Sleep Deprivation: A Mixed Methods Analysis of Cardiovascular Risk, Sleep Perceptions, and Sleep Behaviors among College Students

By India Benson

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Mark Sorensen, Thesis Advisor
Paul Leslie, Reader
Amanda Thompson, Reader

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ABSTRACT

Previous research has shown a correlation between increased levels of inflammatory markers and cardiovascular risk (Danesh et al. 2004). Inflammatory markers are also influenced by an individual's quality of sleep (Hoevenaar-Blom et al. 2011). These associations are highly important for college students who often experience partial ($\leq$ 6 hours of sleep each night) or acute (pulling 'all-nighters') sleep deprivation. The goals of this research are to 1) investigate how sleep practices affect C-reactive protein (CRP), 2) understand students' perception of sleep, and 3) examine behaviors preventing optimal sleep. CRP was measured using two dried blood spots collected one week apart, and sleep was assessed over seven days via actigraphy, questionnaire (Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, and Nap Questionnaire), and ethnographic interviews from 27 undergraduate college students at UNC Chapel Hill. The data indicate that: 1) sleep deprivation results in increased CRP levels, and thus, increases individuals' cardiovascular risk, 2) the perception of sleep is contingent on other school related factors, and 3) homework and studying are the main reported obstacles to sleep. While current research focuses on the sleep behaviors of older individuals, this thesis adds to the current literature by providing a focused analysis on college students ages 17-22 and the impact that short habitual sleep duration may have on their short- and long-term cardiovascular health.

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INTRODUCTION

"If you're not sleep deprived, are you really a college student?"
-UNC Student, Class of 2022

Coronary heart disease is the leading cause of death among adults in the United States as evinced in 2013 where one in every nine deaths were attributed to heart attacks. As the number one cause of death, finding ways to combat heart disease is of high importance to the population as a whole. Since these diseases result from the buildup of plague in the blood vessels over time, the question becomes that of timing – during what age range do behaviors begin to have an impact on future health outcomes later in life? One such behavior is sleep which has been consistently linked to cardiovascular risk. While current research focuses on the sleep behaviors of older individuals who are 50 and older or individuals from their mid-twenties to their mid-thirties, there is little research on the connection between sleep and CVDs for college students ages 17-22. Due to their sleep patterns, research on this cohort would provide beneficial insights on the long term effects of college sleep culture (whether habitual sleep loss, pulling 'all-nighters, or both) on future cardiovascular risk. This thesis, therefore, investigates how sleep affects inflammation markers in the body of undergraduate college students. It will also take an anthropological look into the students' relationship to sleep and how that influences their behaviors and their views of themselves and peers.

Overview of Thesis

The purpose of this research is to examine how sleep quantity and quality affect inflammation levels in the body among 27 undergraduate college students at The University of North Carolina at Chapel Hill. It serves to add to the current sleep literature by providing mixed method research on college aged students ages 17-22, their view and subsequent behavior regarding sleep, and the impact that habitual sleep loss has on their short- and long-term

cardiovascular health. I use the term 'habitual sleep loss' to refer to the short number of sleep hours the participants engaged in outside of a formal laboratory environment. I did use the term 'sleep deprivation' in conversation with students and during interviews due to its everyday use to mean: the restriction of sleep hours on behalf of the sleeper; however, the students were not deprived of sleep in a laboratory setting which would formally label this research as a sleep deprivation study. These associations between sleep behaviors and cardiovascular risk are of high importance for young adults, particularly college-aged students, who often practice acute sleep deprivation ('pulling all-nighters') during their college years. The lasting effects of habitual sleep loss can lead to alterations in CRP which in turn can change the immune system, allowing for increased risk of infection and an increase in risk of CVDs (Van Leeuwen et al. 2009).

This thesis focuses on the relationship between sleep and CRP. It does not seek to verify the 24 hour effect - normally accomplished by 30 minute sampling - observed in prior studies (Vgontzas et al. 2004; Frey, Fleshner, and Wright Jr. 2007) nor does it seek to provide longitudinal data which follows these participants post-college in order to gauge how college sleep habits affect overall cardiovascular risk later in life. Instead, it examines the effects of habitual sleep loss on baseline CRP levels.

Research Questions

My research project began in January of 2018 when I joined one of the inaugural Undergraduate Research Consultant Teams (URCTs) composed of four other undergraduate students (Darien Campisi, Carissa Cueva, Mallory Happ, and Sydney Puerto-Meredith), two graduate students (Jacob Griffin and Gioia Skeltis), and the primary investigator (Dr. Mark Sorensen, Associate Professor in the Department of Anthropology).[1] The research project

[1] In the course of this paper, the larger URCT research project will be referred to as SIPA.

examined the combined effects of psychosocial stress, sleep, and physical activity on inflammation in the body of UNC college students. From this overarching project, I focused on the relationship between sleep and inflammation. I decided to develop my preliminary research into an Honors Thesis and thus extended my research to the fall of 2018 to include blood assay (CRP), sleep surveys (Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, Nap Questionnaire), sleep measurements (actigraphy and Polar Electro HR monitor), and interview data. I was interested in investigating the sleep patterns of students, particularly habitual sleep loss. Hence, I wanted to explore three primary questions: 1.) What was the correlation between CRP and habitual sleep loss; 2.) How was sleep viewed by college students; and, 3.) What behaviors were preventing students from obtaining better sleep hours?

In the following literature review, I provide a summary of the current research on sleep and cardiovascular disease. In order to understand the biocultural approach of my research, an understanding of the biological relationship between sleep and CVDs is necessary. Therefore, this section serves as the foundation for examining one aspect of sleep before an anthropological perspective is considered in Chapter 1.

Literature Review

Sleep and Cardiovascular Disease

Previous research has consistently linked sleep with cardiovascular disease risk (Cappuccio and Miller 2017; Grandner et al. 2013; Hoevenaar-Blom et al. 2011; Van Leeuwen et al. 2009). It has also been linked to chronic diseases such as type 2 diabetes and obesity (Cappuccio et al. 2010; Knutson et al. 2007; Spiegel, Leproult, and Van Cauter 1999; Spiegel et al. 2009). The inflammatory marker C – reactive protein (CRP) has been shown to have an association with sleep (Meier-Ewert et al. 2001) and have a positive correlation with CVDs

(Danesh et al. 2004; Emerging Risk Factors Collaboration 2010). However, there are some studies which find no association between CRP and sleep duration (Leng et al. 2014; Taheri et al. 2007).

Cappuccio and Miller (2017) discovered a consistent and significant association between sleep durations of less than five hours a night and the risk of hypertension. There was also an increased chance of developing hypertension in short sleepers compared to long sleepers. This is significant since hypertension is the number one risk factor for CVDs. Normally, the body's physiological response is for blood pressure (BP) to lower during the night and increase during the day (follows a diurnal pattern). This makes an elevation in BP during the day and night time an increase in one's risk for hypertension. Meta-analyses have provided data that even when there is variability between studies due to differing populations or methods of assessment, individuals with less than six hours of sleep were more likely to experience and die from coronary heart disease (CHDs) compared to individuals with an average of six to eight hours of sleep.

Meier-Ewert et al. (2004) found that both total sleep deprivation (24+ hours) and partial sleep deprivation (< 6.5 hours) results in elevated high-sensitivity concentrations. A meta-analyses of 54 long-term studies found a linear association of CRP levels with several conventional risk factors – blood pressure, BMI, triglycerides, cholesterol, smoking history, alcohol consumption, diabetes history – and inflammatory markers (Emerging Risk Factors 2010). In response to changes to Interleukin 6 (another pro-inflammatory marker) in the body, CRP is synthesized and secreted by the liver. However, while levels of Interleukin 6 (IL-6) fluctuate based on the sleep-wake cycle, CRP lacks a circadian variation. This lack of variation, therefore, makes it a better predictor of cardiovascular risk than IL-6 because there are no times when the body naturally has fluctuating higher or lower levels of CRP in the blood. The stable

nature of CRP also means that blood samples can be taken any time of the day without researchers needing to account for a particular rise or fall in CRP concentration (Meier-Ewert et al. 2001).

These increased inflammatory levels are influenced by an individual's quantity and quality of sleep. Hoevenaar Blom (2011) found that sleep duration and quality related to cardiovascular disease incidence among sleepers who had 6 hours or less of sleep had a higher risk of CVD compared to normal sleepers (classified as 7 hours of sleep). In addition, participants who had poor sleep quality had a higher risk of CVD compared to individuals with good sleep quality. Grandner (2013) analyzed various sleep studies that have linked sleep duration, cardiovascular disease, and inflammatory markers. However, more studies are needed examining habitual sleep duration, the inflammatory markers roles in cardiovascular risk, and their biological pathways.

Naps

While overnight sleep influences bodily functions, daytime naps also affect the body. Currently, there is a debate over whether they are harmful or beneficial in the long term. There are associations between naps and chronic diseases (Guo et al. 2017; Leng et al. 2016 {type 2 diabetes}; Vgontzas et al. 1997) and naps and CVDs (Leng et al. 2014 {CRP}; Mantua and Spencer 2015; Yamada et al. 2015) which show the negative effects of frequent (daily) naps. Conversely, there is research highlighting naps' positive effects on the immune cells (Faraut et al. 2011). There is also research which outlines the nuances that the length of naps, time of day, and age play on how naps work in the body (Milner and Cote 2009).

Naps are a common practice engaged by college students and thus deserve to be further studied in the context of this sleep study. When students nap, how frequent, and the length of

time are a few of the factors in which I assess in my paper in relation to sleep patterns. Thus, naps' connection with sleep will serve as a brief secondary exploration in my paper.

Chapter Layout

In Chapter 1, I explore how sleep is experienced by adults and how the effect of that view impacts sleeping patterns. I do so by focusing on anthropologist Matthew Wolf-Meyer's works regarding sleep's role in American society. In Chapter 2, I present my mixed methods research composed of dried blood spots, sleep and nap questionnaires, sleep monitoring data, and interviews. In Chapter 3, I examine the data which suggests a connection between habitual sleep loss and increased CRP levels. Finally, in Chapter 4, I conclude with an emphasis on the future implications of this sleep behavior.

CHAPTER #1: THE ANTHROPOLOGY OF SLEEP

Introduction

Sleep and the college student are intimately connected. Whether sleep is set as a priority or seen as an agent that can be neglected for a brief time, sleep cannot be completely forgone. It *will* eventually occur. What makes this relationship so intriguing, then, is how it can be manipulated, shifted, or completely altered over the course of the college years. While students may make a decision early on, whether consciously or unconsciously, to conform to certain sleep behaviors, how students may view sleep in their first year can drastically change come graduation. Thus, how sleep is experienced by each student can encapsulate a plethora of responses. Nevertheless, an exploration into the college sleep culture can be explored through a broader analysis of American sleep. Students' perceptions of sleep have not formed in isolation. Instead, societal norms and values serve as overt and covert influencers.

This chapter takes a journey through the origin of sleep to its current place in American society. While nighttime sleep is understood as an ideal 7-8 hour episode, this view does not extend worldwide. The discontinuity of sleep is not regarded as a negative act in all societies. Differences in sleep environments around the world show a range of sleep behaviors that involve differing needs based on that culture (Galinier et al. 2010, 822). Therefore, I begin this chapter by delving into the liminal nature of sleep itself before taking a historical look at sleep's cultural significance in America. I then situate sleep in 21st century society in order to detail how capitalism and medicalization have shaped its public perception. Finally, I conclude with the publically recognized concern of sleep deprivation among college students. Anthropologist Matthew Wolf-Meyer serves as my anthropological lens in which I present my material given the extensive nature of his work regarding American sleep culture.

The Sleep Experience

In what realm does sleep reside if consciousness is partially lost during sleep and its liminal state hovers between the real world and the world of dreams? According to Freud (1973), these two worlds are not isolated from one another, for activities in the real world (e.g. noise) can enter dreams, taking on new meaning (Part II). Sleep, thus, becomes an embodied state given to either a waking or non-waking mode of being. This means that there is always a transitory experience felt by an individual. For instance, a sensual experience like wakefulness can spur one into the conscious world but another like drowsiness can pull one into the world of dreams. There is an organic component to sleep given its involuntary and inevitable nature. Even with its natural component, there still remains at times a struggle as the body slips into sleep before desired (tiredness) or not (insomnia). As much as agency can be found in the wakeful state, one's sense of agency is complicated during sleep when acts like sleepwalking and sleep-talking place individuals in a position where bodily actions are lost. Furthermore, sometimes subtle transitions can occur, as in lucid dreams or in normal dreams themselves, having a realness to them where it feels like an awake state (Williams and Crossley 2008, 2-3).

While voluntary movement is absent during sleep, involuntary acts such as shifting body positions with the change in sleep cycles occurs. There are also eye, facial, and finger movements, all adding to the embodied nature of the sleep experience (during the REM phase). Three main factors lead to or influence the sleep state from a biological perspective – temperature, noise, and light. First is the core body temperature which decreases upon sleep onset and increases upon waking. During the sleep episode, the slow wave brain activity is affected according to the awakening time. This length results in what is known as the S process. The characteristics of sleep are thus formed through the interaction of the circadian rhythm of temperature and the S process time course. Second is noise which serves to either interrupt or

lighten the sleep experience. To what extent sleep is disturbed depends on the time in the sleep cycle the disruption happens. However, according to Libert et al. (1991), in a comparison between temperature and noise, high temperature has a greater impact on sleep than noise. Last is light. Biological rhythms place sleep on a light-dark cycle and environmental alterations to light influences sleep patterns. Middleton, Arendt, and Stone (1996) found that participants formed an extended 24 sleep-wake rhythm under a constant exposure of dim lighting for 21 days (Galinier et al. 2010, 824).

A Brief History of American Sleep

Early ideas about sleep in the 17th century were impacted by the moral and cultural values regarding Christianity and labor. Sleep became linked to the carnal desires of the human flesh which were to be suppressed and avoided at all cost. Carnal desires led to sin, and sin was displeasing to God. Therefore, according to Puritan Minister Cotton Mather, to indulge in sleep was to give in to the temptations of the devil which occurred every day, for there was always a daily enticement to remain in bed longer than one should. As a result, it would prevent an individual from fulfilling their earthly duty by being productive. Benjamin Franklin picked up on this connection between sleep and inefficiency through an economic mindset. If individuals wanted to simultaneously be productive members of society and reduce money spent on candles, they would go to bed when the sun's light disappeared and then rise with the natural morning light (Wolf-Meyer 2015, 54-55).

The presumptions of sleep formed by Mather and Franklin influenced a book titled *Sleep* by William Whitty Hall. He argued for a regular sleep cycle by connecting it to nature – which was seen as efficient – and habitual practice. This meant that the sleep cycle had to be willful practice done by an individual. He viewed sleep as flexible and able to be manipulated; thus, it could be consolidated within a specific time frame where the body would naturally become

sleepy and then awake after receiving a renewing amount of rest. Hall termed these inevitable times as "appointments." This meant that for Hall, naps (or as he termed them "second naps") were problematic because people would override nature's call for sleep, disrupting the natural appointments of the body. In order to avoid such acts an individual should not sleep during the day but instead go to bed early, no later than ten, and rise upon initial awakening. By doing so, one would avoid going back to sleep. The only acceptable nap time frame would be for 10 minutes which would provide an energizer for the person. The responsibility, thus, was placed on the individual to maintain and not deviate from the regular sleep schedule (Wolf-Meyer 2015, 55-58).

By the 19th century, sleep and nature had become intertwined. In scientific thought, sleep was a natural act by humans that served to replenish the body. In William Hammond's *Sleep and Its Derangements*, while sleep was natural, there was a conflict between nature, which made one sleepy, and one's individual will. In the case of insomnia, he viewed it as a state of being where the struggle to go to sleep was the brain rebelling against nature's inevitable call. He also believed the flexibility of sleep arose from the variation between individuals' needs. One's mode of life and habits influenced sleep requirements which in turn impacted brain size. Consequently, sleep and human intelligence were associated with individuals with bigger heads having larger and more well-developed brains than those with smaller heads. There was, then, a greater need for internal repair (more sleep) since more cerebral action had been expensed (Wolf-Meyer 2015, 58-61).

Sleep began to be viewed as a necessary act, which, when engaged with properly, could result in more effective labor practices. Donald Laird had a hand in promoting a positive view of sleep and how good sleep behaviors were beneficial for the body. In his writings, he recommended relaxing techniques to help people fall asleep. Poor sleep then occurred when an

individual did not receive the average amount of sleep for one's age or when an individual had difficulties waking upon receiving the average sleep hours. His arguments for good sleep habits was a given a firm foundation given how, by the 20th century, a model of human sleep had been adequately established in the scientific world. However, sleep and its assumed connection with intelligence remained although shifted somewhat in how it was perceived. While before a person with a well-developed brain required more rest time, during Laird's era individuals of high intelligence were presumed to have a harder time falling asleep because they had a lot to worry about – hence, a predisposition toward insomnia. "Feeble-minded folk," on the other hand, had empty brains which made it easier for them to fall asleep (Wolf-Meyer 2015, 62-64).

The scientific nature of sleep was further ignited in the American public by Professor Nathaniel Kleitman who, in 1938, with a graduate student – Bruce Richardson – stayed in a cave for thirty-two days in order to investigate the changes in their circadian rhythms. This would be verified through changes in their body temperature. Kleitman chose the cave intentionally, wanting to remove external environmental factors that impacted sleep, namely daylight and noise. This experiment gained national attention as Kleitman and Richardson set out a schedule comprised of twenty-eight hour days and six-day weeks. In the end, Richardson was able to acclimate to the time change but Kleitman was not. Interestingly, he did not allow himself to take naps during the 'day' hours he set for himself but instead pushed himself to consolidate his sleep in one episode each 'night.' Through this research, a re-emphasis of individual habits occurred as the results clarified in the professor's mind the flexibility but ultimately inevitability of sleep. While variations between individuals could occur, it should only be done within the confines of the accepted societal norms and practices. For Kleitman, this meant that individual sleep variations needed to follow the pattern of consolidated nightly sleep (evinced through his refusal to nap in the cave). His experiment and future investigations worked to formulate modern ideas

regarding sleep and its regularity. It also continued the idea of sleep's inseparable connection to habits (Wolf-Meyer 2015, 65-66).

Current Sleep Culture

American sleep follows a monophasic sleep pattern where it is consolidated in a single nightly episode. Entangled with this sleeping pattern is the idea of privacy. Individuals learn this private behavior from childhood as they become accustomed to sleeping alone in their own room. It is a solitary act which, for children, takes away the comfort of familial closeness felt during the day. In order to help children get use to this behavior, they may be given a comfort item (e.g. stuffed item, blanket) to aid them during the nightly separation period. When children eventually grown into adults, they have become accustomed to this ritualized behavior. Coupled with the idea of privacy is that of protection – there is a need for security during this unconscious state of mind. The bedroom, more specifically, and the home, more broadly, then serve as protection during the vulnerable time of sleep. This makes acts like public sleeping uncommon, for if sleeping is conceptualized as a private act done in the protection of an enclosed space, sleeping in the open brings about potential danger. This, then, gives insight into why many individuals who regularly engage in public sleeping (e.g. the homeless) suffer from chronic sleep deprivation (Steger and Brunt 2003, 12).

Capitalism

Near the end of the 20th century, some predicted that capitalism would stretch into the night. U.S. businesses would remain open later and thus change the daily sleep patterns of Americans. This transition did not occur, however. Instead, many industry jobs went overseas. Still, the drive to make a profit in a capitalistic world made sleep a hurdle which needed to be overcome. As a result, some individuals were required to work hours normally set aside for sleep (e.g. third shift workers). This placed the onus on them to suppress their urge for sleep in order to

complete their required tasks. Workplace fatigue, then, became a problem even for employees working during the day hours. Institutions, consequently, became involved in the sleep sphere where the private, individualize behavior became incorporated in the corporate world. A sufficient amount of sleep became seen not only as a healthy act for an individual but also as a goal towards healthier working bodies. This was not contained to just the corporate world, but became a public issue as well. A solution to dealing with the sleepy working masses was, for some, to see the potential benefits of naps. A company named Metronaps was established in the early 2000s to provide an off-site place for workers to take naps in a relaxing and anonymous setting. It was designed to remove the shame associated with naps and to separate them from the perception of inefficiency (Wolf-Meyer 2015, 181, 186, 190).

Studies have shown the benefits of napping in relation to job performance during working hours (Takahashi 2003; Takeyama, Tomohide, and Toru 2005; Milner and Cote 2009; Lovato and Lack 2010). There are also current conversations surrounding naps' place in the office and which working populations (e.g. night-shift versus both day- and night-shifts) should be able to take them (Takahashi 2012). Science and economics have entered the sleep realm in various ways. While the science of sleep remains a consistent foundation to debate the benefits of naps, economics have been used for and against that argument. On the one hand, naps are seen as energizers for workers who are otherwise hitting a wall of tiredness during business hours. Therefore, if naps are allowed, the extra boost of energy would make workers more productive and benefit the entire company. This would reduce occupational hazards which could arise due to sleepiness. On the other hand, the initial wake period of a nap has been shown to result in a decrease in alertness and performance (Fallis, McMillan, and Edwards 2011), which could be seen as an economically risky move (Takahashi 2012). Even with studies highlighting the benefits of the 15-20 minute sleep period (Takahashi 2003; Waterhouse et al. 2007; Lahl et al.

2008), creating naps as an American capitalistic norm for the workplace has not fully taken hold although more conversations are occurring.

Medicalization

Sleep has interacted with many facets of American life from religion to the economy; however, perhaps the most intimate realm it has become attached to is the medical one. Once it entered the scientific lens those many centuries ago, it did not depart. Science developed it, and since then sleep has been dominated by its biological, habitual, and naturalistic nature. Yet, even the world of science is not immune to the cultural histories, values, and perceptions of its society. As Anthropologist Matthew Wolf-Meyer notes, "their [Kleitman and Dement's] scientific conceptions of sleep are indebted to long-standing cultural traditions of sleep's interpretations" (Wolf-Meyer 2015, 76). Controlling sleep is in the roots of American cultural norms and it is still present today. Just like in the 17th century where ideas of sleep behavior governed what was seen as normal or abnormal, with moral implications greatly imposing on the latter, today's society, too, seeks to place limitations on sleep by defining normalcy (e.g. normal vs. disordered sleep practices). Thus, in an increasing medicalized world where pharmaceuticals have taken hold in practically all aspects of daily life, from the mundane (e.g. use for a headache) to the extreme (e.g. use after a surgical procedure), it is no surprise that sleep has become engulfed as well. The various sleep medications allow an individual to choose from a range of action - from trying to go to sleep to trying to stay awake. Individuals are in constant battle with bending sleep to their will and regulating what has been seen as an inevitable, involuntary, and natural act. Whereas in the past this management was accomplished through the recommended regulation of sleep hours, now medication provides the answer.

The medicalization process arose when sleep became a sub-discipline of medicine in the late 20th century. The normal rhythms of American sleep were capitalized on by pharmaceutical

companies who rebranded some medications, originally designed for individuals with sleep disorders, for over-the-counter use such as "excessive daytime sleepiness." In many cases, the extent of medicalization is witnessed in disordered sleepers – they do not fit the conventional mold of sleep norms and thus are given medication to adjust their sleep schedule. Even for sleepers categorized as 'normal' have easy access to pharmaceuticals which allow them to stay awake and alert during acceptable times of the day. Capitalistic underpinnings resurface again to influence sleep's medicalization. Capitalism impacts sleep in the regard that it sets the standards for acceptable periods of wakefulness, pushing many to stimulants such as coffee to remain at their most attentive during working hours. It shapes perceptions as sleep goes from a naturally occurring event to one that can be controlled. The flexibility of sleep then becomes limited to public ideals. While the longing to sleep may be viewed and accepted as a natural, biological process, that desire is quenched through stimulants which are seen as acceptable if for the sake of productivity (Wolf-Meyer 2015, 67, 146, 154-158).

Sleep Deprivation and the College Student

While studies have shown the extent of poor sleep patterns among college students (Buboltz, Brown, and Soper 2010; Lund et al. 2010), the problem has not subsided. In a society where productivity is held supreme, it is not surprising that the future workers have adopted this same mentality. In a job market where a college degree is, in most cases, expected from employers, students are faced with the harsh reality of a world where competition will always exist and thus one must do everything in one's power to stand out among the sea of candidates. Therefore, while the college experience is pushed as a time of exploration and discovery, the looming light at the end of the tunnel constantly reminds students that graduation is always creeping forward. Even without post-graduate thoughts, the academic rigor of college demands hours upon hours outside of the classroom. There is, then, a race against time where priorities

must be established. For many college students, sleep takes last place and it is not long until sleep deprivation becomes an almost inescapable cycle.

Technology usage is not without its connections with poor college sleep habits either. However, it is important to realize that while technological devices such as phones and computers have impacted sleeping habits of American society beyond the university setting, they are not the first technological items to impact sleep. William Dement saw the invention of the light bulb as the beginning of a tragedy. He stated: "Our loss of sleep time and natural sleep rhythms is the tragic legacy of a single and profound technological advance— the light bulb…. Edison accomplished something Prometheus could not imagine, because he separated the light from the fire and offered it" (Wolf-Meyer 2011, 962). Moreover, the continuing technological uses of American life, like all night television, pushed back sleep for those individuals who chose to engage in it. As a result, there was a separation of sleep from nature. Individuals could now stay up well beyond the setting of the sun, overriding the natural "appointment" that sleep brings on humans. That, in turn, brings about sleep debt and for some, more severe sleep disorders (Wolf-Meyer 2011, 962). Changes in sleep quantity and/or quality are equally important, as argued in my thesis, thus it is important to note that White, Buboltz, and Igou (2011) found that phone usage among college students affected sleep quality more than sleep quantity. So while the phones were not affecting the number of hours students were sleeping each night, they did affect how well students slept.

How, then, must one approach this dilemma? What is the balance between beneficial, academic rigor which strengthens the mind and results in non-sleep deprived students? As I will show throughout the remainder of my thesis, students are aware of this problem. Their perception of their own sleep habits and those of their peers show that they are not oblivious to the actions in which they are engaging. While sleep studies may provide the tangible evidence of

the detrimental effects of their behaviors, in which the students are at least vaguely aware of good sleep hygiene, that does not take away from the real issue that time is needed to digest complex information, work on homework, and read x amount of pages for each class. If a homework assignment takes until 1:00am to complete, sleep will just be lost that night. So while sleep statistics and other data should be communicated to them, in the end, what they believe is more important (i.e. what takes priority) will win. The focus on priority also becomes relevant when extracurricular and social activities are added into a student's life. The attempt to 'have-it-all' (academically, socially, and personally – involving sleep) is thus, for many, not seen as an attainable goal.

CHAPTER #2: COLLEGE STUDENTS' RELATIONSHIP TO SLEEP

Materials and Methods

Subjects

Twenty-seven healthy undergraduate college students were recruited for this week long study which occurred on the UNC Chapel Hill campus. The participants reported to the Human Biology Laboratory located in the Anthropology Department building for their lab appointments and completed a consent form which outlined the requirements, protocols, and goals of the study. The participants' ages ranged from 18-21 and included individuals from each school year. The ages and years listed in Table 1 are at the time of the study.

While there were twenty-seven participants in this study, some individuals were excluded from certain aspects of data analyses due to cut off points of infection for CRP values and/or lack of data due to technological difficulties. A breakdown of these numbers are found in Table 2 and further explained in the following research method sections.

Table 1. Demographics[2]

Habitual Sleep Loss Participants (HSLP) (n=7)

Name	Age	Year
Participant 1	20	4th
Participant 2	18	1st
Participant 3	20	3rd
Participant 4	20	2nd
Participant 5	19	1st
Participant 6	19	2nd
Participant 7	18	1st

Control (n=20)

Age	Total	Year	Total
18	1	1st	3
19	6	2nd	8
20	7	3rd	2
21	4	4th	5

[2] The demographics for two of the control participants are not listed, so while the table is actually (n=18), the total number of control participants were (n=20).

Table 2. Included participants per research method

	Control	HSLP	Total
CRP Blood Assay	12	7	19
Sleep Surveys	0	7	7
Actigraphy Data	13	0	13
Interviews	0	7	7

Heart Rate data from the Polar Electro HR monitor was not discussed in this thesis

Protocol[3]

Control Group

There was a total of twenty participants recruited in the spring of 2018. They visited the laboratory twice during the course of the week – on day zero and day seven. On day zero, participants arrived at the lab, signed the consent form, and gave their first blood spot. They were then fitted with a heart rate monitor (Polar Electro) and actigraph which they wore for the entire week with minimal removal (e.g. during showers). On day seven, they returned their equipment and gave their final blood spot. The number of hours of sleep per night was not a limiting factor for this group; therefore, during recruitment, individuals with varying levels of nightly sleep hours were gathered. However, based on the actigraphy data, these participants all received an average nightly sleep greater than 6.5 hours. The full lab protocol for the control can be found in Appendix A.

[3] I have two rounds of participants – one from the SIPA study (spring 2018) and the other from my independent research (fall 2018).

Habitual Sleep Loss Participants (HSLP)

There was a total of seven participants recruited in the fall of 2018. Involvement in this round was confined to students who received an average nightly sleep of six hours or less. On day zero, participants arrived at the lab to sign the consent form, complete three sleep questionnaires, give their first blood spot, and receive their polar heart rate monitor and actigraph. The participants wore the sleep monitoring equipment for one week with minimal removal (e.g. during showers) before returning the equipment on day seven where they gave their final blood spot and completed a brief semi-structured interview.

Blood Assay

The blood samples were collected by finger prick using a sterile disposable microlancet onto Whatman No. 903 protein filter paper and allowed to dry overnight before being stored in the Human Biology Laboratory freezer at approximately -25^{o}C until analysis. The dried blood spot samples of 1/8" hole punch size were assayed using a high sensitivity ELISA kit to measure concentrations of C-reactive protein (Item No. DCRP00, R & D Systems, Minneapolis, MN). The microtiter plate contained duplicates of eight standards; three Quantikine controls of low, medium, and high; twenty-eight baseline samples, and nine follow-up samples. The 28th baseline and the 9th follow-up samples were excluded due to age, leaving twenty-seven baseline samples and eight follow-up samples.

CRP values were evaluated based on clinical guideline ranges. A CRP value less than 1.0mg/L was considered to indicate low risk of developing CVD; a range between 1.0mg/L and 3.0mg/L was indicative of average risk; and greater than 3.0mg/L was considered high risk (Salazar et al. 2014). Since CRP was gathered via dried blood spots, the cutoff point was

6.25mg/L or greater. Eight participants from the control were excluded from baseline sample analyses (CRP values ranging from 11.24 to 47.73mg/L). Therefore, twelve control and seven HSLP CRP baseline values were analyzed for a total of nineteen samples. The full lab protocol can be found in Appendix B.

The statistical system of SAS JMP Pro 14.0 for Windows was used to conduct statistical analyses. CRP values were log-transformed for comparison between the control and HSLP.

Surveys

The sleep questionnaires, completed by the HSLP (n = 7), included the Pittsburgh Sleep Quality Index, the Epworth Sleepiness Scale, and a nap questionnaire designed by honors thesis student India Benson. The Pittsburg Sleep Quality Index is designed to measure 7 sleep components (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction) which produce an overall global sleep quality score. Global scores range from 0 to 21 with a score of 5 or greater indicative of poor sleep. Due to the small sample size (n = 7), an analysis to test the reliability of the questionnaire via Cronbach's alpha was not included because the values would produce poor alpha qualities (Bonett 2002).

The Epworth Sleepiness Scale assesses individual sleepiness based on the chance of dozing off in eight different situations. The chance of dozing was on a scale of 0 to 3 with 0 = would never doze; 1 = slight chance of dozing; 2 = moderate chance of dozing; and, 3 = high chance of dozing. A scoring of 0-5 indicates lower normal daytime sleepiness; 6-10: a higher normal daytime sleepiness; 11-12: a mild excessive daytime sleepiness; 13-15: a moderate excessive daytime sleepiness; and, 16-24: a severe excessive daytime sleepiness. The Nap

Questionnaire was an informal, multiple choice survey. The nap questions were as followed: How often do you take naps? What time of the year are you most likely to take a nap? What time(s) during the week are you most likely to take a nap? What time(s) of the day do you take a nap? How long do you usually sleep during your naps? How often do you feel well rested after a nap? And finally, an open ended question asking: "Why do you take naps?" Morning, afternoon, evening, and night times were classified as 8am-12pm, 12pm-6pm, 6-8pm, and 8pm-12am, respectively.

Sleep Recordings

An actigraph and polar heart rate monitor were used to monitor sleep behavior and heart rate. Actigraphy data was only gathered for 13 control participants who completed 7 days of data collection. These participants were asked to continue their usual sleeping routine. Technological difficulties with the actigraphs arose for the HSLP. Only a small amount of data was collected (3 out of the 7 participants) which did not provide a large enough sample representation; as a result, this data was not included in sleep data analyses. Participants' daytime and nighttime heart rates were not examined in this thesis due to the extent of data gathered from the blood assay, survey, actigraph, and interview data.

The actigraphs produced clinical sleep reports for the participants which included a sleep period breakdown based on Cole-Kripke's Sleep Algorithm. The breakdown detailed time in bed, out of bed, latency (min), efficiency, total time in bed (min), Total Sleep Time (TST) (min), Wake After Sleep Onset (WASO), number of awakenings, and the average awakening (min). A distinction was made between nightly sleep (*Table 7*) and naps (*Table 8*) based on the subjective individual analysis of each sleep period regarding time of day, length, as well as prior and

subsequent sleep periods. Some sleep periods were grouped together - meaning they constituted one night of sleep - based on the "In Bed" and "Out Bed" columns, whether it was day or night, and the time between sleep periods (i.e. if a participant slept for 3 hours, woke up for 30 minutes, and then returned to bed to sleep for 5 hours). While there were a few exceptions, generally, periods were classified as 'naps' if they occurred during waking hours (morning, afternoon, evening) and 'nightly sleep' if they spanned from the night time of one day to the day time of the next.

Interviews

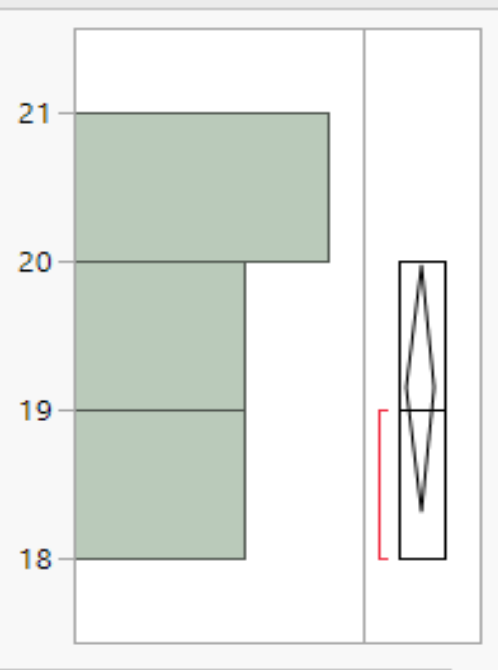
Semi-structured interviews were conducted among the HSLP (n = 7) regarding their perceptions of sleep and their actual sleep behavior. The interviews took place in the Human Biology Laboratory and ranged from 10-25 minutes. Generally, the interviews followed what would traditionally be classified as a structured interview; however, some participants were asked a few extra questions depending on their responses which kept the interview from being completely uniform across every interview. The interview questions were as followed:

- Do you consider yourself sleep deprived? Why?
- How important do you consider sleep (Slightly, Very, Extremely)? Why?
- Do you feel like you get enough sleep each night? How often do you feel well rested?
- In your opinion, what number of hours constitutes a good night's sleep? Where do you receive your information regarding the proper number of hours of sleep? When you achieve this number, do you feel well rested?
- What do you consider more important? Sleep quantity (number of hours) or sleep quality (how well you sleep)?
- What are some experiences that make up quality sleep?
- Do you feel like others around you are getting enough sleep each night? How does that make you feel?
- Do you believe it's possible to achieve good sleep hygiene as a college student?
- Do you see your sleep habits (i.e. number of hours of sleep per night) continuing post-college?

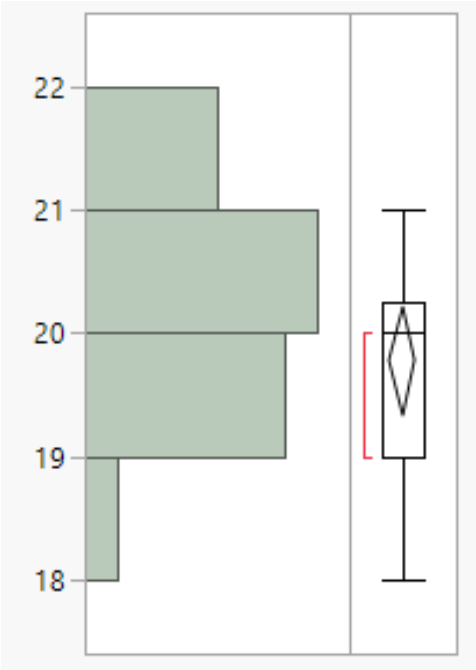
- Did you have similar sleep habits (i.e. number of hours of sleep per night) in high school? What has/has not changed from then till now?
- Do your sleeping patterns stay consistent throughout the year? If not, why? If so, why?
- What is your nightly routine? What sort of activities do you do before going to sleep?
- When are you most likely to engage in all-nighters? Why do you think they're necessary? Are they effective in accomplishing your goal?

Results

Figure 1. Distribution of age among the study participants

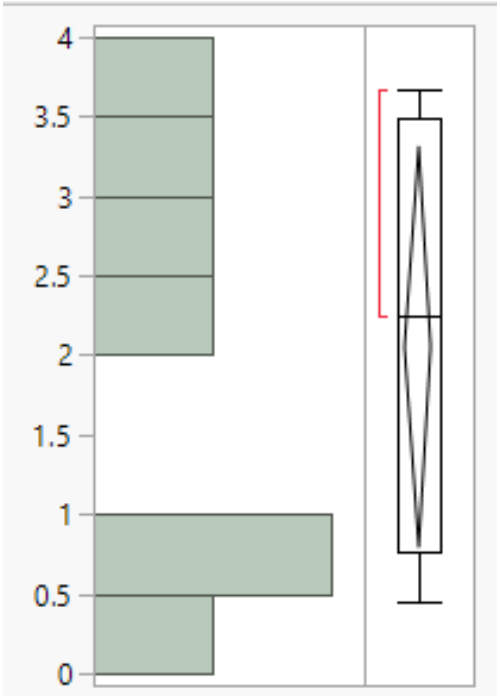


HSLP

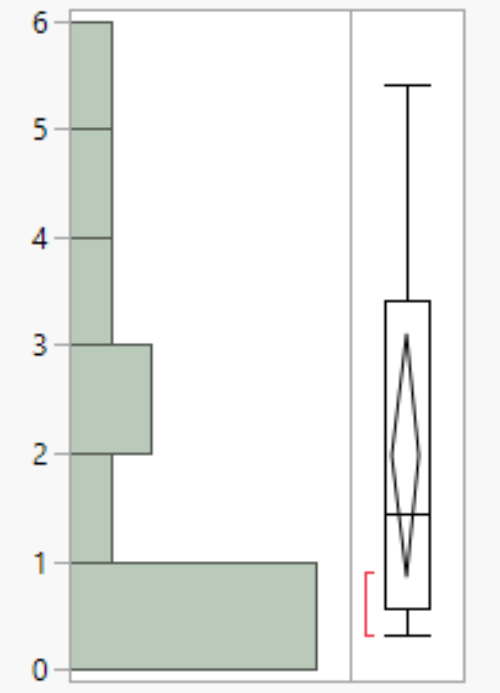


Control Sample

Figure 2. Distribution of raw CRP levels among the study participants



Baseline HSLP Samples



Baseline Control Samples

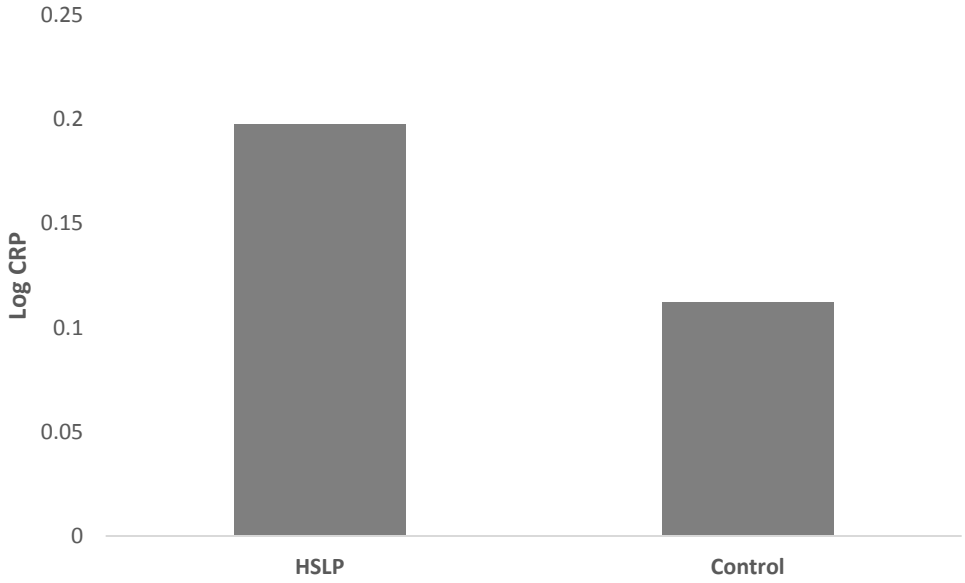
Note different scales between study groups

Table 3. Geometric Median, Standard Deviation, and Logarithmic Values of the raw CRP data

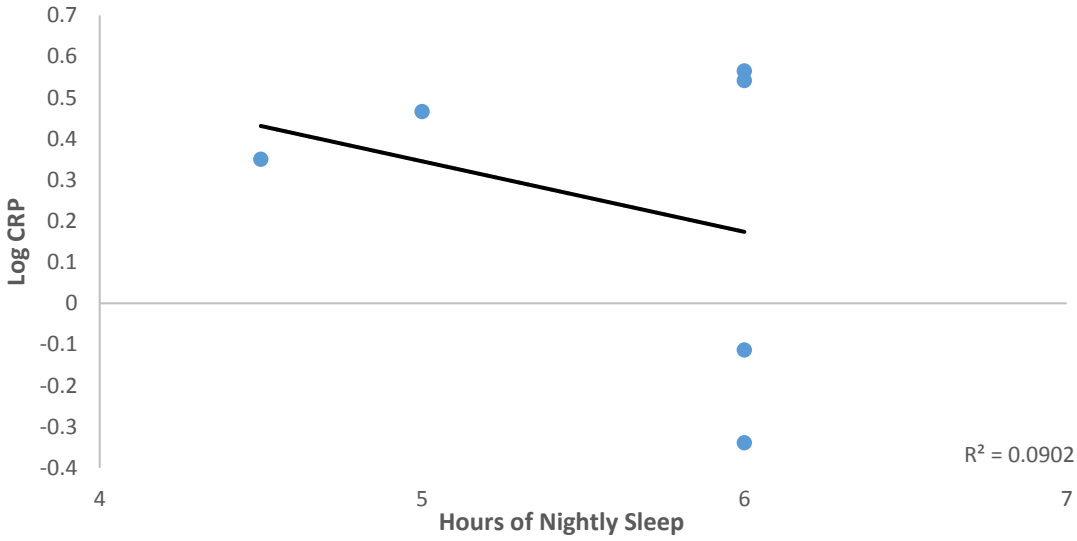
Study Sample	Geometric Mean	Standard Deviation	Log Value
Control (*n = 12*)	1.29	1.77	0.11
HSLP (*n = 7*)	1.58	1.37	0.20

Figure 3. Correlation between Baseline CRP and Habitual Sleep Loss

a. Mean Log Values of the HSLP and Control Groups



b. CRP verses HSLP Self-Reported Nightly Sleep*



Self-Reported Nightly Sleep taken from the PSQI survey

Table 4. Coefficient of Variation in CRP Samples

Single	Duplicate	Coefficient of Variation	
Control			
0.543	0.705		0.184
0.532	0.564		0.041
0.295	0.365		0.151
2.264	2.423		0.048
4.384	3.167		0.228
0.75	0.783		0.031
2.314	1.689		0.221
1.848	2.095		0.088
0.834	0.954		0.095
4.796	4.630		0.025
0.315	0.325		0.022
5.767	5.054		0.093
		Average	0.102 (0.076)
HSLP			
2.228	2.257		0.009
3.208	3.762		0.112
0.711	0.828		0.108
3.929	3.420		0.098
0.458	0.458		0.000
0.811	0.823		0.010
3.615	2.235		0.334
		Average	0.096 (0.116)

Averages are listed as value (SD)

Blood Assay

The distribution of the study participants are detailed in Figure 1 with both HSLP and control participants. The HSLP had a mean age of 19.14 (±0.90) and the control had a mean age of 19.78 (±0.88). The overall range of participants was 18-21 at the time of the study. The CRP distribution (*Figure 2*) was skewed left for the HSLP with a mean value of 2.05 (±1.37) and 25th, 50th, and 75th percentiles of 0.77, 2.24, and 3.49, respectively. The left shift was created by 4 participants who were within the 2.0mg/L – 4.0mg/L range. The remaining 3 participants had CRP values less than 1.0mg/L. The control group was skewed right with a mean value of 1.97 (±1.77) and 25th, 50th, and 75th percentiles of 0.57, 1.42, and 3.42, respectively.

The geometric mean, standard deviation, and log-transformed CRP values were calculated in Table 3. The log-transformed CRP values are plotted in Figure 3a with the HSLP (0.20) displaying a higher CRP value than the control (0.11). The relationship between CRP and average nightly sleep is displayed in Figure 3b. The self-reported nightly sleep hours of the HSLP – taken from the PSQI – is plotted against the log-transformed CRP values of the HSLP and shows a negative correlation ($r = -0.30$).

An analysis of the variance between duplicate blood samples is listed in Table 4. The table was organized by day, sample, and variance average. The coefficient of variance value [CV=standard deviation/mean] represented the variation between the single and duplicate samples. All values received an average variance less than 0.120.

Figure 4. HSLP Epworth Sleepiness Scale (ESS) Scores

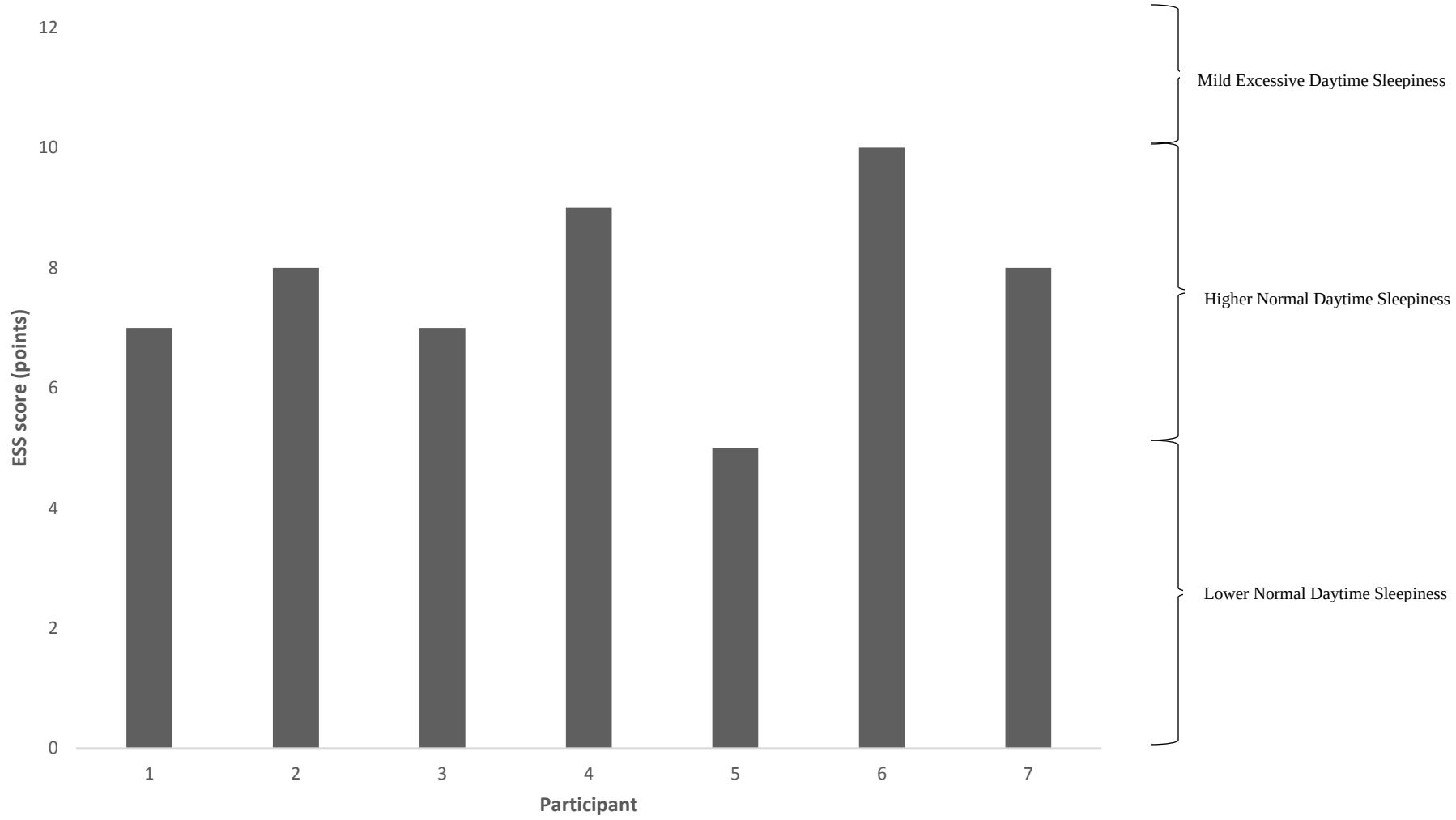
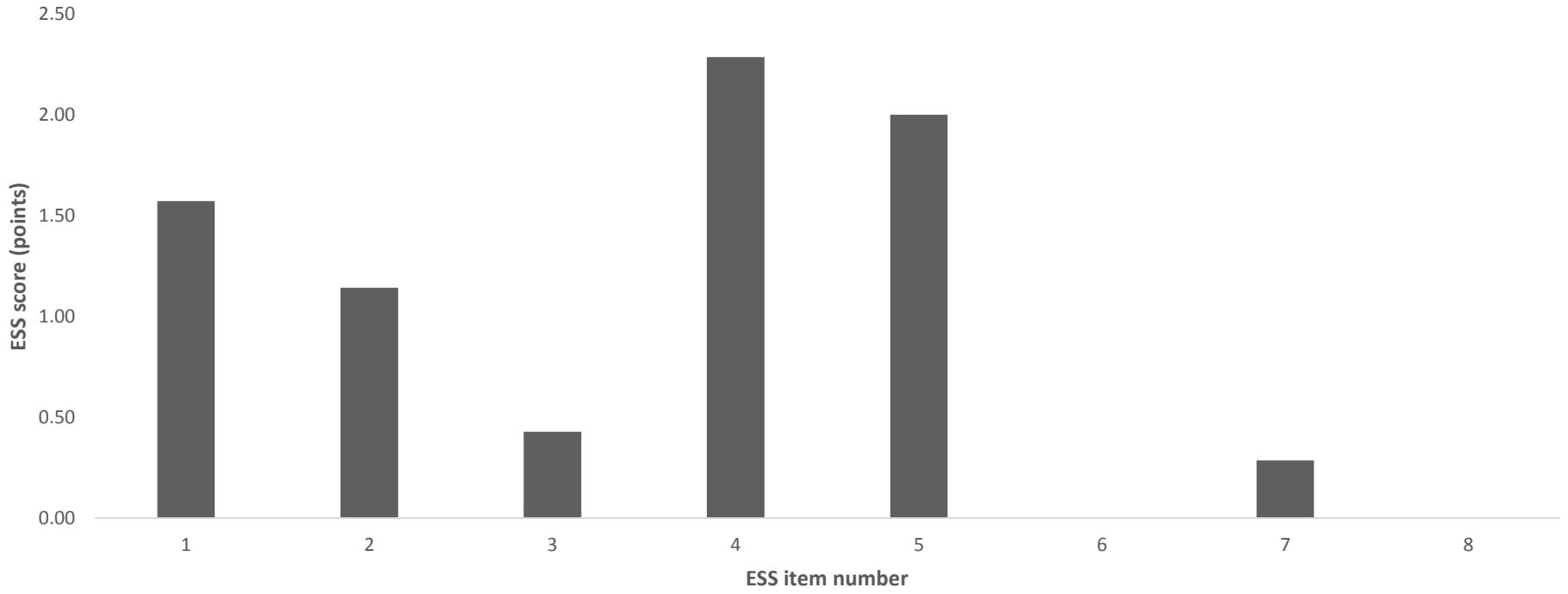


Figure 5. Epworth Sleepiness Survey

a. ESS Score vs. Item Number



b. Rank sum analysis of items of the ESS

Rank Sum	1	2	3	4	5	6	7	8	Total ESS
ESS	Q4 2.29 (0.95)	Q5 2.00 (1.00)	Q1 1.57 (0.98)	Q2 1.14 (0.69)	Q3 0.43 (0.53)	Q7 0.29 (0.49)	Q6 0.00 (0.00)	Q8 0.00 (0.00)	7.72

Data listed as mean (SD)

Table 5. Mean and SD for Each Component Score and Global Sleep Quality Variables for the PSQI

Component Scores (1-7)	Study (*HSLP*, *n = 7*)	
	Mean	SD
1. Subjective sleep quality	1.17	0.41
2. Sleep latency	1.71	0.95
3. Sleep duration	1.29	0.95
4. Habitual sleep efficiency	0.43	0.53
5. Sleep disturbances	0.86	0.38
6. Use of sleeping medication	0.86	1.21
7. Daytime dysfunction	1.67	0.52
Global Sleep Quality	7.57	2.82
Minutes to fall asleep	35.00	15.49
Sleep efficiency in percent	0.88	0.12

Table 6. Nap Specific Questionnaire

Participant	How often do you take naps?	What time of the year are you most likely to take a nap?	What time(s) during the week are you most likely to take a nap?	What time(s) of the day do you take a nap?	How long do you usually sleep during your naps?	How often do you feel well rested after a nap?
1	1 or 2 times a week	College months (fall and spring semesters)	Weekdays	Afternoon	Between 1 and 2 hours	Sometimes
2	1 or 2 times a week	All throughout the year	Weekdays	Afternoon	Between 30 to 60 minutes	Sometimes
3	1 or 2 times a week	All throughout the year	Weekdays and Weekends	Afternoon and Evening	Between 1-2 hours	Sometimes
4	1 or 2 times a week	All throughout the year	Weekdays and Weekends	Morning and Afternoon	Between 30-60 minutes	Sometimes
5	Rarely or never	All throughout the year	Weekdays and Weekends	Morning and Afternoon	Between 30-60 minutes	Often
6	3 to 4 times a week	College months (fall and spring semesters)	Weekdays	Afternoon and Evening	More than 2 hours	Often
7	1 or 2 times a week	All throughout the year	Weekdays	Afternoon	Between 30-60 minutes	Sometimes
Mean	1 or 2 times a week	All throughout the year	Weekdays	Afternoon	Between 30 to 60 minutes	Sometimes

**Morning (8am-12pm); Afternoon (12pm-6pm); Evening (6pm-8pm); Night (8pm-12am)*

Surveys

The Epworth Sleepiness Scale (ESS) scores of the HSLP (*Figure 4*) were in the normal range of daytime sleepiness; however, all but one (score = 5) had totals on the higher normal end. The ESS scores ranged from 5 to 10 with an average ESS score was 7.7. **The ESS score verses item number** (*Figure 5a*) displays the distribution of scores of each ESS item number. All items had values greater than 0 except situations six (*Sitting and talking to someone*) and eight (*In a car, while stopped for a few minutes in the traffic*). Situations 4 and 5 ranked the highest (*As a passenger in a car for an hour without a break* and *Lying down to rest in the afternoon when circumstances permit*, respectfully) with 3 (*Sitting, inactive in a public place (e.g. a theatre or a meeting)* and 7 (*Sitting quietly after a lunch without alcohol*), excluding item numbers 6 and 8. The rank sum analysis of the situations gave a numerical representation of the ESS (*Figure 5b*), showing where the situations fell in the 0-3 dozing off range. Item 4 had the highest rank sum of 2.29 and item 7 had the lowest sum of 0.29, again excluding questions 6 and 8 which received scores of 0.00. The standard deviation for each item numbers was 1.00 or less.

Table 5 displays the average and standard deviations for the seven component scores or categories of the PSQI. The highest mean component score was for sleep latency with a value of 1.71 (±0.95). Daytime dysfunction was the second highest mean score with 1.67 (±0.52). Subjective sleep quality and sleep duration both had mean values below 2.00, and habitual sleep efficiency, sleep disturbances, and use of sleeping medication mean values below 1.00. The lowest mean component score was habitual sleep efficiency with a value of 0.43 (±0.53). The average global sleep quality was 7.57 (±2.82) with individual scores ranging from 5 to 13. The average minutes to fall asleep for the participants was 35.00 (±15.49) and the sleep efficiency percent was 0.88 (±0.12). The global sleep quality was very high compared to Buysse et al.

(1991) where healthy 20-30 year old females had a global sleep score of 1.9 (± 1.4) with an average of 7 hours of sleep. However, Lund et al. (2010) found that 38% (total = 1125) of the college participants had global scores over 7 with an average nightly sleep of 7 hours.

The Nap Specific Questionnaire (*Table 6*) that the habitual sleep loss participants (HSLP), on average, reported taking one to two naps per week. While two participants indicated that they only took naps during the college months, the rest of the participants indicated that they engaged in that behavior throughout the year. The weekdays were noted as the most likely time for napping, and the most common time of day to take naps was in the afternoon. Finally, the average nap duration reported was between 30 to 60 minutes.

Table 7. Comparison of Actigraphy Data from the Control Group to HSLP Self-Reported Sleep Behavior

Question*	Actigraphy Data (Sleep Algorithm: Cole-Kripke)	The Pittsburgh Sleep Quality Index (PSQI)
	Control (n = 13)	HSLP (n=7)
1. When have you usually gone to bed?	12:03 am	1:40 am
3. What time have you usually gotten up in the morning?	11:23 am	7:46 am
4 A. How many hours of actual sleep did you get at night?	10.02 (2.53)	5.93 (1.10)
B. How many hours were you in bed?	11.02 (2.60)	6.86 (1.68)

Data listed as mean (SD)
**Questions (1, 3, 4) taken from The Pittsburgh Sleep Quality Index (PSQI) Questionnaire*

Table 8. Comparison of Actigraphy Data from the Control Group to HSLP Self-Reported Nap Behavior

Question*	Actigraphy Data (Sleep Algorithm: Cole-Kripke)	Nap Questionnaire
	Control (n = 13)	HSLP (n = 7)
1. How often do you take naps?	2 times a week	1 or 2 times a week
2. What time(s) of the day do you take a nap?	Afternoon	Afternoon
3. How long do you usually sleep during your naps?	4 hours	30-60 minutes

**Questions (1-3) taken from the Nap Questionnaire*

Sleep Measurements

Table 7 compares the self-reported sleep behavior of the HSLP to the actigraphy data of the control group (Sleep Algorithm: Cole-Kripke), and the four questions listed were taken from the PSQI Questionnaire. For the question – *When have you usually gone to bed?* – the control group had an average bedtime of 12:03am while the HSLP self-reported an average bedtime of 1:40am. The difference between the two groups was an hour and thirty-seven minutes. For the question – *What time have you usually gotten up in the morning?* – the control group had an average waking time of 11:23am while the HSLP self-reported an average wake time of 7:46am. The difference between the two groups was three hours and thirty-seven minutes. Based on the PSQI, the HSLP averaged 5.93 (±1.10) hours of actual sleep a night with a mean of 6.86 (±1.68) hours in bed. In comparison, the control group had an average of 10.02 (±2.53) hours of actual sleep and 11.02 (±2.60) total hours in bed. There was a difference of 4.09 hours of actual sleep and 4.16 total hours in bed between the groups.

Table 8 details how the self-reported nap behavior of the HSLP compared to the actigraphy data for the control group. The three questions listed were taken from the Nap Questionnaire and the actigraphy data gathered came from the Sleep Algorithm: Cole-Kripke. Over a week period, the control group averaged 2 naps per week which occurred in the afternoons for an average of 4 hours. The HSLP reported that on average they napped 1 or 2 times a week in the afternoon time frame between 30-60 minutes. The length of the naps was the biggest difference with the control averaging about three hours longer than the HSLP.

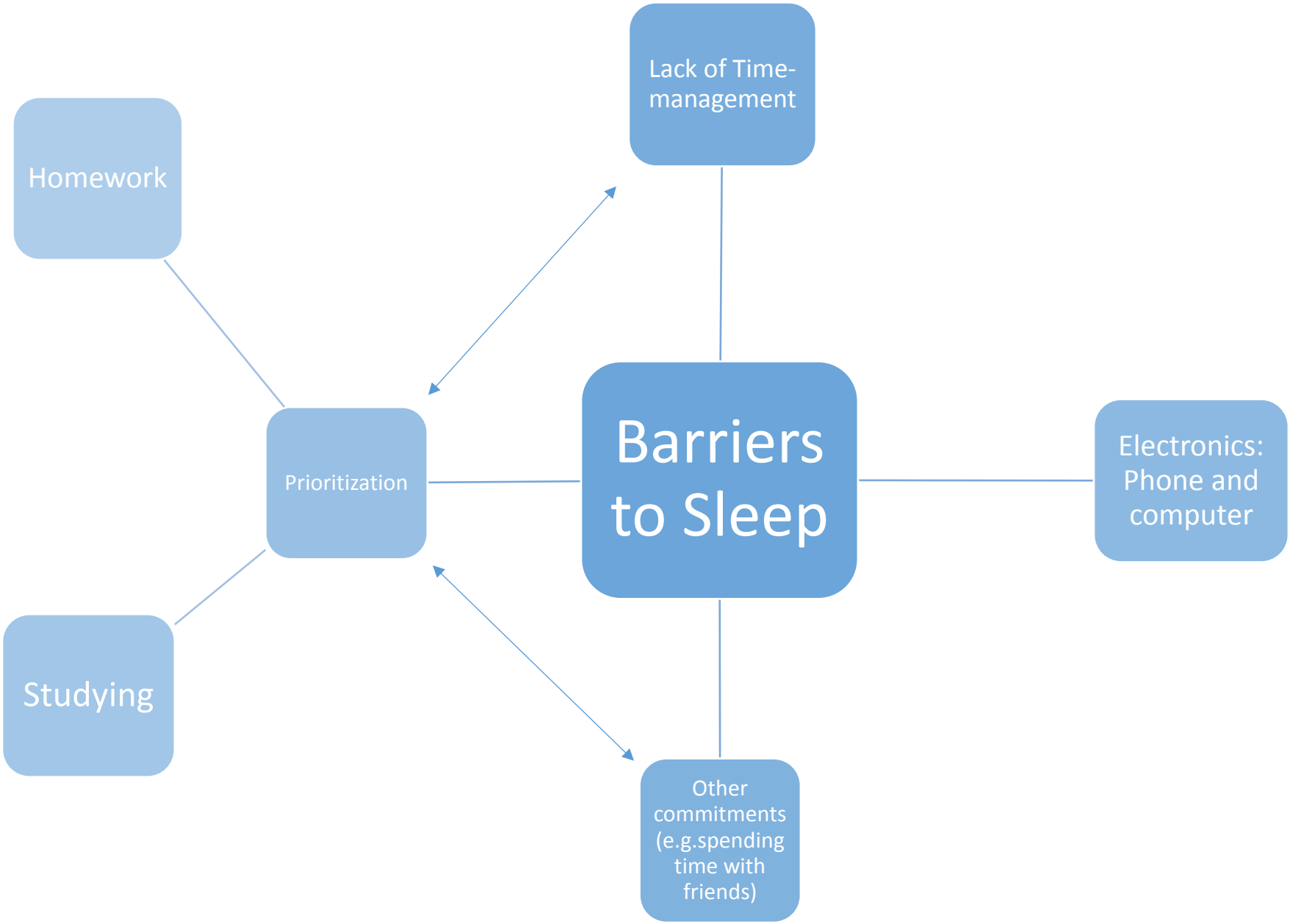
Table 9a. Perception of Sleep

Interview Question Topics	Responses
Importance of sleep	If I don't have a lot of homework or studying to do, it's very important; but, if I have an exam the next day, a quiz, test, or anything, I prioritize that over sleep—all the time…These grades are forever. It'll affect me all throughout college—my GPA—so I try to put more focus on that than getting an extra hour or two of sleep.
Sleep quantity vs. quality	Roughly spit between quantity and quality by the HSLP.
Characteristics of sleep quality	Falling asleep quickly and not waking up in the middle of the night for anything or like no noises waking you up.
Self-Awareness of sleep habits	4 participants self-identified as being sleep deprived 2 participants did not identify as being sleep deprived 1 participant considered her sleep behavior as variable
Hours that constitute a good night's sleep	8 hours
Other students' sleep habits	Most of the people I know are not sleeping enough. They're like pulling all-nighters or at the UL till 3 ol clock in the morning and they have an 8 or 9 am. I know so many people who are like 'yea, I haven't slept…I've slept 5 hours in the past 48 hours'….So I feel like I'm getting much better sleep than some of my peers and I don't feel like I'm getting great sleep.
The potentiality of good sleep hygiene	College students have this expectation to study all the time, to get exceptionally great grades, but then also they're expected to have this social life and go to the gym and just take care of themselves regularly, and it's like how do you make time for these things while still making time for that quality quote on quote 8 hours out of your 24 hour day that you don't have to use, you know?

Table 9b. Sleep Behaviors

Interview Question Topics	Responses
Feeling well rested	I'm always tired when I wake up, just, throughout the day I feel myself…just wanting to go to sleep.
Sleep consistency	I definitely get more sleep over the summers even when I have a part-time job.
Nightly routine (before bed)	If I finish homework, I'm still watching stuff on the computer or I'm still on my phone. I know that's a bad habit because that doesn't help me adjust getting ready to go to bed…I try not to do homework and studying in bed…so if I'm in bed and on the computer, that's recreation.
Pulling all-nighters	Two students' perspectives: Sleep is the sacrifice I'm making…I tend to let things accumulate and then I will like just purge on studying or like binge on studying…I feel like it works for me but it's not the healthiest way to do it….I think just like in the nature of college students, it's inevitably going to happen. I'm apprehensive to all-nighters right now but I could see that changing as my course load gets more rigorous. Hopefully, I still don't do it but I could definitely see myself becoming more prone to all-nighters as I move forward in college.
High school sleep habits	Especially in high school like working a job, doing all these clubs in school as an officer, and just doing multiple varsity sports…my sleep quality was probably less than it is now because I was doing all these things to work up to getting into college.
Anticipated post-college sleep habits	I do think it is possible that it [sleep behavior] can continue just because once you get into a habit of going to sleep at a certain time…I've gotten into a habit of having to do my work at night, I've become more comfortable. I don't want to do my work in the middle of the day; I want to do it at night now. So I could definitely see myself becoming a night worker.

Figure 6. Barriers to Sleep



Interviews

Table 9 contains the semi-structured interview question topics based on the HSLP perception of sleep and sleep behaviors. The responses listed were taken from individual participants but serve to give a general overview of the responses gathered from the rest of the participants (*total, n=7*). The perception of sleep topics (*Table 9a*) involved the participants' personal views of sleep as well as how they perceive the sleep patterns of others. The topics involved the importance of sleep, sleep quantity vs. quality, characteristics of sleep quality, self-awareness of sleep habits, hours that constitute a good night's sleep, other students' sleep habits, and the potentiality of good sleep hygiene (as a college student).

The importance of sleep fell into three categories: 1.) Sleep was viewed as important but sleep behavior did not correlate with that view; 2.) Sleep was viewed as important with a verbal statement of attempting to get better sleep; and, 3.) Sleep was not viewed as important when compared to coursework. Sleep quantity vs. sleep quality received a roughly equal response from the participants as some considered quantity as more important while others saw quality as most important based on personal experiences. Common responses about what was considered features of sleep quality were falling asleep quickly and not waking up in the middle of the night, either in combination or separately. When asked if they considered themselves sleep deprived, four participants stated that they were, two stated that they were not, and one considered her sleep behavior as variable. From the two participants who did not self-identity as sleep deprived, one stated that she did not consider herself as sleep deprived because she stated that she made up on her sleep through naps. The other participant stated that she was taking steps to manage her time so she received a good amount of sleep each night. When questioned about the number of hours that constituted a good night's sleep, every participant mentioned 8 hours, or a 7-8 or 8-9

hour range. The 8 hour mark of good sleep was stated as information coming from high school classes or from parents at home. The perception of how other students' behave, for most of the participants, came through their knowledge of their friends' behaviors. Many responses to this question was comprised of a short anecdote or conversation with friends about their sleeping habits. Each one involved the friends having poor sleep habits. Lastly, the potentiality of good sleep hygiene received a general response of the need for better time-management skills, prioritization, college sleep culture pushing bad sleep habits (i.e. staying out late to party or drink), or the feeling of certain expectations which restricts the ability to get adequate sleep on a consistent basis.

The sleep behavior question topics *(Table 9b*) involved the actual habits of the participants, including those in the past and expected sleep patterns of the future. The question topics included feeling well rested, sleep consistency, nightly routine (before bed), pulling all-nighters, high school sleep habits, and anticipated post-college sleep habits. When asked if they felt well rested after sleep, a general answer of tiredness was stated, although for some it depended on sleep hours (i.e. if they received 5 hours of sleep, the next day they felt tired, compared to receiving 9 hours and feeling well-rested). Sleep behaviors were not consistent as participants stated that they received more sleep over the summer as opposed to the college year. The nightly routine before bed varied based on the weekday or weekend. Homework, studying, and phone/computer use were the common responses given apart from other actions such as showering. The conversation of all-nighters varied between its effectiveness and if it was engaged in. High school sleep habits were stated as being worse in high school compared to college. When questioned if they expected their sleep habits to continue post-college, there was a

split between yes (due to a graduate school, a professional program, or anticipated job type) and hopefully no (based on an anticipated 9am-5pm work schedule).

The most common responses to barriers to sleep are detailed in Figure 6. Prioritization was a common theme among the participants, whether it was verbally stated or implied through other language (i.e. choosing X over sleep). Homework and studying were the main factors which took priority over sleep. Participants described having to make a choice of whether to complete their homework which takes a while to complete or needing to study for an exam. The double arrows from prioritization to "lack of time-management" and "other commitments" is given to display how the areas affect one another. Lack of time-management was another barrier which resulted in prioritization of activities they needed to complete. Other commitments was a barrier during the week and the weekends, as time with friends was a common response as to why they went to bed at or past midnight. Lastly, while phones and computers were not stated as hindering sleep, their use occurred before going to bed which prolonged their waking hours.

CHAPTER #3: ANALYSIS OF MIXED METHODS RESEARCH

Discussion

Cardiovascular Risk in College Students

The CRP distributions of Figure 2 provide an interesting distinction between the control and the HSLP. Since this thesis is investigating habitual sleep behavior (i.e. regularly practiced sleep patterns in a real world setting), the CRP levels provide insight into averages among HSLP and the control. So, while the control group may have had a wider range of CRP values, the overall distribution skewed right with 6 out of the 12 participants below 1.0mg/L. The HSLP had a smaller range of CRP values but had a left skewed distribution. Due to this opposite skew of distribution, the HSLP had a higher baseline CRP than the control (*figure 3a*). In addition, Figure 3b shows that average nightly sleep duration impacts CRP even when comparing a short sleep duration period between 4-6 hours. Therefore, this data suggests that the HSLP may be at a higher risk for cardiovascular disease than the control.

While a short number of sleeping hours may, for some students, remain confined to their college years, the lasting imprint of sleep deprivation is highlighted by Van Leeuwen et al. (2009). This study provides biological evidence that corrects a major misconception believed by many college students - that one can ‘catch up’ after one or more nights of deprived sleep. This misconception was voiced during the interviews, although, it can be seen in the overall college sleep culture as well. One of the HSLP who did not consider herself sleep deprived explained that her self-identification resulted from the many naps she took the next day which she believed made up for the sleep she missed. There are also broader ideas in the college sleep culture that a few nights of deprived sleep can be corrected by subsequent nights of normal sleep. The results of the Van Leeuwen et al. (2009) study, however, found that “immunological changes that [took]

place after multiple nights of short sleep [could not] be restored completely by sleeping normally for a few nights and long-term sleep restriction may lead to an increased risk of developing cardiovascular diseases" (5). This is particularly relevant for many college students who, arguably, spend 4 years of their lives engaging in deprived sleep practices. If they then move into careers where they continue to engage in sleep deprived behaviors, the increased risk of CVDs becomes that much more relevant since prolonged sleep restriction can lead to persistent changes in the immune system such as an increased production of CRP (and IL-17, another pro-inflammatory marker) (Van Leeuwen et al. 2009).

Among the HSLP, there was a prioritization of homework, classes, studying, and cumulative GPA over sleep. There was an interesting pattern that emerged which gave insight into the college student thought process. This was shown through a present and future impact ranking of their GPA and sleep. For instance, while the students stated that they knew their sleep habits were not healthy, the present health impacts due to lack of sleep did not outweigh the importance of classwork. Their GPA affected their future plans (i.e. opportunities) which meant that sleep had to be sacrificed. The long term health risks of poor sleep habits in college was not discussed by 6 participants except in regards to them understanding it was bad for them. The extent to which it was unhealthy for them was not elaborated on in detail either (i.e. a possible response: poor sleep can affect the immune system). The potential cardiovascular disease risk of their continued sleep restrictive behaviors was also not mentioned by any of the participants. This last point is not surprising, however, since cardiovascular risk conversations with physicians, commercial ads, and scientific research studies target middle age to older adults as opposed to young healthy individuals in their prime years.

According to the nap actigraphy data of the control participants and the self-reported nap behavior of the HSLP, there was not a significant difference between the groups over the course of a week (*table 8*). The control averaged two naps per week while the HSLP averaged one or two times a week. While short naps (classified as $\leq$ 30 minutes) have been found to potentially lower cardiovascular disease risk, longer naps ($\geq$ 60 minutes) have been shown to increase one's risk (Yamada et al. 2015). In the HSLP, 2 out of the 7 participants had CRP values greater than 3.00mg/L and self-reports of naps occurring 1 or 2 times a week with an average of 6 hours of nightly sleep. Since the average nap length times of all the control participants were greater than 60 minutes, assessing to what extent naps increase cardiovascular disease risk between short and long duration sleepers is a potential area of future study.

It should be noted that naps are a fluid practice, at least within the confines of the U.S., where individuals may choose to take no naps or more than one in a single day. This is especially true for college students who are faced with class schedules which can potentially affect overall sleep behavior (naps included). As a result, the one week period which was assessed in this thesis may have represented a higher number of naps from students than would normally occur among this cohort.

Perceptions Regarding Sleep, Sleep Habits, and Sleep Quality

The seven interview question topics of Table 9a. give insight into the college view of sleep. As seen in the response to the *Importance of Sleep*, the participant stated that sleep is important if she does not have a lot of homework or studying to do; yet, right when a heavy load is felt, sleep becomes less important. As has been the case throughout the American history of sleep, there is a cultural view, whether positive or negative, attached to the act. Sleep, for college

students, is both a source of unproductivity and blissful rest. The persona in which it takes for students depends on their goals for that day and what they need to accomplish. Sleep must then be forgone in favor of the productive state of staying up and continuing to work into the late night and early morning. This was a common sentiment among many of the HSLP although not all saw the forgoing of sleep as being productive. The blissful side of sleep arose during the conversation of sleep quality. One participant stated that she felt she had a good night's sleep if she had "slept like a baby." Others stated that not waking up in the middle of the night and waking up in the same position they had fallen asleep in were considered good quality sleep.

Sleep for the college student also involved naps which were self-reported as being at least once a week on average (*table 6*). Naps served the purposes of catching-up on sleep, allowing for a short period of rest, responding to feeling tired, and serving as an energizer. They served as a tool for the participants to use when needed. As opposed to nightly sleep, they were a productive method to increase efficiency for studying or focusing in class.

Self-awareness of sleep habits was not surprising as 4 out of the 7 participants considered themselves sleep deprived. Many of the participants also stated that they considered other students (normally within the lens of friends or individuals they knew) as also being sleep deprived. One student, though, stated that there was a range, with some students being able to get more sleep than others due to their majors. *The potentiality of good sleep hygiene* revolved around two key words: prioritization and sacrifice. Either sleep was prioritized, which would allow someone to get a good night's sleep (a consensus of 8 hours was stated by the participants) or some other aspect of a student's life (social, personal, academic) would have to be sacrificed. Only one participant stated that it was possible to prioritize the various aspects of the student life to get a good night's sleep.

Based on the PSQI values in Table 5, sleep latency ranked the highest (1.71) among the HSLP with daytime dysfunction just below it (1.67). This suggests that the HSLP are having trouble falling completely asleep and are waking up feeling tired. Given that these participants not only practice habitual sleep loss but also wake earlier, these results are unsurprising. While it was expected that subjective sleep quality would be self-reported as worse (which would result in a higher score) due to the average nightly sleep hours of 6 or less, this was not the case. Sleep quantity may have been low among the participants but poor sleep quality was not self-reported as such. The low value of habitual sleep efficiency resulted due to the hour or less time it took for participants to fall asleep (average score of 35.00min (±15.49)) and how those times were scored. The global sleep quality score was a high value of 7.57 (±2.82) out of a potential maximum PSQI score of 21, indicating that based on how the participants answered, they were not receiving good quality sleep.

Sleep Behaviors

How is sleep experienced by the college student? Or, more specifically, how is sleep deprivation experienced by the college student? Feelings of tiredness was a common response to the interview question topic regarding *Feeling well rested* in Table 9b. Participants also stated that during the summers they normally received more hours of sleep. This suggests that deprived sleep practices are primarily engaged in during the traditional college months (fall and spring semester). There was an expected correlation between short duration sleep and naps, particularly that when sleep deprivation was more likely (during the college months), naps would also be more common. However, as detailed in Table 6, this was not the case. Instead, taking naps were self-reported as happening all throughout the year. For some of the participants, there was a

connection between *Feeling well rested* and their perception of sleep's most important characteristic (*sleep quantity vs. quality*). Individuals who considered sleep quantity more important meant that getting a certain number of hours resulted in feeling well rested in the morning. For those who considered sleep quality more important, the number of hours did not necessarily result in feeling more or less well-rested. For instance, even without getting 8 or 9 hours, they felt well rested if they considered themselves getting good quality sleep.

Feelings of tiredness, specifically sleepiness, can be visualized and quantified through the Epworth Sleepiness Scale data. Figure 4 shows that out of the eight situations presented to the participants and how they ranked them in regards to chance of dozing off, all of the participants except one (score = 5) had an overall score in the higher normal daytime sleepiness range. "As a passenger in a car for an hour without a break," "Lying down to rest in the afternoon when circumstances permit," and "Sitting and reading" were the top three situations in which dozing off could occur (*figure 5*). "Sitting and talking to someone" and "In a car, while stopped for a few minutes in the traffic" were given a score of 0 across all the participants and were thus not considered moments of sleepiness. The higher than normal daytime sleepiness range of the Epworth Sleepiness Scale adds additional support to the high ranking component score of daytime dysfunction (*table 5*).

The *Nightly routine (before bed)* involved various activities from showering to reading (for class or recreation) to watching shows on a phone or computer. The conversation of all-nighters brought about two main perspectives as seen through the quotes by two students on the issue (*table 9b*). Pulling all-nighters either worked and were effective or had not been engaged in during the college years. It was interesting, though, how one participant who had not pulled an all-nighter mentioned that she could potentially see herself doing one in the future as courses

became more rigorous. The definition of an all-nighter also influenced responses. For example, one participant stated that if she could get 1 hour of sleep during a night of studying then she would. Therefore, if an individual gets any amount of sleep that night, even if it is a small amount, would that person consider that as pulling an all-nighter? This clarifying question was not asked during the interviews. Engaging in this practice was related to school year with the 1st year participants less likely to have pulled one while in college compared to the two participants who were 3rd and 4th years.

Table 7 offers a comparison between self-reported sleep behaviors of the HSLP and the actigraphy data gathered from the control. Overall, the HSLP went to sleep later and awakened earlier than the control (1:40am vs. 12:03am; 7:46am vs. 11:23am) with an average nightly sleep slightly lower than 6 hours compared to the control that was almost exactly at 10 hours. Interestingly, the HSLP had a lower difference between hours in bed and actual sleep (0.93) compared to the control (1.00). This observation could have been due to the later bedtime of the HSLP which could have potentially made them fall asleep faster because of it compared to the control.

The final two interview topics of *High school* and *anticipated post-college sleep habits* listed in Table 9b offer further insight into the HSLP past and future sleep behaviors. For mostly all of the HSLP, while college sleep habits were not ideal, sleep during high school was worse. As mentioned in the quote, high school is a time of countless possibilities as students prepare themselves for applying to college. This knowledge then adds an additional layer to the practice of sleep deprivation and cardiovascular risk when one considers that some students have practicing this behavior between 4-8 years. High school and college are critical times in students' lives and, unfortunately, sleep is being consistently pushed aside – a practice that for some of the

participants will only extend because of additional schooling or type of job. The extension can also occur due to habit, which one of the participants noted, saying: “I don’t want to do my work in the middle of the day; I want to do it at night now. So I could definitely see myself becoming a night worker.”

Barriers to Sleep

Finally, Figure 6 provides a visualization of the barriers of sleep mentioned during the interviews. While a specific question was not asked in regards to activities that prohibited them from receiving 8 hours of sleep, the figure was designed based on the analysis of all the responses. Since the interview centered on sleep perceptions and behaviors, activities mentioned were connected to the habitual sleep loss behavior. Therefore, these answers were classified as barriers.

Overall, there were four main barriers gathered from the HSLP: prioritization; lack of time-management; other commitments; and, electronics. Prioritization was a key word among the interviews and served as connections between the other barriers of sleep (seen through the double headed arrows). Sleep was a factor that had to be ranked against the other tasks that the participants had to navigate. Homework and studying were the top answers to be completed over sleep. As one participant stated: “I try to put more focus on that [GPA] than getting an extra hour or two of sleep.” Thus, the task seen as more important in that particular moment wins out in the end. Procrastination was mentioned although not all the participants who mentioned prioritization stated that they had to do so because they procrastinated on their homework. Lack of time-management regarding planning out the completion of various tasks resulted in a barrier too. The lack of time to complete those activities consequently led to the prioritization of X task

over sleep. Likewise, the ‘other commitments’ category led to a ranking based on priority. The last barrier was ‘electronics’ with phone and computer usage extending the awake time of the participants. They were also used in bed.

Technology use is a common barrier to sleep. When studying first semester college students, Adams et al. (2017) found that social/technological distractions impacted sleep along with socializing and the fear of missing out. Paterson et al. (2017) found that technology use, time demands (e.g. spending time with friends), unpredictable routines, and difficulty winding down served as barriers to sleep among young adults (16-25 years old).

Limitations

There were several limitations of this study. First, there were no male participants that took part in either the control or HSLP which could have provided an examination between male and female CRP distributions and future cardiovascular risk. Second, the sample of HSLP was small (n = 7). The intended goal was to have an equal number for both samples; however, two barriers made this objective unobtainable due to time constraints. The first was overall recruitment. In trying to gather participants, there was a disconnect between individuals who self-identified as getting an average of 6 hours or less of sleep each night and those who were able to participate. The finger prick also deterred some participants. The second barrier was the loss of participants before a full week’s worth of data plus a second blood spot could be retrieved.

Third, IL-6 was not included in the study which would have introduced an analysis of how the pro-inflammatory marker fluctuated due to sleep deprivation and circadian rhythm. Fourth, there were technological problems in retrieving the actigraphy data for half of the HSLP; as a result, the self-reported information was used to compare between them and the control.

Finally, the classification of sleep and naps was a subjective process that generally produced clear sleep and nap periods. There were, however, some periods that had a long sleep time where a subjective decision had to be made on whether to classify it as sleep or a nap since these periods coincided with data representing nighttime sleep. In the end, these periods were categorized as ‘long naps.’

CHAPTER #4: IMPLICATIONS OF THE CURRENT COLLEGE SLEEP CULTURE

Sleep deprivation is a real problem many college students face not only at UNC but at other universities across the United States. While long term sleep restriction has lasting effects, habitual sleep loss is viewed by those who engage in it as a necessary practice to complete coursework. This thesis sought to investigate how habitual sleep loss affected cardiovascular risk, to explore students' perception of sleep, and to examine behaviors inhibiting sleep. The goal of this research was to emphasize the importance of a 'good night's sleep;' to show that even confining sleep deprivation to one's college years can have a lifelong impact; and, to lay the groundwork for further discussions and action plans regarding college student sleep behaviors.

The overall results of this study showed that an average of 6 hours of sleep or less each night resulted in higher baseline and follow-up CRP levels compared to participants who received more than 6 hours of sleep each night. The potential effect of naps on these values could not be gathered from the low participant sample; however, while nap frequency and time of day were similar among the control and the HSLP, the control had a higher average nap length period. Perceived sleep ideas fell into three categories: 1.) Sleep was viewed as important but sleep behavior did not correlate with that view; 2.) Sleep was viewed as important with a verbal statement of attempting to get better sleep; and, 3.) Sleep was not viewed as important when compared to coursework. Students' view of the sleep experience had a juxtaposing nature as either a restful state or an obstacle to completing coursework. Naps, then, worked as a mediating factor in order to get enough sleep to function throughout the next day. The major consequence of deprived sleep was fatigue which naps helped remedy. Not all the HSLP engaged in all-nighters; but, those who did stated that they were effective. Lastly, the barriers to sleep involved prioritization, lack of time-management, other commitments, and electronics.

While this study adds to sleep literature regarding habitual sleep loss among college students, more studies are needed. Not only do habitual sleep studies work well for college students who typically cannot spend many days in a sleep lab but they also allow researchers to examine the normal, everyday sleep patterns of students instead of prescribing bedtimes. This thesis focused on the potential cardiovascular risk of habitual sleep loss; however, sleep behaviors can also influence stress, diet, physical activity as well as other factors which will affect more than just pro-inflammatory markers in the body. Therefore, studying the relationships between various factors is also important in understanding the far reaching impacts of sleep loss.

Other avenues that future sleep studies can investigate, particularly in regards to habitual sleep loss, involve: the relationship between habitual sleep loss and procrastination, the effects of nap length on cardiovascular risk, and the relationship between habitual sleep loss and psychosocial stress among college students. While an examination of psychosocial stress was not explored in this thesis, high stress levels can be an additive to cardiovascular risk. This means there can be an even greater risk among individuals who experience high levels of stress and habitual sleep loss. Many college students face these unhealthy habits as poor sleep behaviors can be argued to result from stressful situations (e.g. classes, homework) or lead to high stress.

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Appendix A

FULL LAB PROTOCOL FOR SIPA RESEARCH PROJECT

Day One

- Arrive at the lab and sign the consent form
- Collect first blood spot
- Complete stress and physical activity surveys
    - Minnesota Leisure Time Physical Activities
    - Short Form Health Survey 36 Questionnaire
    - 11-item College Student Stress Scale
    - Traditional College Student Stress Scale
    - College Student's Stressful Event Checklist.
- Fit with a Polar Electro heart rate monitor and actigraph to monitor sleep and physical activity

Day Seven

- Return sleep monitoring equipment
- Complete the remaining surveys
    - 3-Day Physical Activity Recall
    - Cohen Perceived Stress Scale
    - International Physical Activity Questionnaire
- Collect final blood spot
- Gather anthropometric measurements via anthropometer and scale
    - Stature
    - Weight
    - Triceps
    - Biceps
    - Subscapular
    - Suprailiac skinfolds

Appendix B

ELISA ASSAY FOR CRP LAB PROTOCOL USING QUANTIKINE KIT

SAMPLE PREPARATION: DAY 1

Time Stamp: 11:45am-2:30pm on November 13, 2018

Bloodspot Samples: Preparation

1. Check kit expiration
2. Set RD5P concentrate out to room temperature for later preparation of Calibrator Diluent
3. Lay out dust-free surface paper absorbent side down
4. Bring out bloodspot samples from refrigerator
5. Label bloodspot card ID numbers on small 2mL closing tubes and place in stand

Calibrator Diluent: Preparation

1. Combine 5mL of Calibrator Diluent RD5P Concentrate with 20mL DI H2O to make 25mL of Calibrator Diluent
2. Add 20mL of DI H2O into 50mL beaker
3. Forward pipette 1mL*5 of Calibrator Diluent RD5P Concentrate into beaker
    a. Used 100-1000µL pipette
4. Mix solution with pipette by injecting and releasing under surface to avoid bubbles
    a. Note: Sample preparation instructions by Quantikine suggests 100 fold dilutions, but we used the diluted Calibrator Diluent to elute the blood spots to reconstructed whole blood (based on McDade's email instructions)

Bloodspot Samples: Punching Bloodspots & Vortexing

1. Select largest bloodspot from sample card
2. Use 1/8 inch hole punch on center of bloodspot
3. Release the circle onto the dust-free paper
4. Use tweezers to place the punched out bloodspot into the correctly labeled 2mL tubes
5. Vortex every tube
6. Place dilute in the fridge.

SAMPLE PREPARATION: DAY 2

Time Stamp: 6:55am-5:50pm on November 14, 2018

<u>Preparation</u>

Bloodspot Samples

1. Place blood spots out on the counter
2. Place the RD5P bottles in the top drawer

Water Buffer

1. Warm wash buffer to room temperature to dissolve crystals
2. Invert wash buffer container to gently mix

3. Add 20mL of wash buffer in 50mL graduated cylinder
4. Add wash buffer to 600mL beaker
5. Add 480mL of DI H2O to 500mL line
6. Pour into new squirt bottle

Substrate Solution

1. Mix Color Reagents A and B together in equal volumes within 15 minutes of use
2. Protect from light
3. 200µL of the resultant mixture was required per well

Calibrator Diluent RD5P (diluted 1:5)

1. Add 20mL of Calibrator Diluent RD5P to 80mL of DI H2O

Human CRP Standard

1. Label 6 standard 2mL capped tubes
2. Pipette 200µL of Calibrator Diluent RD5P (diluted 1:5) into each tube
3. Pipette 200µL of Standard concentrate into 25ng/mL tube and vortex for 1 minute
4. The Human CRP Standard (50ng/mL) served as the high standard
5. The Calibrator Diluent RD5P (diluted 1:5) served as the zero standard (0 ng/mL)

Procedure

Assay

1. Add 100µL of Assay Diluent RD1F to each well using repeat pipette
    a. Used 5mL tip (48 repeats for 100µL)
    b. Dispensed first two and last four
        i. Checked accuracy with H2O on scale to test
    c. Vortexed each sample for 20s
2. Pipette 50µL of standard, control, or sample into well
    a. Used 20-200µL pipette
3. Cover with adhesive strip
4. Incubate for 2 hours in drawer
5. Aspirate each well and wash, repeating the process 3x for a total of four washes
    a. Used plate washer for aspiration step
        i. Saved program:
            1. Prewash → EBV (3x) → 400
    b. Removed remaining wash buffer by inverting plate and blotting it against clean lint-free white paper towels
6. Add 200µL CRP Conjugate to each well
    a. Used 100-1000µL pipette
7. Cover plate with new adhesive strip
8. Incubate for 2 hours inside drawer
9. Repeat the aspiration as in Step #5
10. Add 200µL of Substrate Solution to each well.
    a. Substrate Solution was equal parts Reagent A and B (10,000µL of each) and vortexed to thoroughly mix

11. Incubate for 30 minutes in drawer
12. Add 50µL of Stop Solution to each well
    a. Used 20-200µL pipette
    b. The blue color in the wells turned to yellow
13. Place in microplate reader
    a. Elx800 microplate reader
    b. Note: Determine the optical density of each well within 30 minutes, using a microplate reader set to 450nm

Cleanup

1. Well samples placed in insulation packet then put in drawer
2. Blood samples placed in the fridge

MICROPLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD1 0 standards	STD1 0 standards	CTL1	CTL1	SPL6	SPL6	SPL15	SPL15	SPL24	SPL24	SPL35	SPL35
B	STD2 0.78 standards	STD2 0.78 standards	CTL2	CTL2	SPL7	SPL7	SPL16	SPL16	SPL25	SPL25	SPL36	SPL36
C	STD3 1.56 standards	STD3 1.56 standards	CTL3	CTL3	SPL8	SPL8	SPL17	SPL17	SPL28	SPL28	SPL37	SPL37
D	STD4 3.12 standards	STD4 3.12 standards	SPL1	SPL1	SPL9	SPL9	SPL18	SPL18	SPL29	SPL29	SPL38	SPL38
E	STD5 6.25 standards	STD5 6.25 standards	SPL2	SPL2	SPL11	SPL11	SPL19	SPL19	SPL31	SPL31	SPL39	SPL39
F	STD6 12.5 standards	STD6 12.5 standards	SPL3	SPL3	SPL12	SPL12	SPL20	SPL20	SPL32	SPL32	SPL40	SPL40
G	STD7 25 standards	STD7 25 standards	SPL4	SPL4	SPL13	SPL13	SPL21	SPL21	SPL33	SPL33	SPL41	SPL41
H	STD8 50 standards	STD8 50 standards	SPL5	SPL5	SPL14	SPL14	SPL22	SPL22	SPL34	SPL34	SPL42	SPL42

WAVELENGTH & CONCENTRATION TABLES

450

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.046	0.048	0.416	0.446	0.450	0.472	1.490	1.495	0.575	0.642	0.189	0.174
B	0.224	0.221	1.020	1.185	0.710	0.569	2.131	2.056	0.208	0.227	0.127	0.125
C	0.359	0.357	1.388	1.440	0.212	0.221	0.752	0.737	0.662	0.602	0.700	0.725
D	0.597	0.615	0.173	0.204	1.655	1.640	1.242	1.238	0.162	0.160	1.227	1.210
E	0.828	0.845	0.173	0.180	1.602	1.420	0.131	0.136	0.225	0.225	0.193	0.193
F	1.379	1.225	0.128	0.149	0.468	0.368	0.850	0.780	0.630	0.452	0.295	0.277
G	1.792	1.821	1.973	2.060	0.394	0.433	1.614	1.802	0.272	0.136	1.192	1.015
H	2.263	1.962	1.636	1.610	0.231	0.252	0.463	0.476	1.230	1.278	0.708	0.684

[Concentration]450

	1	2	3	4	5	6	7	8	9	10	11	12
A	<0.000	<0.000	2.006	2.215	2.243	2.401	16.437	16.565	3.188	3.748	0.623	0.543
B	0.814	0.798	7.799	10.222	4.357	3.140	49.486	42.535	0.726	0.831	0.304	0.294
C	1.627	1.614	14.041	15.215	0.748	0.798	4.757	4.612	3.923	3.409	4.265	4.498
D	3.368	3.518	0.538	0.704	21.271	20.772	11.188	11.117	0.481	0.470	10.926	10.637
E	5.527	5.709	0.538	0.575	19.566	14.753	0.324	0.348	0.820	0.820	0.644	0.644
F	13.847	10.892	0.309	0.414	2.372	1.685	5.763	5.033	3.645	2.257	1.227	1.119
G	26.555	27.873	36.365	42.869	1.857	2.123	19.938	27.001	1.090	0.348	10.337	7.733
H	>52.500	35.642	20.641	19.813	0.854	0.973	2.336	2.430	10.978	11.837	4.339	4.119

Standard Curve

StdCurve