels of inflammation (Miller et al., 2009; Patel et al., 2009); however, not all studies are consistent (Prather et al., 2009) and often vary by inflammatory marker (Patel et al., 2009).

Mounting laboratory evidence shows that peripheral inflammatory mediators act on the brain to regulate aspects of sleep (Imeri & Opp, 2009), making it is impossible to discern the direction of associations between sleep and levels of systemic inflammation using cross-sectional data. While inflammation went unmeasured during the vaccination phase of the present study, the prospective design would aid in disentangling important temporal links among systemic

inflammation, sleep, and antibody response. Accordingly, longitudinal data collection tracking underlying neuroendocrine, autonomic, and immune parameters over time is necessary to characterize biological mechanisms that are responsible for coordinating antibody production and vary by fluctuations in sleep behavior.

7.4.2 Genetics

Growing evidence indicates that several sleep dimensions are, in part, genetically determined. For instance, studies have found the genetic contributions to chronotypes (heritability estimates for usual bedtime=0.23-0.47; Klei et al., 2005; Vink, Groot, Kerkhof, & Boomsma, 2001) and sleep duration (heritability (h) estimates= 0.23-0.40; Heath, Kendler, Eaves, & Martin, 1990; Klei et al., 2005; Watson, Buchwald, Vitiello, Noonan, & Goldberg) to be sizeable. In much the same way, antibody response to the hepatitis B vaccination series is heritable (h=0.61), with 25% of the genetic contribution accounted for by genes integral to antigen-presentation (Hohler et al., 2002). Circadian clock genes are proposed to contribute to individual differences in sleep by affecting circadian rhythmicity at the level of the SCN (Franken & Dijk, 2009). Moreover, variability in clock genes have also been observed in peripheral immune cells, raising the possibility that individual differences in clock gene activity contribute to both sleep and immune function. In this regard, individuals with 4/5 or 5/5 54-base pair tandem repeat in *Period (Per)*3 clock gene, which has been related to variability in sleep structure (Viola et al., 2007), displayed elevated circulating IL-6 relative to those with the 4/4 genotype (Guess et al., 2009). Other studies support *Period* clock genes as integral to the regulation of innate immunity (Arjona & Sarkar, 2005, 2006). To date, no study has explored the association between clock genes and aspects of the adaptive immune system; however, it is

plausible that variability in clock gene activity may have contributed to both sleep and antibody production in this study.

7.4.3 Psychological Stress

Psychological stress is the best researched psychosocial correlate of antibody production. Several comprehensive reviews support an inverse relationship between stress and antibody levels, including responses to the hepatitis B vaccine (Burns, Carroll, Ring et al., 2003; Cohen et al., 2001; Pedersen et al., 2009). This raises the question of whether the influence of sleep on antibody response in this study merely reflects variation in psychological stress. Indeed, sleep is readily disrupted at times of acute and chronic stress, potentially leading to clinical sleep disorders (e.g. primary insomnia; Spielman, Caruso, & Glovinsky, 1987). Conversely, poor sleep has been shown to foster heightened stress reactivity (Franzen, Buysse, Dahl, Thompson, & Siegle, 2009; Yoo, Gujar, Hu, Jolesz, & Walker, 2007) and diminished coping (Morin et al., 2003), suggesting a dynamic bidirectional relationship.

Several studies have failed to demonstrate that sleep mediates the association of stress with antibody response (Burns et al., 2002; Kiecolt-Glaser et al., 1996); in part, this may be due to the fact that researchers have relied on broad measures of habitual sleep duration, which is subject to recall bias, thus supporting the need for objective measures of sleep to be assessed over multiple nights. Using serial measurement of stress and sleep, Miller and colleagues (2004) found that sleep duration, averaged over 12 days, was marginally associated with antibody response to influenza vaccination and partially mediated the relationship between stress, averaged over the same 12 days, and antibody production (Miller et al., 2004). In addition, because they had multiple days of assessment, they were able to examine the direction of these

associations. Consistent with bidirectional influences, they found that shorter nighttime sleep predicted greater stress the following day, controlling for the stress the previous day. Similarly, higher levels of stress during the day predicted fewer hours of sleep that night, after controlling for sleep quantity the prior night. Accordingly, it will be important in future work to monitor sleep and stress longitudinally across the vaccination series to explore potential additive and synergistic effects of the sleep-stress relationship on antibody production.

7.5 STUDY LIMITATIONS

There are a number of study limitations that must be considered when interpreting these findings. First, actigraphy, which served as a more objective measure of sleep behavior, was only assessed at the first vaccination period. Furthermore, this period of sleep assessment was interrupted by the vaccination itself. While our data suggested that any effects of the vaccination on sleep were negligible, it is impossible to disentangle subtle influences that may have contributed to observed associations between sleep and antibody response. Relatedly, our serial sleep data suggests that at least 6 days are necessary to obtain a reliable average for most sleep parameters, which is consistent with prior studies (Knutson, Rathouz et al., 2007; van Hilten et al., 1993); however, other measures of temporal stability suggest that several weeks of data are needed (Wohlgemuth, Edinger, Fins, & Sullivan, 1999). Accordingly, our findings would have been strengthened by additional nights of consecutive actigraphy measurement prior to each of the vaccination periods. Furthermore, it is important to recognize that actigraphy assesses activity, not sleep, and merely capitalizes on the fact that sleep is characterized by extended periods of inactivity. As such, a confirmatory study utilizing home-based polysomnography is

indicated. That said, actigraphy is much less cumbersome than PSG and potentially provides a better window into natural sleep patterns (Littner et al., 2003).

There were a number of limitations to the electronic sleep diaries in the present study. First, we failed to discriminate between "going to bed" and "attempting to go to sleep (i.e. lights out)." As such, an individual who reported going to bed and watched television for 2 hours would have an inflated measure of sleep duration. This limitation also affected actigraphy scoring, which relied on the electronic diary data to establish "rest intervals." In addition, we did not include an estimate of minutes awake after sleep onset in our set of diary questions. This likely resulted in an overestimation of diary-based sleep efficiency, as any sleep loss was related to sleep onset latency. Furthermore, napping was not assessed in this study, potentially obscuring our interpretation of sleep duration and efficiency that can be influenced substantially by chronic napping behavior (Ancoli-Israel & Martin, 2006). Relatedly, this study would be strengthened by a measure of perceived sleep need and daytime sleepiness, as it would improve our ability to differentiate individuals who are sleep deprived versus those with a lower sleep need (Anderson & Horne, 2008; Dinges, 2005; Klerman & Dijk, 2005). Finally, we did not distinguish between sleep occurring on weekdays or weekends in our analyses. This is important because individuals may attempt to "pay off" their sleep debt from the week on weekend nights (Dinges, 2005). That said, this omission is likely of little consequence when considering measures of sleep across all days of measurement, as each vaccination time point spanned 7 days; however, it is plausible that a portion of pre-vaccination averages included weekend sleep, which may differentially influence immune function.

The present study is strengthened by its prospective design; however, in the absence of an experimental sleep manipulation and proper control conditions, we cannot determine

conclusively that variation in sleep leads to alterations in primary or secondary antibody responses to the hepatitis B vaccination. It is plausible that a third unmeasured variable, such as personality characteristics, psychological stress, or genetics may be related to both to sleep disturbance and diminished antibody response. Furthermore, clinical protection conferred by the hepatitis B vaccination cannot be verified without experimentally exposing participants to the hepatitis B virus; however, epidemiologic studies support clinical protection over time among "protected" individuals at high risk of hepatitis B exposure (Hadler et al., 1986).

Finally, the presence of clinical sleep disorders, such as obstructive sleep apnea (OSA), was not assessed in the current sample. Epidemiological evidence suggests that approximately 6% of the U.S. population suffers with sleep apnea (Gliklich, Taghizadeh, & Winkelman, 2000). Given the high correlation between obesity and clinical sleep disorders, adjustment for BMI in our regression analyses would have limited the influence of OSA on our outcomes. Moreover, a recent study found no differences in antibody responses to the influenza vaccination between untreated patients with OSA and normal sleepers (Dopp et al., 2007). Nevertheless, OSA has been associated with modulation in immune function, including elevated inflammation (Mohamed-Ali et al., 1997), which may, in turn, influence antibody production.

7.6 FUTURE DIRECTIONS AND IMPLICATIONS

There are several directions in which to take this work. First and foremost, study replication addressing the limitations listed above is needed. If research continues to support sleep duration, efficiency, and intra-individual variability in sleep duration as predictive of antibody production, future work is warranted to better identify "critical periods" when sleep

may exert influence on vaccination response. In this study, we began to explore these temporal associations; however, a lack of actigraphy sleep measures at the second vaccination period limited our ability to appropriately test independent effects of objectively assessed sleep parameters at specific time periods on antibody production.

Poor sleep continuity prior to the initial vaccination predicted a more robust primary antibody response; however, our understanding of the underlying mechanisms are unclear. While Edwards and colleagues posit that activation of inflammatory pathways prime aspects of the innate and adaptive immune system to respond more vigorously to antigenic challenge, evidence is limited to acute stress and exercise (Edwards, Burns, Carroll et al., 2007). The extent to which inefficient or fragmented sleep may produce similar immune effects under laboratory conditions is unknown. Further exploration of this potential pathway will contribute to growing knowledge about psychosocial correlates of vaccination response.

Epidemiologic studies show that approximately 20-30% of individuals mount low, transient antibody responses to the hepatitis B vaccination series (Averhoff et al., 1998; Pasko & Beam, 1990). Moreover, individuals who initially mount a protective response show greater declines in antibody levels over time, with a steeper decline associated with increasing age (Zuckerman, 2006). Accordingly, beyond establishing a relationship between sleep behavior and antibody response, determining how variation in sleep affects antibody maintenance is warranted.

Shorter sleep duration and night-to-night variability in duration prospectively predicted lower secondary antibody responses and decreased likelihood of being clinically protected at the conclusion of the hepatitis B vaccination series, raising questions about ways to improve vaccine efficacy. On one hand, efficacy could be improved among poor sleepers by withholding the

vaccine until their sleep improved. On the other hand, disturbed sleep is a risk factor for several medical conditions, suggesting that sleep interventions may confer the most long term benefit. In this regard, several pharmacologic and behavioral therapies have been developed to treat sleep complaints with good success (Edinger, Wohlgemuth, Radtke, Marsh, & Quillian, 2001; Smith et al., 2002). Behavioral sleep interventions may be particularly helpful for older adults who, as a demographic, report more disturbed sleep, show greater immunosenescence, and are more likely than younger adults to have medical co-morbidities potentially complicating pharmacologic treatment.

8.0 CONCLUSIONS

The goal of this study was broadly to investigate how natural variation in sleep influences the magnitude of antibody response to vaccination, an *in vivo* measure related to susceptibility to infectious disease. Recognizing the constraints of prior laboratory research, we employed unique strategies to prospectively investigate the influence of sleep in the field, measured objectively and subjectively using actigraphy and electronic diaries, on an integrated and clinically relevant measure of immune function, namely antibody response to the hepatitis B vaccine. This study revealed several intriguing relationships between sleep and antibody production. In this regard, shorter sleep duration was associated with both lower secondary antibody levels and decreased likelihood of being clinically protected at the conclusion of the vaccination series. These findings were independent of the effects of age, gender, race, body mass index and consistent with a larger epidemiologic literature describing the negative health correlates of habitually short sleep. Additionally, we provide preliminary evidence that night-to-night variability in sleep duration is related to magnitude of antibody response, suggesting that variability in sleep may be another sleep dimension that affects health.

Contrary to our hypotheses, poorer sleep continuity, particularly poor sleep efficiency, predicted a greater likelihood of mounting a detectable response to the first vaccination, which is consistent with an emerging literature that immune activation may prime the adaptive immune

system to respond more robustly. However, these findings did not withstand covariate adjustment. As such, future research is needed to clarify this association.

Taken together, our findings provide preliminary evidence supporting variation in sleep as a significant contributor to an integrated immune response. If replicated, future work identifying "critical periods" when sleep may exert a disproportionate influence on immunity is warranted. An examination of the biological and psychological pathways that contribute to sleep-related variation in antibody response would also be interesting and may assist in the development of therapeutic strategies, including targeted behavioral sleep interventions to improve vaccination efficacy among vulnerable populations, including elderly, chronically-ill, and otherwise immunocompromised individuals.

APPENDIX A

Appendix A 1: Unadjusted and adjusted logistic and linear regressions using total sleep time (TST) to test hypotheses 1, 4, and 7 from the primary study analyses

	B	SE	Wald	p-value	Odds Ratio	95% C.I.
DV: Responder Status (hypothesis 1)						
Total Sleep Time (TST) (n=89)						
1. V1 actigraphy pre-vac. TST	-.01	.01	2.68	.10	.99	.98-1.00
After covariate adjustment						
1. Covariates[a]						
2. V1 actigraphy pre-vac. TST	-.01	.01	1.51	.22	.99	.98-1.00

	B	SE	p-value	R^2	ΔR^2
DV: Secondary antibody levels (hypothesis 4)					
Total Sleep Time (TST) (n=85)					
1. V1 actigraphy TST	.007	.004	.06	.04	---
After covariate adjustment					
1. Covariates[b]				.17	---
2. V1 actigraphy TST	.007	.004	.09	.19	.02
DV: Secondary antibody levels (hypothesis 7)					
Total Sleep Time (TST) (n=83)					
1. V2 diary pre-vac. sleep duration				.00	---
2. V1 actigraphy pre-vac. TST	.007	.004	.07	.02	.02
After covariate adjustment					
1. Covariates[b]				.18	---
2. V2 diary pre-vac. sleep duration				.18	.00
3. V1 actigraphy pre-vac. TST	.006	.004	.11	.20	.02

[a] age, gender, race, BMI; [b] age, gender, race, BMI, responder status to initial vaccination

APPENDIX B

Appendix B 1: Unadjusted and adjusted logistic regression analyses examining whether pre-vaccination sleep duration, efficiency, and quality predicts likelihood of mounting detectable antibodies (i.e. being a responder) in response to the first hepatitis B injection. Analyses based on imputed data.

	B	SE	Wald	p-value	Odds Ratio	95% C.I.
DV: Responder Status						
Sleep Duration (n=90)						
1. V1 actigraphy pre-vac. sleep duration	-.12	.31	.14	.71	.89	.49-1.63
After covariate adjustment						
1. Covariates[a]						
2. V1 actigraphy pre-vac. sleep duration	-.08	.33	.05	.82	.93	.49-1.75
Sleep Efficiency (n=90)						
1. V1 actigraphy pre-vac. sleep efficiency	-.08	.03	6.17	.03	.93	.87-.98
After covariate adjustment						
1. Covariates[a]						
2. V1 actigraphy pre-vac. sleep efficiency	-.07	.03	3.65	.06	.94	.88-1.00
3. V1 act. pre-vac. sleep efficiency (quadratic effect)	.18	.29	.39	.53	1.20	.68-2.12
Sleep Quality (n=117)						
1. V1 diary pre-vac. sleep quality	-.41	.42	.97	.33	.66	.29-1.51
After covariate adjustment						
1. Covariates[a]						
2. V1 diary pre-vac. sleep quality	-.31	.44	.51	.48	.73	.31-1.72

[a]age, gender, race, BMI

Appendix B 2: Unadjusted and adjusted linear regression analyses examining whether actigraphy-based measures of sleep predicts secondary antibody levels following the second hepatitis B vaccination. Analyses based on imputed data.

	B	SE	p-value	R^2	ΔR^2
DV: Secondary Antibody Levels (nat. log)					
Sleep duration (n=87)					
1. V1 actigraphy sleep duration	.57	.24	.02	.05	---
After covariate adjustment					
1. Covariates[a]				.17	---
2. V1 actigraphy sleep duration	.49	.22	.03	.21	.04
Sleep efficiency (n=87)					
1. V1 actigraphy pre.-vac. sleep efficiency	.02	.02	.45	.00	---
After covariate adjustment					
1. Covariates[a]				.17	---
2. V1 actigraphy pre.-vac. sleep efficiency	.03	.02	.19	.18	.01

[a] age, gender, race, BMI, and responder status to initial vaccination

Appendix B 3: Unadjusted and adjusted linear regression analyses examining whether actigraphy and diary-based measures of sleep, assessed prior to the first vaccination, predict secondary antibody levels after controlling for the effects of sleep occurring prior to the second vaccination. Analyses used imputed data.

	B	SE	p-value	R^2	ΔR^2
DV: Secondary Antibody Levels (nat. log)					
Sleep duration (n=84)					
1. V2 diary pre-vac. sleep duration				.00	---
2. V1 actigraphy pre-vac. sleep duration	.47	.22	.04	.03	.03
After covariate adjustment					
1. Covariates[a]				.19	---
2. V2 diary pre-vac. sleep duration				.19	.00
3. V1 actigraphy pre-vac. sleep duration	.39	.21	.07	.21	.02
Sleep efficiency (n=84)					
1. V2 diary pre-vac. sleep efficiency				.00	---
2. V1 actigraphy pre-vac. sleep efficiency	.02	.02	.47	.00	.00
After covariate adjustment					
1. Covariates[a]				.19	---
2. V2 diary pre-vac. sleep efficiency				.19	.00
3. V1 actigraphy pre-vac. sleep efficiency	.03	.02	.19	.20	.01
Sleep quality (n=107)					
1. V2 diary pre-vac. sleep quality				.00	---
2. V1 diary pre-vac. sleep quality	.00	.42	.99	.00	.00
After covariate adjustment					
1. Covariates[a]				.23	---
2. V2 diary pre-vac. sleep quality				.22	-.01
3. V1 diary pre-vac. sleep quality	.18	.37	.63	.22	.00

[a] age, gender, race, BMI, and responder status to initial vaccination

BIBLIOGRAPHY

Acebo, C., Sadeh, A., Seifer, R., Tzischinsky, O., Wolfson, A. R., Hafer, A., et al. (1999). Estimating sleep patterns with activity monitoring in children and adolescents: how many nights are necessary for reliable measures? *Sleep, 22*(1), 95-103.

Ancoli-Israel, S., Cole, R., Alessi, C., Chambers, M., Moorcroft, W., & Pollak, C. P. (2003). The role of actigraphy in the study of sleep and circadian rhythms. *Sleep, 26*(3), 342-392.

Ancoli-Israel, S., & Martin, J. L. (2006). Insomnia and daytime napping in older adults. *J Clin Sleep Med, 2*(3), 333-342.

Anderson, C., & Horne, J. A. (2008). Do we really want more sleep? A population-based study evaluating the strength of desire for more sleep. *Sleep Med, 9*(2), 184-187.

Arjona, A., & Sarkar, D. K. (2005). Circadian oscillations of clock genes, cytolytic factors, and cytokines in rat NK cells. *J Immunol, 174*(12), 7618-7624.

Arjona, A., & Sarkar, D. K. (2006). The circadian gene mPer2 regulates the daily rhythm of IFN-gamma. *J Interferon Cytokine Res, 26*(9), 645-649.

Arnardottir, E. S., Mackiewicz, M., Gislason, T., Teff, K. L., & Pack, A. I. (2009). Molecular signatures of obstructive sleep apnea in adults: a review and perspective. *Sleep, 32*(4), 447-470.

Averhoff, F., Mahoney, F., Coleman, P., Schatz, G., Hurwitz, E., & Margolis, H. (1998). Immunogenicity of hepatitis B Vaccines. Implications for persons at occupational risk of hepatitis B virus infection. *Am J Prev Med, 15*(1), 1-8.

Ayas, N. T., White, D. P., Al-Delaimy, W. K., Manson, J. E., Stampfer, M. J., Speizer, F. E., et al. (2003). A prospective study of self-reported sleep duration and incident diabetes in women. *Diabetes Care, 26*(2), 380-384.

Ayas, N. T., White, D. P., Manson, J. E., Stampfer, M. J., Speizer, F. E., Malhotra, A., et al. (2003). A prospective study of sleep duration and coronary heart disease in women. *Arch Intern Med, 163*(2), 205-209.

Babyak, M. A. (2005). *Some key developments in data analysis*. Paper presented at the American Psychosomatic Society.

Baldwin, D. C., Jr., & Daugherty, S. R. (2004). Sleep deprivation and fatigue in residency training: results of a national survey of first- and second-year residents. *Sleep, 27*(2), 217-223.

Benca, R. M., & Quintas, J. (1997). Sleep and host defenses: a review. *Sleep, 20*(11), 1027-1037.

Bock, H. L., Kruppenbacher, J., Sanger, R., Hobel, W., Clemens, R., & Jilg, W. (1996). Immunogenicity of a recombinant hepatitis B vaccine in adults. *Arch Intern Med, 156*(19), 2226-2231.

Bonnet, M. H., & Arand, D. L. (2003). Clinical effects of sleep fragmentation versus sleep deprivation. *Sleep Med Rev, 7*(4), 297-310.

Born, J., Lange, T., Hansen, K., Molle, M., & Fehm, H. L. (1997). Effects of sleep and circadian rhythm on human circulating immune cells. *J Immunol, 158*(9), 4454-4464.

Born, J., Uthgenannt, D., Dodt, C., Nunninghoff, D., Ringvolt, E., Wagner, T., et al. (1995). Cytokine production and lymphocyte subpopulations in aged humans. An assessment during nocturnal sleep. *Mech Ageing Dev, 84*(2), 113-126.

Brown, R., Pang, G., Husband, A. J., & King, M. G. (1989). Suppression of immunity to influenza virus infection in the respiratory tract following sleep disturbance. *Reg Immunol, 2*(5), 321-325.

Bryant, P. A., Trinder, J., & Curtis, N. (2004). Sick and tired: Does sleep have a vital role in the immune system? *Nat Rev Immunol, 4*(6), 457-467.

Burns, V. E., Carroll, D., Drayson, M., Whitham, M., & Ring, C. (2003). Life events, perceived stress and antibody response to influenza vaccination in young, healthy adults. *J Psychosom Res, 55*(6), 569-572.

Burns, V. E., Carroll, D., Ring, C., & Drayson, M. (2003). Antibody response to vaccination and psychosocial stress in humans: relationships and mechanisms. *Vaccine, 21*(19-20), 2523-2534.

Burns, V. E., Carroll, D., Ring, C., Harrison, L. K., & Drayson, M. (2002). Stress, coping, and hepatitis B antibody status. *Psychosom Med, 64*(2), 287-293.

Buysse, D. J. (2005). Introduction. In D. J. Buysse (Ed.), *Sleep Disorders and Psychiatry* (pp. 1-28). Arlington, VA: American Psychiatric Publishing Inc.

Buysse, D. J., Cheng, Y., Germain, A., Moul, D. E., Franzen, P. L., Fletcher, M., et al. (in press). Night-to-night sleep variability in older adults with and without chronic insomnia. *Sleep Med, 11*(1), 56-64.

Cappuccio, F. P., D'Elia, L., Strazzullo, P., & Miller, M. A. (*in press-a*). Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care, 33*(2), 414-420.

Cappuccio, F. P., D'Elia, L., Strazzullo, P., & Miller, M. A. (*in press-b*). Sleep duration and all-cause mortality: a systematic review and meta-analysis of prospective studies. *Sleep, 33*(5), 585-592.

Carskadon, M. A., & Dement, W. C. (2000). Normal human sleep: an overview. In M. H. Kryger, T. Roth & W. C. Dement (Eds.), *Principles and practice of sleep medicine* (3rd ed.). Philadelphia: W.B. Saunders Company.

CDC. (1987). Leads from the MMWR. Recommendations of the immunization practices advisory committee update on hepatitis B prevention. *JAMA, 258*(4), 437, 444-435, 449.

Cohen, S., Doyle, W. J., Alper, C. M., Janicki-Deverts, D., & Turner, R. B. (2009). Sleep habits and susceptibility to the common cold. *Arch Intern Med, 169*(1), 62-67.

Cohen, S., Doyle, W. J., Skoner, D. P., Rabin, B. S., & Gwaltney, J. M., Jr. (1997). Social ties and susceptibility to the common cold. *JAMA, 277*(24), 1940-1944.

Cohen, S., Miller, G. E., & Rabin, B. S. (2001). Psychological stress and antibody response to immunization: a critical review of the human literature. *Psychosom Med, 63*(1), 7-18.

Colwell, C. S., & Michel, S. (2003). Sleep and circadian rhythms: do sleep centers talk back to the clock? *Nat Neurosci, 6*(10), 1005-1006.

Czeisler, C. A., & Klerman, E. B. (1999). Circadian and sleep-dependent regulation of hormone release in humans. *Recent Prog Horm Res, 54*, 97-130; discussion 130-132.

de Souza, L., Benedito-Silva, A. A., Pires, M. L., Poyares, D., Tufik, S., & Calil, H. M. (2003). Further validation of actigraphy for sleep studies. *Sleep, 26*(1), 81-85.

Delves, P. J., & Roitt, I. M. (2000a). The immune system. First of two parts. *N Engl J Med, 343*(1), 37-49.

Delves, P. J., & Roitt, I. M. (2000b). The immune system. Second of two parts. *N Engl J Med, 343*(2), 108-117.

Dew, M. A., Hoch, C. C., Buysse, D. J., Monk, T. H., Begley, A. E., Houck, P. R., et al. (2003). Healthy older adults' sleep predicts all-cause mortality at 4 to 19 years of follow-up. *Psychosom Med, 65*(1), 63-73.

Dhabhar, F. S. (2002). Stress-induced augmentation of immune function--the role of stress hormones, leukocyte trafficking, and cytokines. *Brain Behav Immun, 16*(6), 785-798.

Diehl, S., & Rincon, M. (2002). The two faces of IL-6 on Th1/Th2 differentiation. *Mol Immunol, 39*(9), 531-536.

Dimitrov, S., Benedict, C., Heutling, D., Westermann, J., Born, J., & Lange, T. (2009). Cortisol and epinephrine control opposing circadian rhythms in T cell subsets. *Blood, 113*(21), 5134-5143.

Dimitrov, S., Lange, T., Nohroudi, K., & Born, J. (2007). Number and function of circulating human antigen presenting cells regulated by sleep. *Sleep, 30*(4), 401-411.

Dimitrov, S., Lange, T., Tieken, S., Fehm, H. L., & Born, J. (2004). Sleep associated regulation of T helper 1/T helper 2 cytokine balance in humans. *Brain Behav Immun, 18*(4), 341-348.

Dinges, D. F. (2005). Can habitual sleep duration harbor sleep debt? *Sleep, 28*(10), 1209-1210.

Dopp, J. M., Wiegert, N. A., Moran, J. J., Muller, D., Weber, S., & Hayney, M. S. (2007). Humoral immune responses to influenza vaccination in patients with obstructive sleep apnea. *Pharmacotherapy, 27*(11), 1483-1489.

Drake, C., Richardson, G., Roehrs, T., Scofield, H., & Roth, T. (2004). Vulnerability to stress-related sleep disturbance and hyperarousal. *Sleep, 27*(2), 285-291.

Dyugovskaya, L., Lavie, P., & Lavie, L. (2005). Lymphocyte activation as a possible measure of atherosclerotic risk in patients with sleep apnea. *Ann N Y Acad Sci, 1051*, 340-350.

Edinger, J. D., Wohlgemuth, W. K., Radtke, R. A., Marsh, G. R., & Quillian, R. E. (2001). Cognitive behavioral therapy for treatment of chronic primary insomnia: a randomized controlled trial. *JAMA, 285*(14), 1856-1864.

Edwards, K. M., Burns, V. E., Allen, L. M., McPhee, J. S., Bosch, J. A., Carroll, D., et al. (2007). Eccentric exercise as an adjuvant to influenza vaccination in humans. *Brain Behav Immun, 21*(2), 209-217.

Edwards, K. M., Burns, V. E., Carroll, D., Drayson, M., & Ring, C. (2007). The acute stress-induced immunoenhancement hypothesis. *Exerc Sport Sci Rev, 35*(3), 150-155.

Edwards, K. M., Burns, V. E., Reynolds, T., Carroll, D., Drayson, M., & Ring, C. (2006). Acute stress exposure prior to influenza vaccination enhances antibody response in women. *Brain Behav Immun, 20*(2), 159-168.

Ensrud, K. E., Blackwell, T. L., Redline, S., Ancoli-Israel, S., Paudel, M. L., Cawthon, P. M., et al. (2009). Sleep disturbances and frailty status in older community-dwelling men. *J Am Geriatr Soc, 57*(11), 2085-2093.

Everson, C. A. (1993). Sustained sleep deprivation impairs host defense. *Am J Physiol, 265*(5 Pt 2), R1148-1154.

Fiorentini, A., Valente, R., Perciaccante, A., & Tubani, L. (2007). Sleep's quality disorders in patients with hypertension and type 2 diabetes mellitus. *Int J Cardiol, 114*(2), E50-52.

Francis, D. P., Hadler, S. C., Thompson, S. E., Maynard, J. E., Ostrow, D. G., Altman, N., et al. (1982). The prevention of hepatitis B with vaccine. Report of the centers for disease control multi-center efficacy trial among homosexual men. *Ann Intern Med, 97*(3), 362-366.

Franken, P., & Dijk, D. J. (2009). Circadian clock genes and sleep homeostasis. *Eur J Neurosci, 29*(9), 1820-1829.

Franzen, P. L., Buysse, D. J., Dahl, R. E., Thompson, W., & Siegle, G. J. (2009). Sleep deprivation alters pupillary reactivity to emotional stimuli in healthy young adults. *Biol Psychol, 80*(3), 300-305.

Fujino, Y., Iso, H., Tamakoshi, A., Inaba, Y., Koizumi, A., Kubo, T., et al. (2006). A prospective cohort study of shift work and risk of ischemic heart disease in Japanese male workers. *Am J Epidemiol, 164*(2), 128-135.

Gangwisch, J. E., Heymsfield, S. B., Boden-Albala, B., Buijs, R. M., Kreier, F., Pickering, T. G., et al. (2006). Short sleep duration as a risk factor for hypertension: analyses of the first National Health and Nutrition Examination Survey. *Hypertension, 47*(5), 833-839.

Gangwisch, J. E., Heymsfield, S. B., Boden-Albala, B., Buijs, R. M., Kreier, F., Pickering, T. G., et al. (2007). Sleep duration as a risk factor for diabetes incidence in a large U.S. sample. *Sleep, 30*(12), 1667-1673.

Glaser, R., Kiecolt-Glaser, J. K., Bonneau, R. H., Malarkey, W., Kennedy, S., & Hughes, J. (1992). Stress-induced modulation of the immune response to recombinant hepatitis B vaccine. *Psychosom Med, 54*(1), 22-29.

Glaser, R., Kiecolt-Glaser, J. K., Malarkey, W. B., & Sheridan, J. F. (1998). The influence of psychological stress on the immune response to vaccines. *Ann N Y Acad Sci, 840*, 649-655.

Gliklich, R. E., Taghizadeh, F., & Winkelman, J. W. (2000). Health status in patients with disturbed sleep and obstructive sleep apnea. *Otolaryngol Head Neck Surg, 122*(4), 542-546.

Goodwin, K., Viboud, C., & Simonsen, L. (2006). Antibody response to influenza vaccination in the elderly: a quantitative review. *Vaccine, 24*(8), 1159-1169.

Grandner, M. A., & Kripke, D. F. (2004). Self-reported sleep complaints with long and short sleep: a nationally representative sample. *Psychosom Med, 66*(2), 239-241.

Guess, J., Burch, J. B., Ogoussan, K., Armstead, C. A., Zhang, H., Wagner, S., et al. (2009). Circadian disruption, Per3, and human cytokine secretion. *Integr Cancer Ther, 8*(4), 329-336.

Hadler, S. C., Francis, D. P., Maynard, J. E., Thompson, S. E., Judson, F. N., Echenberg, D. F., et al. (1986). Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. *N Engl J Med, 315*(4), 209-214.

Hall, M. H., Muldoon, M. F., Jennings, J. R., Buysse, D. J., Flory, J. D., & Manuck, S. B. (2008). Self-reported sleep duration is associated with the metabolic syndrome in midlife adults. *Sleep, 31*(5), 635-643.

Heath, A. C., Kendler, K. S., Eaves, L. J., & Martin, N. G. (1990). Evidence for genetic influences on sleep disturbance and sleep pattern in twins. *Sleep, 13*(4), 318-335.

Heiser, P., Dickhaus, B., Schreiber, W., Clement, H. W., Hasse, C., Hennig, J., et al. (2000). White blood cells and cortisol after sleep deprivation and recovery sleep in humans. *Eur Arch Psychiatry Clin Neurosci, 250*(1), 16-23.

Heslop, P., Smith, G. D., Metcalfe, C., Macleod, J., & Hart, C. (2002). Sleep duration and mortality: The effect of short or long sleep duration on cardiovascular and all-cause mortality in working men and women. *Sleep Med, 3*(4), 305-314.

Hohler, T., Reuss, E., Evers, N., Dietrich, E., Rittner, C., Freitag, C. M., et al. (2002). Differential genetic determination of immune responsiveness to hepatitis B surface antigen and to hepatitis A virus: a vaccination study in twins. *Lancet, 360*(9338), 991-995.

Hui, S. L., Chu, L. W., Peiris, J. S., Chan, K. H., Chu, D., & Tsui, W. (2006). Immune response to influenza vaccination in community-dwelling Chinese elderly persons. *Vaccine, 24*(25), 5371-5380.

Imeri, L., & Opp, M. R. (2009). How (and why) the immune system makes us sleep. *Nat Rev Neurosci, 10*(3), 199-210.

Irwin, M. (2002). Effects of sleep and sleep loss on immunity and cytokines. *Brain Behav Immun, 16*(5), 503-512.

Irwin, M., Clark, C., Kennedy, B., Christian Gillin, J., & Ziegler, M. (2003). Nocturnal catecholamines and immune function in insomniacs, depressed patients, and control subjects. *Brain Behav Immun, 17*(5), 365-372.

Irwin, M., Mascovich, A., Gillin, J. C., Willoughby, R., Pike, J., & Smith, T. L. (1994). Partial sleep deprivation reduces natural killer cell activity in humans. *Psychosom Med, 56*(6), 493-498.

Irwin, M., McClintick, J., Costlow, C., Fortner, M., White, J., & Gillin, J. C. (1996). Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. *FASEB J, 10*(5), 643-653.

Irwin, M., Thompson, J., Miller, C., Gillin, J. C., & Ziegler, M. (1999). Effects of sleep and sleep deprivation on catecholamine and interleukin-2 levels in humans: clinical implications. *J Clin Endocrinol Metab, 84*(6), 1979-1985.

Irwin, M., Wang, M., Campomayor, C. O., Collado-Hidalgo, A., & Cole, S. (2006). Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. *Arch Intern Med, 166*(16), 1756-1762.

Jabaaij, L., Grosheide, P. M., Heijtink, R. A., Duivenvoorden, H. J., Ballieux, R. E., & Vingerhoets, A. J. (1993). Influence of perceived psychological stress and distress on antibody response to low dose rDNA hepatitis B vaccine. *J Psychosom Res, 37*(4), 361-369.

Jabaaij, L., van Hattum, J., Vingerhoets, J. J., Oostveen, F. G., Duivenvoorden, H. J., & Ballieux, R. E. (1996). Modulation of immune response to rDNA hepatitis B vaccination by psychological stress. *J Psychosom Res, 41*(2), 129-137.

Jean-Louis, G., Kripke, D. F., & Ancoli-Israel, S. (2000). Sleep and quality of well-being. *Sleep, 23*(8), 1115-1121.

Jennings, J. R., Muldoon, M. F., Hall, M., Buysse, D. J., & Manuck, S. B. (2007). Self-reported sleep quality is associated with the metabolic syndrome. *Sleep, 30*(2), 219-223.

Jones, B. E. (2005). Basic mechanisms of sleep-wake states. In M. H. Kryger, T. Roth & W. C. Dement (Eds.), *Principles and Practice of Sleep Medicine* (4th ed.). Philadelphia, PA: Elsevier Saunders.

Kiecolt-Glaser, J. K., Glaser, R., Gravenstein, S., Malarkey, W. B., & Sheridan, J. (1996). Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proc Natl Acad Sci U S A, 93*(7), 3043-3047.

Kin, N. W., & Sanders, V. M. (2006). It takes nerve to tell T and B cells what to do. *J Leukoc Biol, 79*(6), 1093-1104.

Klei, L., Reitz, P., Miller, M., Wood, J., Maendel, S., Gross, D., et al. (2005). Heritability of morningness-eveningness and self-report sleep measures in a family-based sample of 521 hutterites. *Chronobiol Int, 22*(6), 1041-1054.

Klerman, E. B., & Dijk, D. J. (2005). Interindividual variation in sleep duration and its association with sleep debt in young adults. *Sleep, 28*(10), 1253-1259.

Knutson, K. L., Rathouz, P. J., Yan, L. L., Liu, K., & Lauderdale, D. S. (2007). Intra-individual daily and yearly variability in actigraphically recorded sleep measures: the CARDIA study. *Sleep, 30*(6), 793-796.

Knutson, K. L., Spiegel, K., Penev, P., & Van Cauter, E. (2007). The metabolic consequences of sleep deprivation. *Sleep Med Rev, 11*(3), 163-178.

Kripke, D. F., Garfinkel, L., Wingard, D. L., Klauber, M. R., & Marler, M. R. (2002). Mortality associated with sleep duration and insomnia. *Arch Gen Psychiatry, 59*(2), 131-136.

Kushida, C. A., Chang, A., Gadkary, C., Guilleminault, C., Carrillo, O., & Dement, W. C. (2001). Comparison of actigraphic, polysomnographic, and subjective assessment of sleep parameters in sleep-disordered patients. *Sleep Med, 2*(5), 389-396.

Lange, T., Dimitrov, S., & Born, J. (in press). Effects of sleep and circadian rhythm on the human immune system. *Ann N Y Acad Sci, 1193*(1), 48-59.

Lange, T., Perras, B., Fehm, H. L., & Born, J. (2003). Sleep enhances the human antibody response to hepatitis A vaccination. *Psychosom Med, 65*(5), 831-835.

Lauderdale, D. S., Knutson, K. L., Yan, L. L., Rathouz, P. J., Hulley, S. B., Sidney, S., et al. (2006). Objectively measured sleep characteristics among early-middle-aged adults: the CARDIA study. *Am J Epidemiol, 164*(1), 5-16.

Leineweber, C., Kecklund, G., Janszky, I., Akerstedt, T., & Orth-Gomer, K. (2003). Poor sleep increases the prospective risk for recurrent events in middle-aged women with coronary disease. The Stockholm Female Coronary Risk Study. *J Psychosom Res, 54*(2), 121-127.

Lichstein, K. L., Durrence, H. H., Riedel, B. W., Taylor, D. J., & Bush, A. J. (2004). *Epidemiology of Sleep: Age, Gender, and Ethnicity*. Mahwah, N.J.: Lawrence Erlbaum Associates.

Lissoni, P., Rovelli, F., Brivio, F., Brivio, O., & Fumagalli, L. (1998). Circadian secretions of IL-2, IL-12, IL-6 and IL-10 in relation to the light/dark rhythm of the pineal hormone melatonin in healthy humans. *Nat Immun, 16*(1), 1-5.

Little, R. J. A., & Rubin, D. A. (1987). *Statistical analysis with missing data*. New York: John Wiley & Sons.

Littner, M., Kushida, C. A., Anderson, W. M., Bailey, D., Berry, R. B., Davila, D. G., et al. (2003). Practice parameters for the role of actigraphy in the study of sleep and circadian rhythms: an update for 2002. *Sleep, 26*(3), 337-341.

Maier, S. F., & Watkins, L. R. (1998). Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychol Rev, 105*(1), 83-107.

Mallon, L., Broman, J. E., & Hetta, J. (2002). Sleep complaints predict coronary artery disease mortality in males: a 12-year follow-up study of a middle-aged Swedish population. *J Intern Med, 251*(3), 207-216.

Mallon, L., Broman, J. E., & Hetta, J. (2005). High incidence of diabetes in men with sleep complaints or short sleep duration: a 12-year follow-up study of a middle-aged population. *Diabetes Care, 28*(11), 2762-2767.

Marsland, A. L., Bachen, E. A., Cohen, S., & Manuck, S. B. (2001). Stress, Immunity, and Susceptibility to Infectious Disease. In A. Baum, T. A. Revenson & J. E. Singer (Eds.), *Handbook of Health Psychology* (pp. 683-695). Mahwah, N.J.: Lawrence Erlbaum Associates.

Marsland, A. L., Cohen, S., Rabin, B. S., & Manuck, S. B. (2001). Associations between stress, trait negative affect, acute immune reactivity, and antibody response to hepatitis B injection in healthy young adults. *Health Psychol, 20*(1), 4-11.

Marsland, A. L., Cohen, S., Rabin, B. S., & Manuck, S. B. (2006). Trait positive affect and antibody response to hepatitis B vaccination. *Brain Behav Immun, 20*(3), 261-269.

Marsland, A. L., Herbert, T. B., Muldoon, M. F., Bachen, E. A., Patterson, S., Cohen, S., et al. (1997). Lymphocyte subset redistribution during acute laboratory stress in young adults: mediating effects of hemoconcentration. *Health Psychol, 16*(4), 341-348.

Matthews, K. A., Caggiula, A. R., McAllister, C. G., Berga, S. L., Owens, J. F., Flory, J. D., et al. (1995). Sympathetic reactivity to acute stress and immune response in women. *Psychosom Med, 57*(6), 564-571.

Matzinger, P. (2002). The danger model: a renewed sense of self. *Science, 296*(5566), 301-305.

Meisinger, C., Heier, M., Lowel, H., Schneider, A., & Doring, A. (2007). Sleep duration and sleep complaints and risk of myocardial infarction in middle-aged men and women from the general population: the MONICA/KORA Augsburg cohort study. *Sleep, 30*(9), 1121-1127.

Mezick, E. J., Matthews, K. A., Hall, M., Kamarck, T. W., Buysse, D. J., Owens, J. F., et al. (2009). Intra-individual variability in sleep duration and fragmentation: associations with stress. *Psychoneuroendocrinology, 34*(9), 1346-1354.

Miller, G. E., Cohen, S., Pressman, S., Barkin, A., Rabin, B. S., & Treanor, J. J. (2004). Psychological stress and antibody response to influenza vaccination: when is the critical period for stress, and how does it get inside the body? *Psychosom Med, 66*(2), 215-223.

Miller, M. A., Kandala, N. B., Kivimaki, M., Kumari, M., Brunner, E. J., Lowe, G. D., et al. (2009). Gender differences in the cross-sectional relationships between sleep duration and markers of inflammation: Whitehall II study. *Sleep, 32*(7), 857-864.

Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., et al. (1997). Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *J Clin Endocrinol Metab, 82*(12), 4196-4200.

Mohren, D. C., Jansen, N. W., Kant, I. J., Galama, J., van den Brandt, P. A., & Swaen, G. M. (2002). Prevalence of common infections among employees in different work schedules. *J Occup Environ Med, 44*(11), 1003-1011.

Moldofsky, H. (1993). Fibromyalgia, sleep disorder and chronic fatigue syndrome. *Ciba Found Symp, 173*, 262-271; discussion 272-269.

Moldofsky, H. (1995). Sleep and the immune system. *Int J Immunopharmacol, 17*(8), 649-654.

Moldofsky, H., Lue, F. A., Davidson, J. R., & Gorczynski, R. (1989). Effects of sleep deprivation on human immune functions. *FASEB J, 3*(8), 1972-1977.

Mongrain, V., Carrier, J., & Dumont, M. (2006). Circadian and homeostatic sleep regulation in morningness-eveningness. *J Sleep Res, 15*(2), 162-166.

Mongrain, V., Lavoie, S., Selmaoui, B., Paquet, J., & Dumont, M. (2004). Phase relationships between sleep-wake cycle and underlying circadian rhythms in Morningness-Eveningness. *J Biol Rhythms, 19*(3), 248-257.

Morin, C. M. (1993). *Insomnia: Psychological assessment and management.* . New York-London: The Guilford Press.

Morin, C. M., Rodrigue, S., & Ivers, H. (2003). Role of stress, arousal, and coping skills in primary insomnia. *Psychosom Med, 65*(2), 259-267.

Nathan, C. (2002). Points of control in inflammation. *Nature, 420*(6917), 846-852.

Ohayon, M. M. (2005). Prevalence and correlates of nonrestorative sleep complaints. *Arch Intern Med, 165*(1), 35-41.

Ohayon, M. M., Caulet, M., & Guilleminault, C. (1997). How a general population perceives its sleep and how this relates to the complaint of insomnia. *Sleep, 20*(9), 715-723.

Ohayon, M. M., & Zulley, J. (2001). Correlates of global sleep dissatisfaction in the German population. *Sleep, 24*(7), 780-787.

Opp, M. R., Born, J., & Irwin, M. R. (2007). Sleep and the immune system. In R. Ader (Ed.), *Psychoneuroimmunology* (4th ed., pp. 570-618). New York, NY: Academic Press.

Paine, S. J., Gander, P. H., & Travier, N. (2006). The epidemiology of morningness/eveningness: influence of age, gender, ethnicity, and socioeconomic factors in adults (30-49 years). *J Biol Rhythms, 21*(1), 68-76.

Palmblad, J., Petrini, B., Wasserman, J., & Akerstedt, T. (1979). Lymphocyte and granulocyte reactions during sleep deprivation. *Psychosom Med, 41*(4), 273-278.

Pasko, M. T., & Beam, T. R., Jr. (1990). Persistence of anti-HBs among health care personnel immunized with hepatitis B vaccine. *Am J Public Health, 80*(5), 590-593.

Patel, S. R., Ayas, N. T., Malhotra, M. R., White, D. P., Schernhammer, E. S., Speizer, F. E., et al. (2004). A prospective study of sleep duration and mortality risk in women. *Sleep, 27*(3), 440-444.

Patel, S. R., Zhu, X., Storfer-Isser, A., Mehra, R., Jenny, N. S., Tracy, R., et al. (2009). Sleep duration and biomarkers of inflammation. *Sleep, 32*(2), 200-204.

Patil, S. P., Schneider, H., Schwartz, A. R., & Smith, P. L. (2007). Adult obstructive sleep apnea: pathophysiology and diagnosis. *Chest, 132*(1), 325-337.

Pavlov, V. A., & Tracey, K. J. (2004). Neural regulators of innate immune responses and inflammation. *Cell Mol Life Sci, 61*(18), 2322-2331.

Pedersen, A. F., Zachariae, R., & Bovbjerg, D. H. (2009). Psychological stress and antibody response to influenza vaccination: A meta-analysis. *Brain Behav Immun, 23*(4), 427-433.

Petrie, K. J., Booth, R. J., Pennebaker, J. W., Davison, K. P., & Thomas, M. G. (1995). Disclosure of trauma and immune response to a hepatitis B vaccination program. *J Consult Clin Psychol, 63*(5), 787-792.

Petry, L. J., Weems, L. B., 2nd, & Livingstone, J. N., 2nd. (1991). Relationship of stress, distress, and the immunologic response to a recombinant hepatitis B vaccine. *J Fam Pract, 32*(5), 481-486.

Phillips, A. C., Carroll, D., Burns, V. E., & Drayson, M. (2005). Neuroticism, cortisol reactivity, and antibody response to vaccination. *Psychophysiology, 42*(2), 232-238.

Phillips, A. C., Carroll, D., Burns, V. E., Ring, C., Macleod, J., & Drayson, M. (2006). Bereavement and marriage are associated with antibody response to influenza vaccination in the elderly. *Brain Behav Immun, 20*(3), 279-289.

Phillips, B., & Mannino, D. (2005). Correlates of sleep complaints in adults: the ARIC study. *J Clin Sleep Med, 1*(3), 277-283.

Prather, A. A., & Marsland, A. L. (2008). Immunological Functioning II: Field measures and viral challenge. In L. J. Lueken & L. C. Gallo (Eds.), *Handbook of Physiological Research Methods in Health Psychology* (pp. 235-256): Sage Press.

Prather, A. A., Marsland, A. L., Hall, M., Neumann, S. A., Muldoon, M. F., & Manuck, S. B. (2009). Normative variation in self-reported sleep quality and sleep debt is associated with stimulated pro-inflammatory cytokine production. *Biol Psychol, 82*(1), 12-17.

Pressman, S. D., Miller, G. E., & Cohen, S. (2005). *Sleep and immunization response*. Paper presented at the American Psychosomatic Society.

Prinz, P. N. (1995). Sleep and sleep disorders in older adults. *J Clin Neurophysiol, 12*(2), 139-146.

Rabin, B. (1999). *Stress, Immune Function, and Health: the connection*. New York: Wiley-Liss.

Rechtschaffen, A., & Bergmann, B. M. (2002). Sleep deprivation in the rat: an update of the 1989 paper. *Sleep, 25*(1), 18-24.

Rechtschaffen, A., Bergmann, B. M., Everson, C. A., Kushida, C. A., & Gilliland, M. A. (1989). Sleep deprivation in the rat: X. Integration and discussion of the findings. *Sleep, 12*(1), 68-87.

Redline, S., Kirchner, H. L., Quan, S. F., Gottlieb, D. J., Kapur, V., & Newman, A. (2004). The effects of age, sex, ethnicity, and sleep-disordered breathing on sleep architecture. *Arch Intern Med, 164*(4), 406-418.

Renegar, K. B., Crouse, D., Floyd, R. A., & Krueger, J. (2000). Progression of influenza viral infection through the murine respiratory tract: the protective role of sleep deprivation. *Sleep, 23*(7), 859-863.

Renegar, K. B., Floyd, R. A., & Krueger, J. M. (1998). Effect of sleep deprivation on serum influenza-specific IgG. *Sleep, 21*(1), 19-24.

Renegar, K. B., Floyd, R. A., & Krueger, J. M. (1998). Effects of short-term sleep deprivation on murine immunity to influenza virus in young adult and senescent mice. *Sleep, 21*(3), 241-248.

Resta, O., Foschino Barbaro, M. P., Bonfitto, P., Giliberti, T., Depalo, A., Pannacciulli, N., et al. (2003). Low sleep quality and daytime sleepiness in obese patients without obstructive sleep apnoea syndrome. *J Intern Med, 253*(5), 536-543.

Roenneberg, T., & Merrow, M. (2007). Entrainment of the human circadian clock. *Cold Spring Harb Symp Quant Biol, 72*, 293-299.

Rogers, A. E., Caruso, C. C., & Aldrich, M. S. (1993). Reliability of sleep diaries for assessment of sleep/wake patterns. *Nurs Res, 42*(6), 368-372.

Roome, A. J., Walsh, S. J., Cartter, M. L., & Hadler, J. L. (1993). Hepatitis B vaccine responsiveness in Connecticut public safety personnel. *JAMA, 270*(24), 2931-2934.

Sadeh, A., Hauri, P. J., Kripke, D. F., & Lavie, P. (1995). The role of actigraphy in the evaluation of sleep disorders. *Sleep, 18*(4), 288-302.

Sadeh, A., Keinan, G., & Daon, K. (2004). Effects of stress on sleep: the moderating role of coping style. *Health Psychol, 23*(5), 542-545.

Sadeh, A., Sharkey, K. M., & Carskadon, M. A. (1994). Activity-based sleep-wake identification: an empirical test of methodological issues. *Sleep, 17*(3), 201-207.

Schafer, J. L., & Olsen, M. K. (1998). Multiple imputation for multivariate missing-data problems:a data analyst's perspective. *Multivariate behavioral research, 33*, 545-571.

Scheen, A. J., & Van Cauter, E. (1998). The roles of time of day and sleep quality in modulating glucose regulation: clinical implications. *Horm Res, 49*(3-4), 191-201.

Segerstrom, S. C., & Miller, G. E. (2004). Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychol Bull, 130*(4), 601-630.

Seneviratne, U., & Puvanendran, K. (2004). Excessive daytime sleepiness in obstructive sleep apnea: prevalence, severity, and predictors. *Sleep Med, 5*(4), 339-343.

Shamsuzzaman, A. S., Gersh, B. J., & Somers, V. K. (2003). Obstructive sleep apnea: implications for cardiac and vascular disease. *JAMA, 290*(14), 1906-1914.

Smith, M. T., Perlis, M. L., Park, A., Smith, M. S., Pennington, J., Giles, D. E., et al. (2002). Comparative meta-analysis of pharmacotherapy and behavior therapy for persistent insomnia. *Am J Psychiatry, 159*(1), 5-11.

Spiegel, K., Leproult, R., & Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. *Lancet, 354*(9188), 1435-1439.

Spiegel, K., Sheridan, J. F., & Van Cauter, E. (2002). Effect of sleep deprivation on response to immunization. *JAMA, 288*(12), 1471-1472.

Spielman, A. J., Caruso, L. S., & Glovinsky, P. B. (1987). A behavioral perspective on insomnia treatment. *Psychiatr Clin North Am, 10*(4), 541-553.

Stamatakis, K. A., & Punjabi, N. M. (*in press*). Effects of sleep fragmentation on glucose metabolism in normal subjects. *Chest, 137*(1), 95-101.

Stone, A. A., Schwartz, J. E., Neale, J. M., Shiffman, S., Marco, C. A., Hickcox, M., et al. (1998). A comparison of coping assessed by ecological momentary assessment and retrospective recall. *J Pers Soc Psychol, 74*(6), 1670-1680.

Tiemeier, H., Pelzer, E., Jonck, L., Moller, H. J., & Rao, M. L. (2002). Plasma catecholamines and selective slow wave sleep deprivation. *Neuropsychobiology, 45*(2), 81-86.

Unruh, M. L., Redline, S., An, M. W., Buysse, D. J., Nieto, F. J., Yeh, J. L., et al. (2008). Subjective and objective sleep quality and aging in the sleep heart health study. *J Am Geriatr Soc, 56*(7), 1218-1227.

Usui, A., Ishizuka, Y., Obinata, I., Okado, T., Fukuzawa, H., & Kanba, S. (1998). Validity of sleep log compared with actigraphic sleep-wake state. *Psychiatry Clin Neurosci, 52*(2), 161-163.

Usui, A., Ishizuka, Y., Obinata, I., Okado, T., Fukuzawa, H., & Kanba, S. (1999). Validity of sleep log compared with actigraphic sleep-wake state II. *Psychiatry Clin Neurosci, 53*(2), 183-184.

Uthgenannt, D., Schoolmann, D., Pietrowsky, R., Fehm, H. L., & Born, J. (1995). Effects of sleep on the production of cytokines in humans. *Psychosom Med, 57*(2), 97-104.

Van Cauter, E., Holmback, U., Knutson, K., Leproult, R., Miller, A., Nedeltcheva, A., et al. (2007). Impact of sleep and sleep loss on neuroendocrine and metabolic function. *Horm Res, 67 Suppl 1*, 2-9.

van Hilten, J. J., Braat, E. A., van der Velde, E. A., Middelkoop, H. A., Kerkhof, G. A., & Kamphuisen, H. A. (1993). Ambulatory activity monitoring during sleep: an evaluation of internight and intrasubject variability in healthy persons aged 50-98 years. *Sleep, 16*(2), 146-150.

Vedhara, K., Cox, N. K., Wilcock, G. K., Perks, P., Hunt, M., Anderson, S., et al. (1999). Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination. *Lancet, 353*(9153), 627-631.

Vedhara, K., Fox, J. D., & Wang, E. C. (1999). The measurement of stress-related immune dysfunction in psychoneuroimmunology. *Neurosci Biobehav Rev, 23*(5), 699-715.

Vgontzas, A. N., Papanicolaou, D. A., Bixler, E. O., Lotsikas, A., Zachman, K., Kales, A., et al. (1999). Circadian interleukin-6 secretion and quantity and depth of sleep. *J Clin Endocrinol Metab, 84*(8), 2603-2607.

Vgontzas, A. N., Zoumakis, E., Bixler, E. O., Lin, H. M., Follett, H., Kales, A., et al. (2004). Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. *J Clin Endocrinol Metab, 89*(5), 2119-2126.

Vink, J. M., Groot, A. S., Kerkhof, G. A., & Boomsma, D. I. (2001). Genetic analysis of morningness and eveningness. *Chronobiol Int, 18*(5), 809-822.

Viola, A. U., Archer, S. N., James, L. M., Groeger, J. A., Lo, J. C., Skene, D. J., et al. (2007). PER3 polymorphism predicts sleep structure and waking performance. *Curr Biol, 17*(7), 613-618.

Walker, M. P., & Stickgold, R. (2004). Sleep-dependent learning and memory consolidation. *Neuron, 44*(1), 121-133.

Watson, N. F., Buchwald, D., Vitiello, M. V., Noonan, C., & Goldberg, J. (*in press*). A twin study of sleep duration and body mass index. *J Clin Sleep Med, 6*(1), 11-17.

Weinberger, B., Herndler-Brandstetter, D., Schwanninger, A., Weiskopf, D., & Grubeck-Loebenstein, B. (2008). Biology of immune responses to vaccines in elderly persons. *Clin Infect Dis, 46*(7), 1078-1084.

Wilson, K. G., Watson, S. T., & Currie, S. R. (1998). Daily diary and ambulatory activity monitoring of sleep in patients with insomnia associated with chronic musculoskeletal pain. *Pain, 75*(1), 75-84.

Wohlgemuth, W. K., Edinger, J. D., Fins, A. I., & Sullivan, R. J., Jr. (1999). How many nights are enough? The short-term stability of sleep parameters in elderly insomniacs and normal sleepers. *Psychophysiology, 36*(2), 233-244.

Wood, R. C., MacDonald, K. L., White, K. E., Hedberg, C. W., Hanson, M., & Osterholm, M. T. (1993). Risk factors for lack of detectable antibody following hepatitis B vaccination of Minnesota health care workers. *JAMA, 270*(24), 2935-2939.

Yoo, S. S., Gujar, N., Hu, P., Jolesz, F. A., & Walker, M. P. (2007). The human emotional brain without sleep--a prefrontal amygdala disconnect. *Curr Biol, 17*(20), R877-878.

Zeeshan, M., Jabeen, K., Ali, A. N., Ali, A. W., Farooqui, S. Z., Mehraj, V., et al. (2007). Evaluation of immune response to Hepatitis B vaccine in health care workers at a tertiary care hospital in Pakistan: an observational prospective study. *BMC Infect Dis, 7*, 120.

Zinkernagel, R. M., Bachmann, M. F., Kundig, T. M., Oehen, S., Pirchet, H., & Hengartner, H. (1996). On immunological memory. *Annu Rev Immunol, 14*, 333-367.

Zisapel, N. (2007). Sleep and sleep disturbances: biological basis and clinical implications. *Cell Mol Life Sci, 64*(10), 1174-1186.

Zuckerman, J. N. (2006). Protective efficacy, immunotherapeutic potential, and safety of hepatitis B vaccines. *J Med Virol, 78*(2), 169-177.