Acknowledgments:

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Introduction

Background and Objective

In recent decades, population health studies have recognized the utility of biomarkers as a method for illuminating how experience "gets under the skin" and is reflected into physiological outcomes. C-reactive protein (CRP), a highly sensitive marker of systemic inflammation, has been recognized as a valuable biomarker for long-term health risk (Worthman and Costello 2009). Studies have linked CRP with a variety of health outcomes, including the Metabolic Syndrome, hypertension, and various cardiovascular diseases (Després 2012).

Cardiovascular disease is the leading cause of death globally, accounting for over 17 million deaths per year- a figure that is expected to rise to almost 24 million by 2030 (Benjamin et al. 2018); the prevalence of cardiovascular disease highlights the importance of analysis of CRP in population studies examining lived experience and differential well-being.

Several anthropometric and psychosocial variables have been shown to mediate circulating levels of CRP. Recent research has recognized the active role of adipose tissue as an endocrine organ involved in metabolic signaling and inflammatory processes. Studies have shown that visceral adipose tissue, and obesity more generally, influences circulating levels of CRP (Dandona et al. 2004). Stress is also a major driver of chronic, low-grade inflammation due to its biochemical effects on pro-inflammatory cytokines (Mortensen 2001). The associations between BMI, psychosocial stress, and inflammation have been examined in populations of middle-aged and older adults; however, few studies have considered the influence of these variables in a population of young, otherwise healthy individuals. Biomarkers have been shown to "track" over time, such that a child's risk of developing disease compared to their peers remains consistent over the lifecourse (McDade 2009; Seeman et al. 2014). In this manner, biomarker measurement in early adulthood may provide insight into pre-disease pathways and disease risk (McDade 2009). Studies that begin to examine these variables in younger individuals may provide additional insight into the origins of differential health outcomes such as cardiovascular disease early in the lifecourse of at-risk individuals.

The purpose of this study is to examine pathways between psychosocial stress and overweight/obesity in mediating levels of CRP in a population of university students. This study investigates daily stressors, levels of perceived stress, anthropometrics, and levels of inflammation. This study aims to expand upon limited research conducted on associations between psychosocial stress, BMI, and CRP in younger adults in order to assess potential risk factors for long-term health outcomes related to chronic, low-grade inflammation. In addition, this study aims to elucidate the particular sources and levels of stress that characterize the daily lives of university students.

Hypotheses

Hypothesis 1a: Students with higher scores on the PSS will have higher levels of CRP in young adulthood. This effect will be linear and will be will be evident in both men and women.

1b: Women will report higher perceived stress, on average, than men, and will have a higher average CRP at baseline.

1c. Frequencies of reported sources of perceived stress will vary in correlation with levels of perceived stress, such that certain factors are present only in those within the highest category of perceived stress.

Hypothesis 2: Students with higher BMIs will have higher levels of CRP in young adulthood. This association will be evident in those with BMIs in the overweight/obese category, but may not be evident in those within the “healthy” range, demonstrating a graded effect.

Hypothesis 3a: The impact of high BMI and high perceived stress will be additive, such that individuals with high BMI and high perceived stress will have the most elevated levels of CRP.

Hypothesis 3b: Higher levels of perceived stress will be positively associated with higher BMI, but only among those in the upper quartile of stress.

Hypothesis 4: Frequently listed sources of perceived stress will be uniform among men and women and among various age groups, but will be unique when compared with sources of stress identified by the general population.

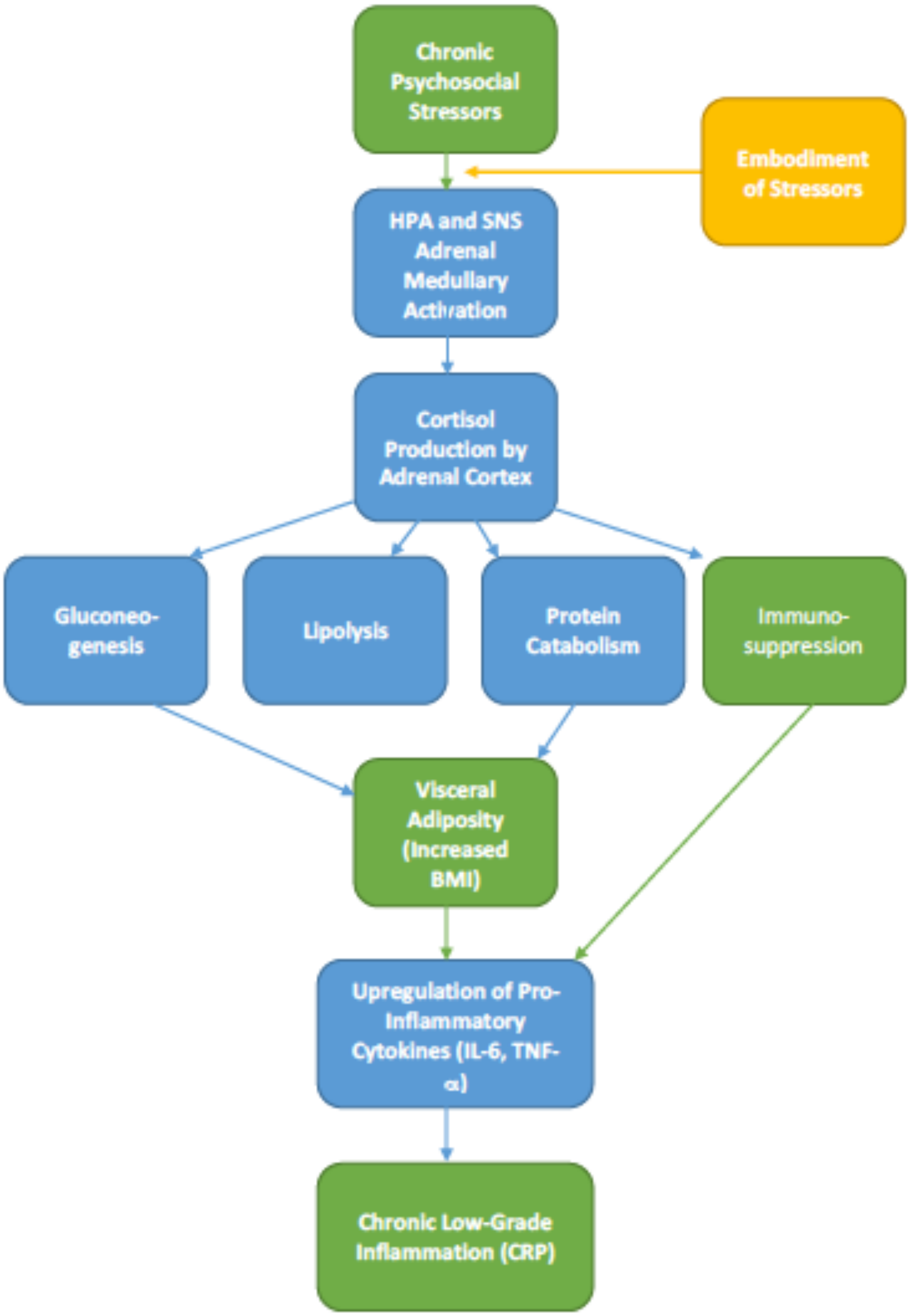

Figure 1. Flow diagram outlining the intersection of daily experience and pathophysiology as a framework for this thesis

Utility of C-reactive Protein

Biomarkers can be utilized to better understand the relationships between context, in this case a university environment, and physiological and psychological outcomes (McDade, Williams, and Snodgrass 2007). In this study, context is principally elucidated by self-reported sources of stress and corresponding levels perceived stress, which reflect the unique experiences of students in a university setting. CRP has been utilized frequently in population-level studies in the past decade, primarily because it is relatively inexpensive to measure using high-sensitivity assay techniques, has been found stable over long periods of time, and has no diurnal variation (Rifai 2003; Meier-Ewert et al. 2001).

CRP upregulation by hepatocytes is one of the multiple changes in protein synthesis and secretion following activation of the innate immune system and complement cascade (Mortensen 2001). At the site of injury or infection, macrophage activity leads to an increase in pro-inflammatory cytokines, including tumor neurosis factor-alpha (TNF-α) and interleukin-6, (IL-6), which stimulate synthesis and secretion of CRP (Rifai 2003; Ledue and 2003). CRP primarily acts as a protective molecule with the ability to identify certain pathogens (Burger and Dayer 2002). Recent studies have recognized the impact CRP may have on pathogenesis of disease, demonstrating its role beyond a nonspecific marker of inflammation (Mortensen 2001).

CRP may be used to index system burden and predict future risk of cardiovascular disease by reflecting an ongoing inflammatory response to cardiovascular wear (Ridker and Silvertown 2008). Moreover, CRP can index risk for diabetes and mortality more generally, since it indicates level of inflammatory responses to pathogenic conditions (Ridker and Silvertown 2008). CRP has been recognized as an indicator for allostatic load, and is typically elevated with increased adiposity, in part due to the production of pro-inflammatory cytokines such as IL-6 by adipocytes (Ridker 2008). The increased production of pro-inflammatory cytokines thus leads to an increase in hepatic CRP production, which contributes to low-grade inflammation (Ridker 2008). Due to its rather extensive general application as a predictor of inflammatory response, CRP is useful not only in considering future development of cardiovascular disease and diabetes, but also as a broader indicator of future health outcomes and accumulated stress burden (Worthman and Costello 2009). Figure 2 below, adopted from Libby and Ridker, displays the relationship between inflammation and the development of atherosclerosis and related coronary artery diseases.

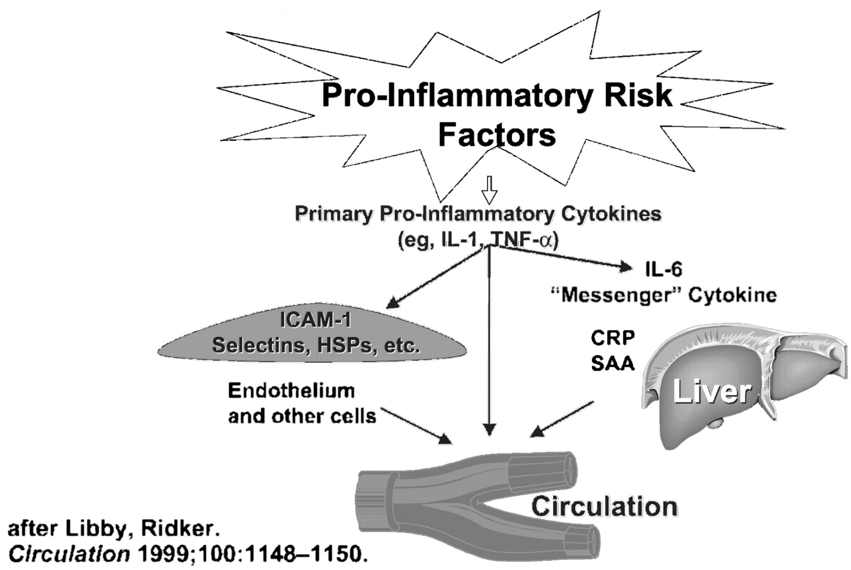

Figure 2. The inflammatory cascade. IL indicates interleukin; ICAM, intercellular adhesion molecule; and HSP, heat shock protein. Adapted from Libby and Ridker. *Circulation* 1999; 100:1148-1150

In some studies, women have been shown to have higher levels of CRP than men, though this finding varies (McDade et al. 2006). Clinical guidelines suggest that hs-CRP levels of less than 1mg/L indicate lower risk of coronary heart disease, 1-3mg/L indicates moderate risk, and a level greater than 3mg/L is indicative of higher risk, with levels of 10 mg/L adopted as a general cut-off for current infection (Salazar et al. 2014). Few studies have examined CRP levels in a population of young adults, particularly through the context of psychosocial stress and BMI. Since this population is expected to be relatively healthy, mean CRP levels are hypothesized to be in the lowest category of less than or approximately 1mg/L.

Defining Overweight/Obesity

Worldwide obesity prevalence has nearly tripled since 1975 and is associated with a range of health risks, including cardiovascular disease and Type II diabetes (WHO, "Obesity and Overweight" 2018). In correspondence with this global statistic, obesity rates in young adults in the United States have also risen in the past several decades (WHO, "Obesity and Overweight" 2018). This study will analyze overweight/obesity according to the guidelines established by the Centers for Disease Control and Prevention (CDC, "Body Mass Index" 2017). Individuals with a body mass index (BMI) of 18.5 to less than 25 are classified as normal weight, 25.0 to less than 30 are classified as overweight, and 30.0 or higher is classified as obese (CDC, "Body Mass Index" 2017). Obesity is then divided into three subcategories: Class 1- BMI of 30 to less than 35, Class 2- BMI of 35 to less than 40 and Class 3- BMI of 40 or higher (CDC, "Body Mass Index" 2017). For the purpose of this study, classifications will only consider overweight/obesity in general. BMI has been shown to be an indicator of overall health, and is moderately associated with other measures of body fat including skinfolds. BMI is also easily calculated from height and weight, and is less invasive than other measures of body fat.

Psychosocial Stress in University Students

The term "stress" may be used to describe adverse environmental conditions, discomfort, psychological, emotional, and physical problems, or a physiological outcome resulting from biological processes (McDade 2009). As proposed in Hans Selye's original model for stress research developed in the 1930s, stress incorporates the following: 1. A stressor, 2. A response, 3. Consequences, and 4. Moderators (McDade 2009). Stressors will be self-reported in this study, and potential consequences may be interpreted through an evaluation of responses on the Cohen-PSS scale (Cohen, Kamarck, & Mermelstein, 1983). Individuals may vary in their appraisal of events as stressful or non-stressful, and this variation is of interest for this study. Additionally, stressors may be classified according to duration as acute, subacute, and chronic, and may also vary in severity and intensity (McDade 2009). Both chronic and acute psychosocial stress can "fatigue" the HPA-axis, resulting in a decreased anti-inflammatory reaction, allowing pro-inflammatory responses to go unregulated (McEwen 2008). Chronic psychosocial stressors are frequently studied in association with long-term health outcomes particularly through the actions of glucocorticoids (McEwen 2008). Activation of the HPA axis pathway promotes the release of glucocorticoids from the adrenal cortex, which can suppress the production of pro-inflammatory cytokines and induce an anti-inflammatory response (McEwen 2008). Moreover, these glucocorticoids stimulate gluconeogenesis in the liver, which increases blood sugar levels; chronically, this may lead to an increase in visceral adiposity due to reabsorption of the glucose (McEwen 2008). This study will examine proximate daily stressors and perceived stress over the course of a month.

Analysis of stress in university students provides researchers with the ability to examine potential differences between stress during and after a university environment through comparative population-level analyses. University students face a unique variety of stressors, some of which have been identified in previous research studies. For many young adults, the transition to a university setting increases daily pressures and demands, ranging from academia and workload to social acclimatization. The long-term accumulation of these daily stressors has been correlated with negative psychological and physiological effects, including stroke, cardiovascular disease, depression, and problem drinking (McEwen 2008). Previous research has examined the relationship between perceived stress and mental health in college students, demonstrating that high levels of perceived stress are associated with poor mental health, lower physical activity, and lower academic achievement when compared to peers with less perceived stress (Leppink et al., 2016). Though investigations of stress in university settings are relatively limited, one study in Australia found that the majority of university students reported elevated levels of distress that were significantly greater than those reported in the general population (Stallman 2010). Stallman found that self-reported rates of mental health issues were higher among university students than the general population, suggesting higher levels of perceived stress (2010). Stress research has found that not all students feel or express stress in the same manner, with gender, personality and temperament contributing to variation in stress response and coping (Leppink et al. 2016; Barbosa-Leiker 2014; Stallman 2010).

Psychosocial stress may influence physiology through both direct and indirect pathways. The impact of stress on the regulation of neuroendocrine activity via the hypothalamic adrenal axis as well as sympathoadrenal system serves as a direct pathway for the embodiment of individuals' lived experiences with stress (McEwen 2008). Stress may also affect physiology indirectly by affecting health behaviors such as diet, eating behaviors, exercise, and alcohol and drug use (Boyce and Kuijer 2015). Research investigating the relationship between stress and obesity has similarly produced variable findings. Wardle and colleagues conducted a meta-analysis in order to analyze these associations in a systematic report of 14 studies conducted in the United States and England. Their findings show that more analyses demonstrated a significant positive effect of stress on adiposity (25%) than those that demonstrated a significant negative effect; however, the majority of studies (69%) found a null effect (Wardle et al. 2010). Their synthesis found in more studies acute stress and the development of obesity was more strongly correlated in men than women, though the pathway that differentiate individuals based on sex is unclear. Previous studies have demonstrated that men typically have higher physiological responses to acute-stress than women, despite findings that women generally report higher levels of perceived stress than men (Stoney, Davis, and Matthews 1987).

This study utilizes the Cohen 10-item perceived stress scale as a measure of perceived stress, which includes items designed to measure how unpredictable, uncontrollable, and overloaded respondents find their lived experiences over a period of one-month (Cohen, Kamarck, and Mermelstein 1983). The PSS contains positively-worded items aimed to offer insight into perceptions of ability to counter stress or cope with unexpected situations. This study additionally included a free-write component in which individuals were asked to list five sources of daily stress in their lives. This study will be one of the first to combine the qualitative analyses utilized in studies of perceived stress with quantitative analysis of inflammation to examine these relationships.

Influence of Stress and Overweight/Obesity on Inflammation

Previous studies investigating factors affecting inflammation have examined both stress and obesity; however, far fewer studies have considered the impact of these two variables in conjunction. Black and colleagues show that stress hormones including glucagon and corticosteroids may mimic the acute phase response generated in the body following a sustained injury (Black 2002). Research has demonstrated that interleukin-6, one of the pro-inflammatory cytokines responsible for increasing levels of CRP, may act as the central mediator of the acute phase response (Black 2002). Perceived stress has also been associated with impaired immune function in correlation with increased levels of pro-inflammatory cytokines including interleukin-6 and tumor necrosis factor-alpha, both of which are associated with the production of CRP (Barbosa-Leiker et al 2014). Despite the associations between perceived stress and interleukin-6, data analyzing the associations between perceived stress and CRP has yielded more mixed results. In an analysis of this relationship, Barbsoa-Leiker and colleagues used a longitudinal study design to examine patterns of perceived stress and CRP (2014). Despite previous literature demonstrating higher levels of perceived stress reported by women, their study did not find any sex differences in stress scores at baseline (2014). The study did report higher levels of CRP in women across the entire 4-year span, but did not control for age. The predominant finding was a positive relationship between BMI and CRP, which was consistent in both sexes, and aligns with previous literature (2014). While the study found that stress and CRP did correlate at the 4-year mark in women, this finding is very limited and does not provide a robust view of the pathways between CRP and stress that are more evident at four years as opposed to earlier time frames. Yudkin and colleagues also reported associations between IL6 and psychosocial stress, finding that IL6 is elevated in individuals who are experiencing stressors related to their sociocultural environments including socioeconomic grade, job stress, and migration (Yudkin et al. 2000).

Obesity has been shown to have an influence on circulating levels of CRP. Studies have shown that production of pro-inflammatory cytokines, and thus CRP, increase with adiposity; moreover, nearly one-third of circulating IL-6 may originate from adipose tissue (Yudkin et al. 2000). Yudkin and colleagues demonstrated that differences in levels of CRP can be contextualized through the application of anthropometric measures of obesity (2000). Increasingly, research has emphasized the crucial role of adipose in regulating and influencing metabolic signaling and inflammatory processes. CRPs associations with chronic disease can partially be explained by obesity, as studies have shown that obesity alone is associated with a myriad of diseases including Type 2 diabetes mellitus and cardiovascular disease, collectively known as Metabolic Syndrome (Yudkin et al. 2000).

Excess adiposity, unlike an acute stimulant or wound, induces a state of chronic, low-grade inflammation within the body (Johnson, Milner, and Makowski 2012). While studies have linked the distinct relationship between obesity and inflammation, few have investigated the relationship among these variables in a young population. This study hopes to illuminate the extent of influence BMI has on overall levels of inflammation in otherwise healthy individuals.

These variables have do not operate in isolation from one another, but are instead part of a complex and multifactorial psychophysiological network. This study analyzes psychosocial stressors as a proxy for an environmental context that may lead to unique physiological outcomes, including obesity and inflammation. While, stress and adiposity may impact inflammation, both of these variables may also impact one another, as higher levels of perceived stress and mental illness have been correlated with higher BMI and are linked through the direct and indirect mechanisms previously outlined (Isasi et al. 2015). Similarly, chronic low-grade inflammation and obesity in individuals may impact perceived stress, particularly if individuals are coping with disease and illness.

Summary

Few studies have examined inflammation in younger populations, leading to a limitation in the literature regarding the emergence of health differences in younger individuals. This study seeks to combine previous research on stress and inflammation with research on BMI and inflammation in order to examine the potential additive impacts of these variables. Notably, this study also seeks to examine sources of stress associated with collegiate experiences in order to illuminate potential differences in perceived stress and the proximate ecology of stress for students. This study takes a unique, biocultural approach through the combination of both quantitative and qualitative methods with the addition of free-listing regarding sources of stress. This research aims to contribute to our understanding of the interactions between stress and body composition on inflammation and metabolic risk in young adults.

Chapter 2

Methods

Subjects and Study Design

Data used for this project was gathered through free flu clinics held at the University of North Carolina at Chapel Hill over the course of three years from 2015-2017. The data used in the present analysis was collected in conjunction with a project analyzing the associations between psychosocial stress, BMI, and immune resolution using the flu shot as a proxy for the latter variable. Subjects were recruited through convenience sampling after receiving a flu shot, at which time the data used for this study was collected. Participation was limited to undergraduate, graduate, and professional students over the age of 18 at the university. In total, 106 individuals participated in the study, ranging in age from 18-36. Demographic questions were limited to sex and age for the scope of this study. Height and weight were measured to calculate BMI.

Assay Methods

Blood samples for analysis of c-reactive protein were obtained using dried blood spots (DBS) collected using non-invasive finger stick and Whatman No. 903 protein filter paper. For the present sub-study, blood samples were collected one time from each subject. Samples were stored at approximately -25 degrees Celsius until the time of assay analysis. Assays were conducted in two phases, the first in 2015, and the second in March of 2018 at the Human Biology Laboratory at the University of North Carolina at Chapel Hill. Analyses were conducted using an enzyme-linked immunosorbent assay technique with a R&D systems human C-reactive protein Quantikine ELISA kit. DBS samples were prepared for analysis using a 1/8" punch of the sample placed into standard test tubes, and immersed in elution buffer. Samples were incubated overnight and stored at approximately 38 degrees Fahrenheit. Blood spots that were too small to extract an appropriately sized punch, or that were not sufficient in quality were excluded from the study (15 samples were excluded).

Following incubation, 50 µL of either standard, control, or sample were added to the wells. Standards were prepared through a serial dilution of knowns provided with the ELISA kit. The microtiter plate contained eight standards and ten samples run as duplicates, as well as three Quantikine controls run as duplicates for low, middle, and high concentrations of cytokines. To quantify the concentration of CRP the wells of the plate were pre-coated with anti-CRP antibody to which the CRP in the sample binds. This allows the CRP to remain in the well while the rest of the sample is removed through a series of washes. A second antibody, conjugated to an enzyme that catalyzes a color change following the addition of its substrate is then added to the wells. Subsequently, the enzymes substrate is added and the sample will change color from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of CRP, and can be detected using a microplate absorbance reader. For this assay, optical densities were calculated using a Biotek ELx800 absorbance microplate at 450nm and 630nm, for corrective purposes. Standards for which there is a known concentration of CRP are used to compare the color change of the standards with that of the samples through the construction of a calibration curve. Gen5-Version 2 software by Biotek technologies was used to complete this step of the procedure. CRP cut points for low (<1 mg/L), moderate (1 to 3 mg/L) and elevated inflammation (3 to 10mg/L) are taken from clinical practice (Pearson et al. 2003). For a full lab protocol, see Appendix A.

Anthropometry

Anthropometric measurements were taken using standard techniques. Stature was measured to the nearest 1mm using a portable stadiometer; weight was measured using a digital scale and recorded to the nearest kg. Body mass index (BMI) was calculated as weight(kg)/height(m^2). Cut-points for BMI classification were adopted from the CDC guidelines (CDC "Body Mass Index" 2017). Individuals in the "overweight" or "obese" category have higher accumulations of fat, which may impair their immune function and their overall health. It should be noted that BMI is the common standard; however, it does not measure precise adiposity in individuals.

Surveys and Free-Listed Stressors

Participants completed a demographic and disease symptom history survey to collect relevant demographic and health information.

The 10-item Cohen Perceived Stress Scale (PSS-10) was used to assess perceived stress of participants (Cohen, Kamarck, and Mermelstein 1983). Four out of the ten items of the PSS-10 are positively stated and the remaining six are negative. Each item was rated on a five-point scale (0=never to 4=very often). Total scores are calculated by reversing positive items' scores and then summing across all items. Possible total scores for the PSS-10 range from 0 to 40 with higher scores indicating higher perceived stress.

The present study utilized free-list response methods in order to analyze the top five daily sources of stress in students' lives. Free-list responses were coded and analyzed using MAXQDA qualitative data analysis software. Mixed-methods analyses examining associations between PSS-global scores and reported factors of stress were also conducted using MAXQDA software.

Statistical Analysis

CRP levels were log-transformed in order to normalize the distribution prior to analysis. Regressions analyses were used to measure potential associations between variables for this study. CRP was used as the dependent variable for the quantitative analyses. Age, PSS-10 global scores, BMI, and sex were analyzed as independent variables in this context. Quantitative analyses were conducted using SAS JMP 13 software for Mac. Results were considered statistically significant at $P < 0.05$.

Chapter 3

Results

BMI and Overweight/Obesity Prevalence

Table 1 shows summary statistics for BMI data by sex. The mean BMI for female participants was 23.8 ± 5.9, while mean BMI for male participants was 23.1± 2.9. There was no statistically significant difference between BMI in men and women in our study (p=0.754). Figure 1 displays BMI distributions by sex for our sample. As is evidenced in the scatterplot and the box and whiskers plot, most women (68%) are clustered at BMI's of 20.0-25.0, with a few outlying points around 35.0, and an outlier at 53.6. Male BMIs were less variable, as is expected given the sex-ratio difference between women and men in our study.

Mean Values and Classifications for BMI

	Men	Women
N	25	50
Mean	23.1	23.8
Standard Deviation	2.9	5.9
Median	23	22.3
Underweight (BMI <18.5)	1	1
Healthy weight (BMI 18.5-24.9)	19	39
Overweight (BMI 25.0-29.9)	5	4
Obese (BMI >30.0)	0	6

Table 1. Summary statistics and BMI Classification (according to CDC 2017 Guidelines) for male and female participants

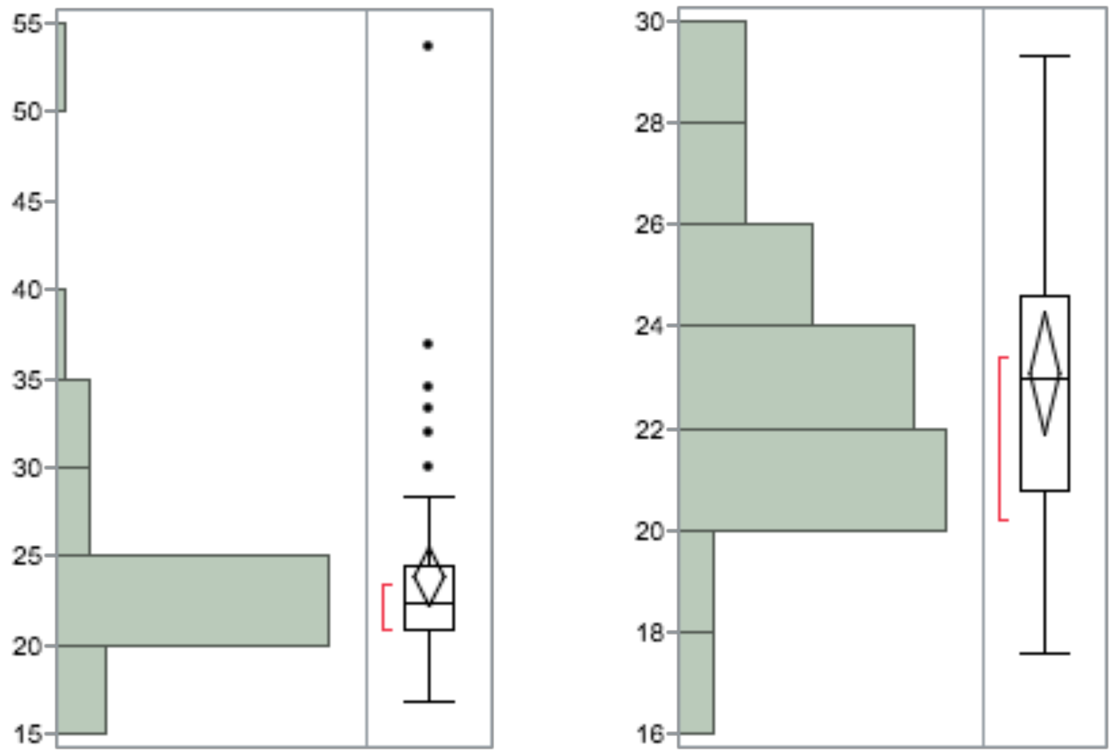

Figure 1. BMI distribution for women (left), and distribution for men (right)

Men in our sample had a more normal distribution, while women had a more skewed distribution overall. From our sample, the majority of men and women were classified within the "healthy" BMI range (n=20 and n=20, respectively). Five men (20%) and four women (8%) were classified as "overweight", and six women were classified as "obese" (12%).

Analysis of BMI as a dependent variable against age produced no significant correlations.

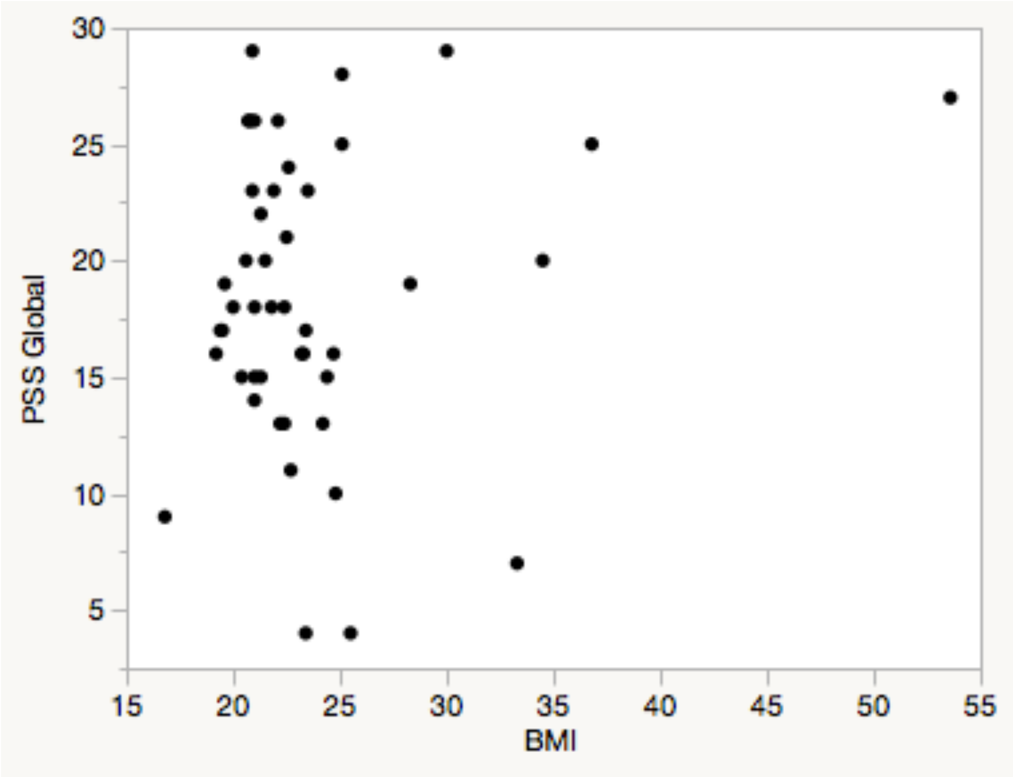

Figure 2. Scatterplot of PSS Global against BMI for female students, r=0.181. p=0.229,

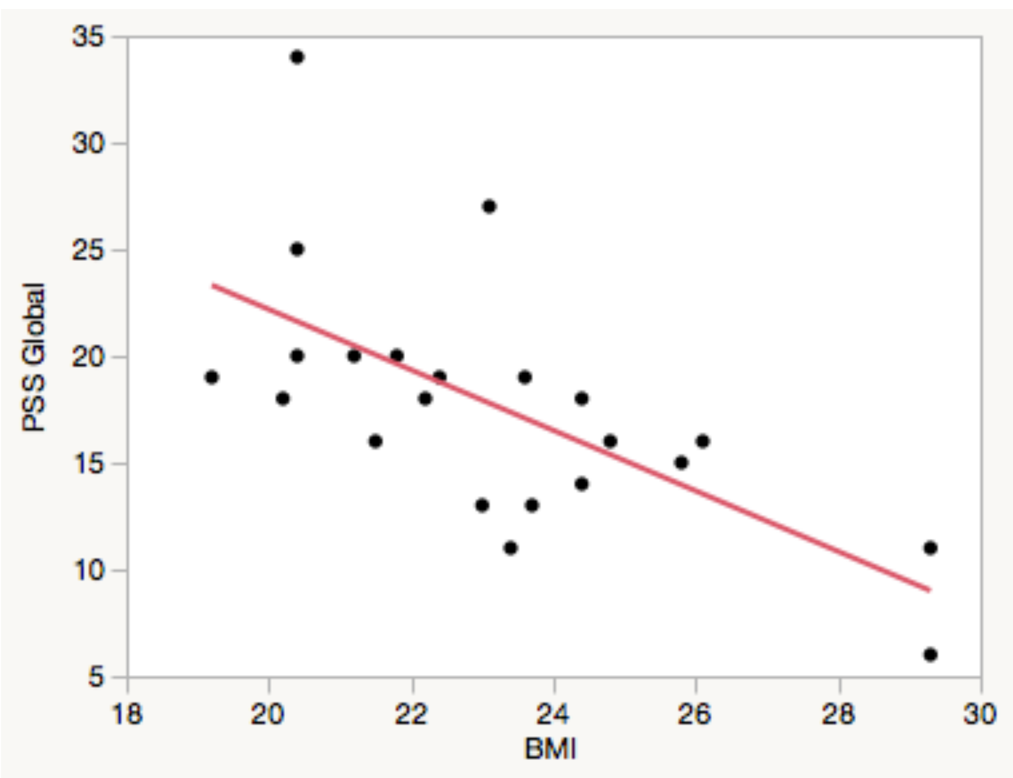

Figure 3. Scatterplot of PSS Global against BMI for male students, r=0.653, p=0.001

The scatterplots above (Figures 2 and 3) demonstrate the correlation between BMI and PSS global score, which is statistically significant in male students (p=0.001), but not in female students. The correlation in male students is negative and linear, indicating that those with higher BMIs had lower levels of perceived stress.

Cohen PSS-10 Perceptions of Stress

Table 2 displays summary statistics for the PSS-10 (PSS) by sex. The mean PSS score for women was 17.3 ± 5.9, while mean PSS for men was 18.6 ±6.2. Contrary to Hypothesis 1, there was no statistically significant difference between men and women in our study ($p=0.191$). Figure 4 shows PSS distributions by sex for men and women, respectively. The overall distribution for men and women was similar, with the exception of a male outlier with a PSS score of 34.

	Men	Women
N	25	50
Median	18	18
Mean	17.3	18.6
Standard Deviation	5.9	6.2

Table 2. PSS Summary Data by Sex

The median PSS score for men and women was the exact same, at 18. These data are similar to reference values reported by Cohen and Janicki-Deverts collected in 2009; comparisons of this data with normative population data are expounded upon in the PSS section of the discussion chapter (Cohen and Janicki-Deverts 2012).

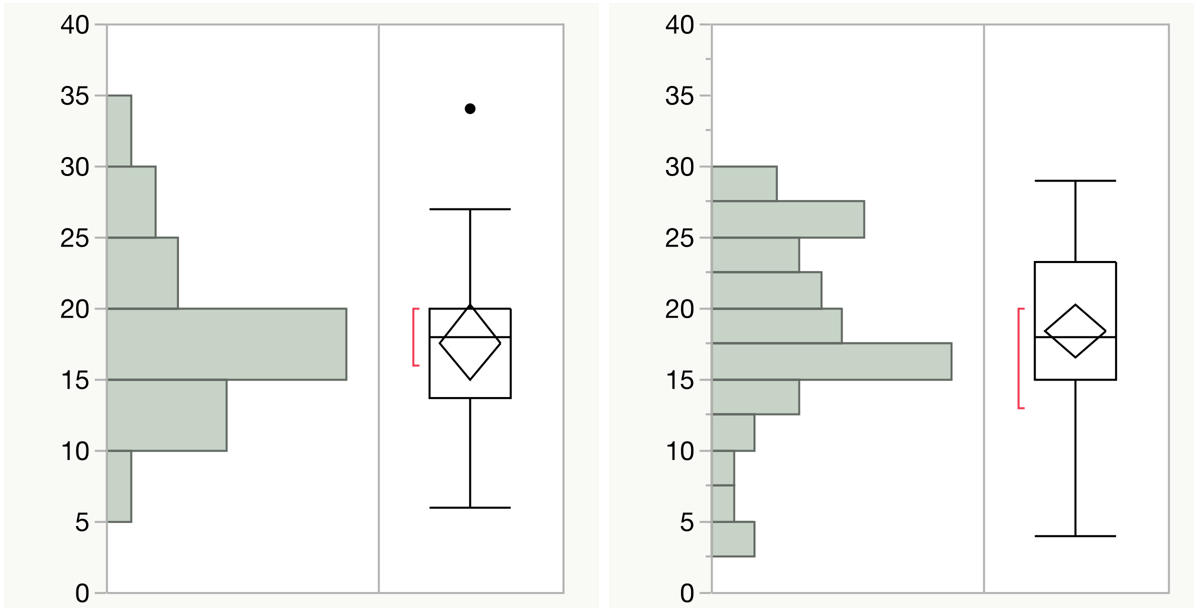

Figure 4. PSS Distribution by sex, with men displayed on the left and women on the right

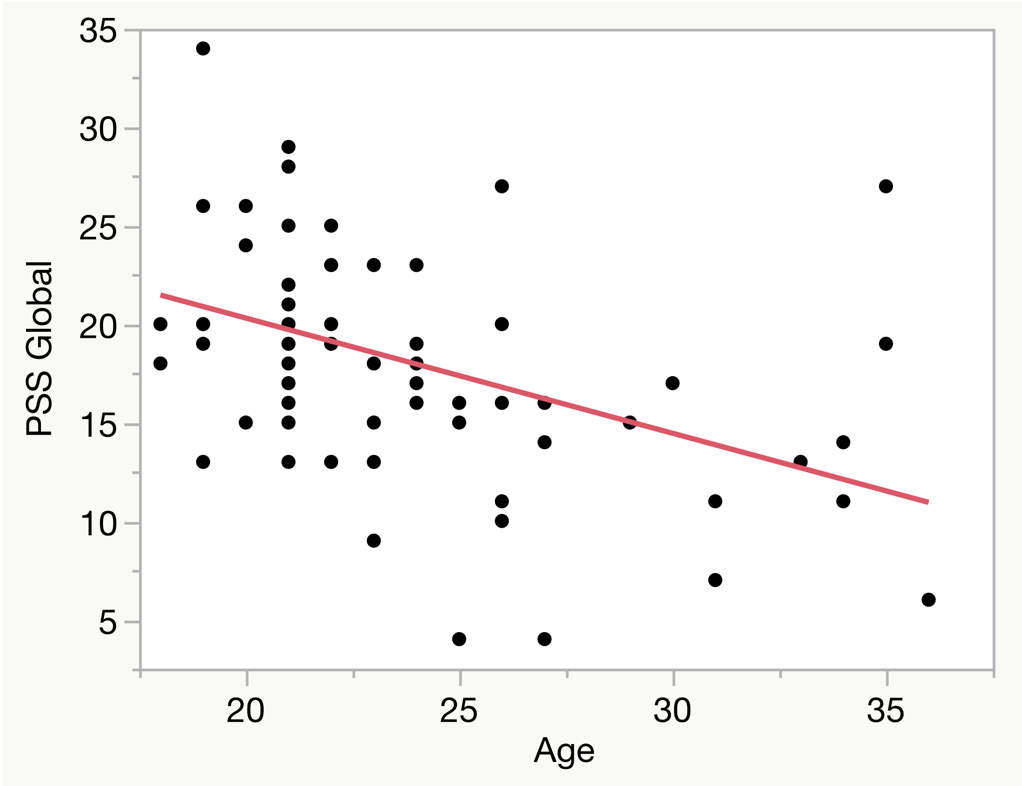

Figure 5. Bivariate analysis of PSS vs Age; r =-0.431, p=0.0002

Previous studies have found associations between age and PSS, which were examined in this study. Regression analysis of PSS global against age does show a statistically significant negative correlation between the variables (-0.431, p=0.0002).

Free-Listed Factors of Stress

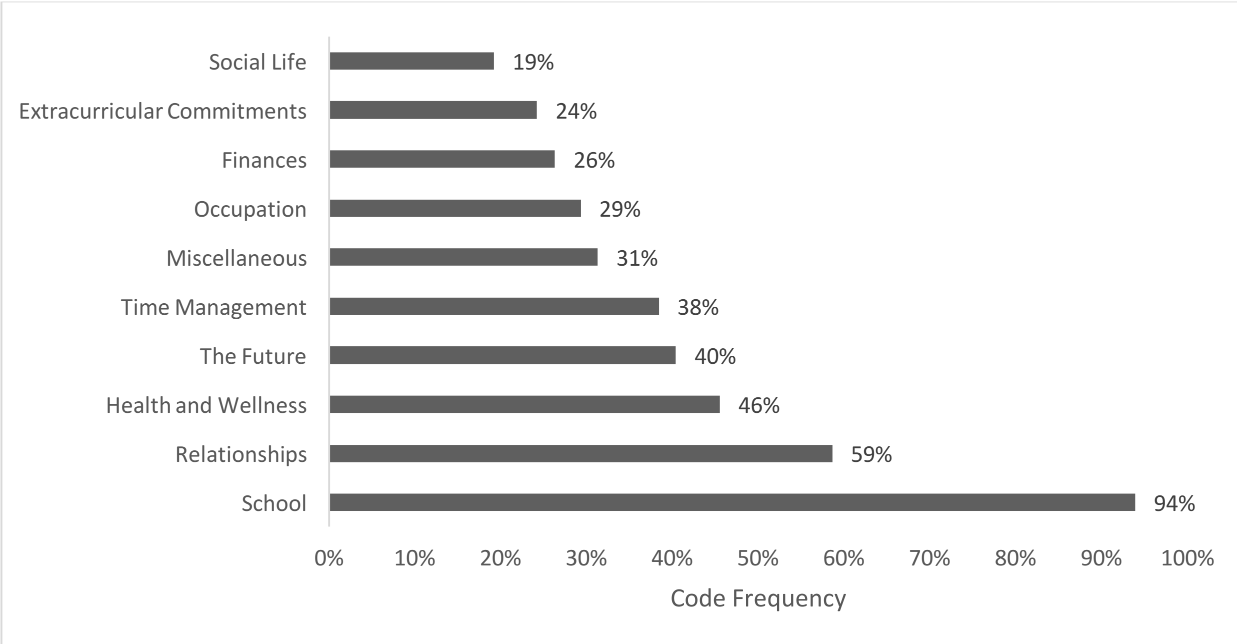

Figure 6. Code Frequency for Free-List Data

Figure 6 displays the most frequently recurring code, which included the following: "school", "relationships", "health and wellness", "the future", "time management", "occupation", "finances", "extracurricular commitments", "social life", and a "miscellaneous" code that included items that fell into individualized categories. From these codes several subcodes were identified, which are discussed below.

The coded data demonstrates the emergence of five frequently listed sources of stress for participants in this study. "School" was the most frequent code, and was utilized in 93.9% of respondents' free-lists; moreover, "school" was shown to be the most salient code; for a visual display of code salience see Appendix B, Figure 2. School was expected to be both frequent and salient considering that the study occurred on a university campus and was limited to undergraduate or graduate/professional students. Given the academic environment in which students are immersed, it is not surprising that “school” was by far the most prevalent and salient source of stress for students. This code was broken down into four prominent subcodes, which are discussed later in this section.

Following school, “Relationships” was the second most employed code, with a frequency of 59%; again, this code was broken down into subcodes, discussed later. “Health and wellness”, “the future”, and “time management” were the next most employed codes with frequencies of 46%, 40%, and 38%, respectively. Other codes utilized in the study had frequencies below 1/3, which signifies the distinction between the “top 5” stressors, and the other code items listed.

Subcode Frequencies

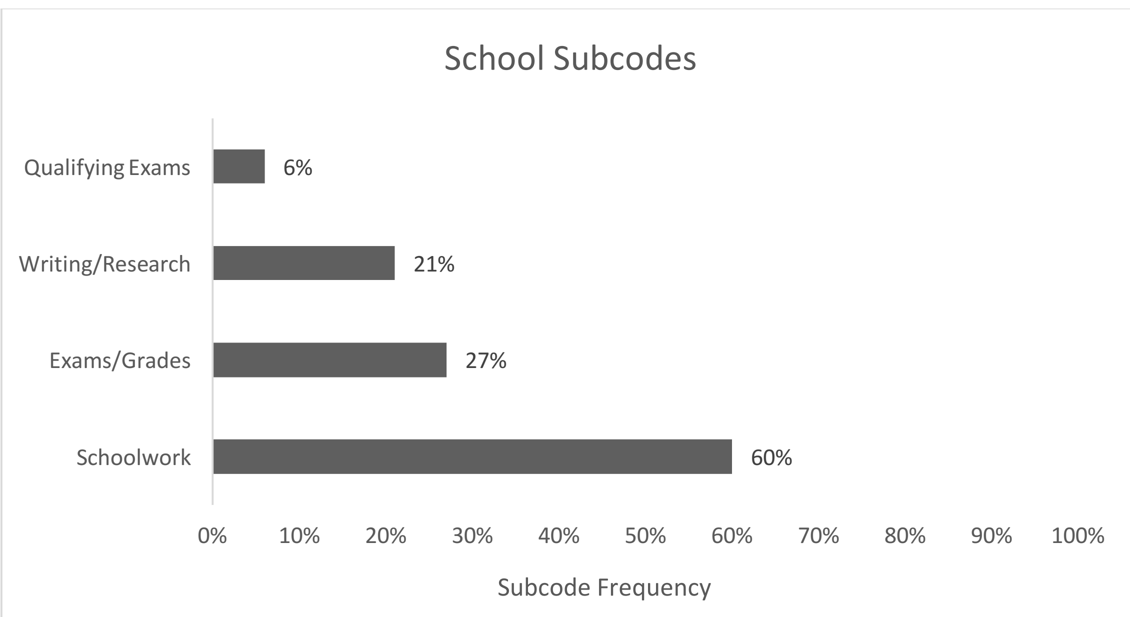

Figure 7. Subcode Frequencies for Code “School”

Figure 7 displays frequencies for subcodes within the “school” domain. Notably, some free-list items may be categorized into multiple subcodes, for instance a response that lists “School (exams, grades, assignments etc.)” would be coded based on specificity to “School”, with subcodes “Exams/Grades” and “Schoolwork”. Frequencies for subcodes of “School” are as follows: “Schoolwork” (60%), “Exams/Grades” (27%), “Writing/Researching” (21%), and “Qualifying Exams” (6%).

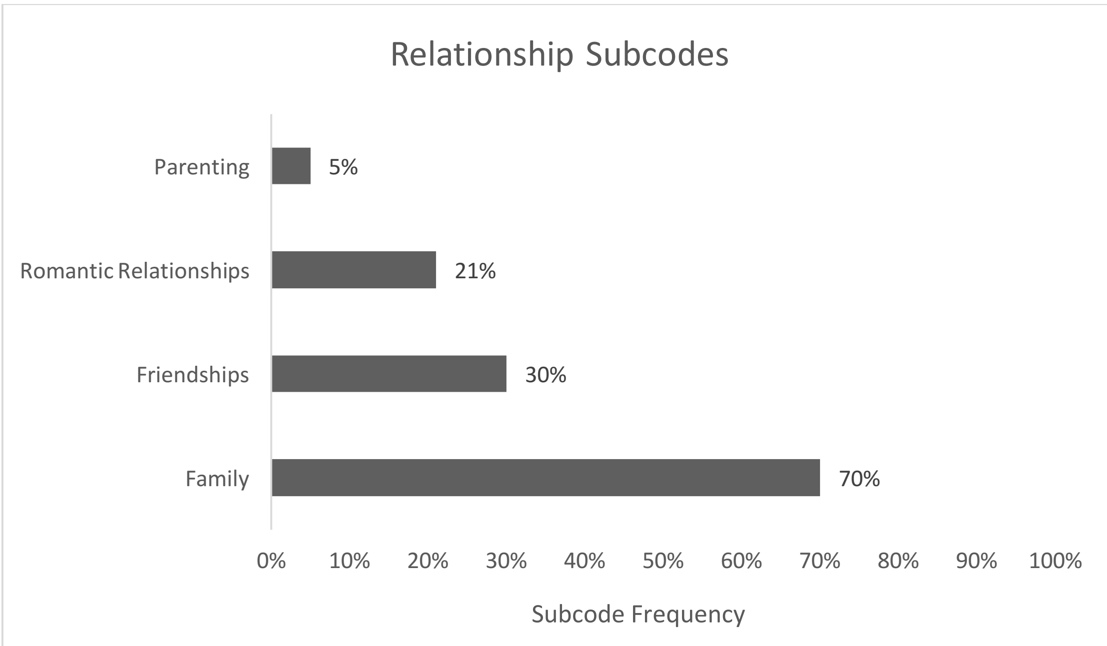

Figure 8. Subcode Frequencies for Code “Relationships”

Similarly, Figure 8 displays frequencies for subcodes of “Relationships”, which are as follows: “Family” (69.9%), “Friendships” (30.2%), “Romantic Relationships” (20.9%), and Parenting (4.7%). Much like the responses within the codes for “school” many respondents’ answers were multifaceted and often included a connotation of worry or anxiety-associated stress, as will be discussed in later sections.

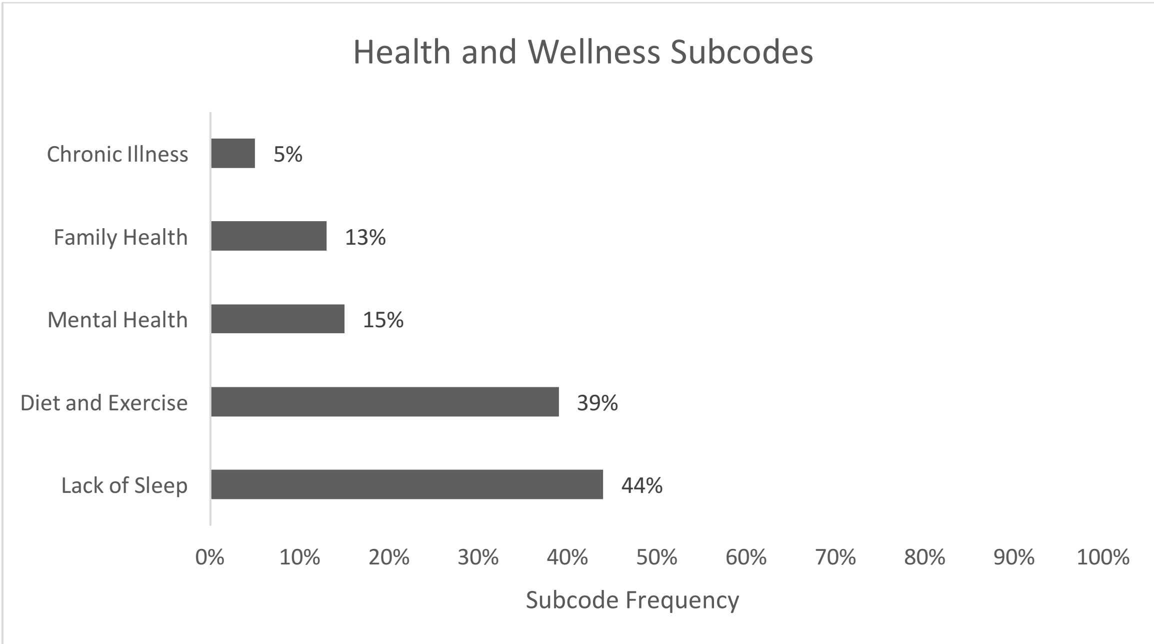

Figure 9. Subcode Frequencies for "Health and Wellness"

Recurring subcodes that emerged within the "Health and Wellness" category are as follows: "Lack of Sleep" (44%), "Diet and Exercise" (39%), "Mental Health" (15%), "Family Health" (13%), and "Chronic Illness" (5%). Again many of these items were associated with words such as "worry" and "anxiety", which provides a more nuanced meaning to the subcodes.

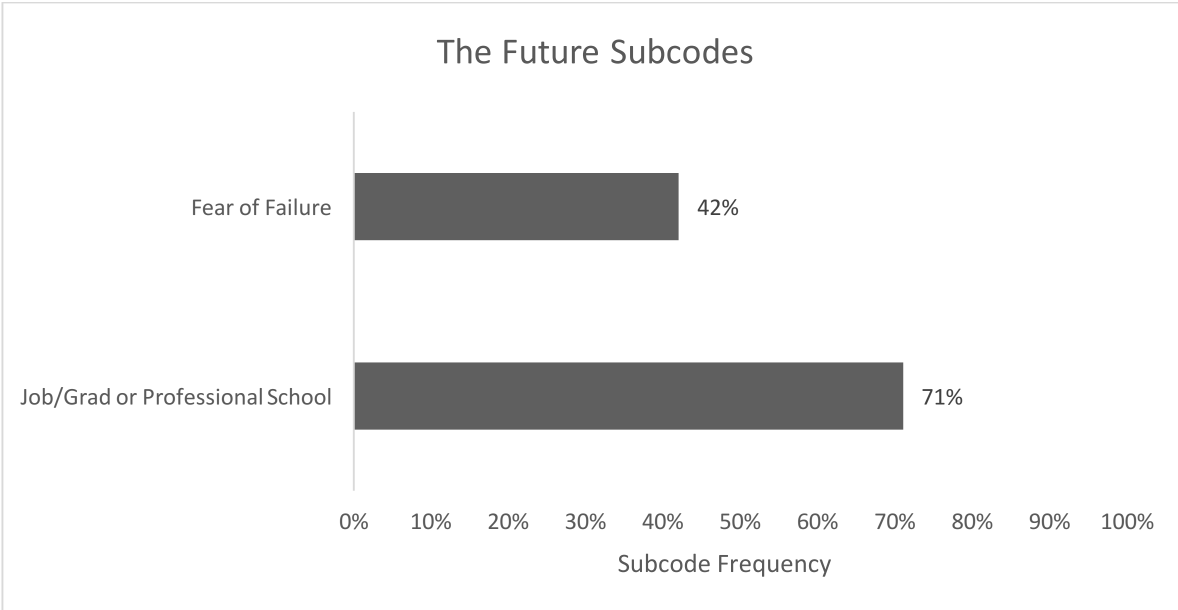

Figure 10. Subcode Frequencies for “The Future”

Of the “top 5” coded stressors, “The Future” was most often mentioned outright, and therefore, was only broken down into two recurring subcodes, “Job Prospects/Graduate or Professional School” and a “Fear of Failure”, with frequencies of 71% and 42% respectively. While the former is defined clearly in terms of a “future prospect”, the latter subcode is more difficult to define, but was most often mentioned outright as a “fear of failure” or “being successful”; these complexities are parceled out more in the discussion section, with additional examples provided in the chart below.

Multi-Method Analysis of Free-List Stressors

	Total % of Low to Mod PSS Individuals	Total % of High PSS Individuals
School	93	100
Relationships	59	57
Health and Wellness	44	57
The Future	41	36
Time Management	39	36
Schoolwork	38	43
Family	**33**	**14**
Social Life	**17**	**36**
Exams/Grades	**14**	**36**
Writing/Researching	13	14
Commuting	**13**	**0**
Friendships	12	21
Romantic Relationships	**5**	**29**
Living Situation	**2**	**29**
Mental Health	**2**	**29**

Table 3. Abbreviated Frequency of Code Occurrence by PSS; for full comparison see Appendix B

Table 3 displays a comparison between participants categorized as low-moderate stress and individuals categorized as high stress. Though the Cohen-PSS does not have an explicit categorization, these categories were determined from the normative data collected by Cohen and Devert (2012). For the purpose of this study, "High Stress" individuals had PSS values higher than one standard deviation from the reference data, and therefore had scores of 25 or higher. All other individuals were lumped into a low to moderate category of stress. Using these categories, 14/75 (19%) of individuals were categorized as experiencing "high stress". By separating individuals in this manner, similarities and differences between coded item frequencies between these groups can be observed. The most frequent overall codes remain fairly similar between both groups; however, several prominent differences are also evident.

As expected, individuals in the high stress group had a significantly higher frequency of "Mental Health" as a subcode (29%) than those in the low-moderate stress category (2%). The specificity of "Mental Health" coded items including "depression" and "anxiety" are likely indicative of increased perceived stress, as demonstrated by previous studies (Stallman 2010; Leppink et al. 2016). Another significant difference occurs within the subcode "Exams/Grades", with low-moderate PSS frequency of 14% as compared to 36% in high PSS individuals. The increased specificity of the subcode may suggest that individuals who perceive school to be a source of stress because of exams and grades experience higher perceived stress than those who perceive school as a more general stressor. Examples of the free-list that support this hypothesis include those that mention exams and grades in the context of anxiety or worry, as documented by one participant with a PSS score of 25 who explicitly listed "schoolwork/worrying about grades (not enough hours in the day)" as their most salient stressor.

Frequency of the subcode "Romantic Relationships" also differs between low-moderate and high PSS participants, with a frequency of 6% among low-moderate stress individuals and a frequency of 29% among high stress individuals. Again, the increased specificity of "Romantic Relationships" may indicate that individuals who see this specific type of relationship as a stressor may experience higher perceived stress than those who listed "Family", for instance. In conjunction with the aforementioned observations, the code "Social Life" was more frequent among high PSS participants (36%) than low-moderate PSS participants (17%). Similarly, the subcode "Living Situation" was significantly more frequent among high PSS participants (29%) than low-moderate PSS participants (2%).

	Total % of Women	Total % of Men
School	96	90
Relationships	57	62
Health and Wellness	46	45
The Future	40	41
Time Management	39	38
Schoolwork	36	45
Finances	**31**	**14**
Exams/Grades	**21**	**7**
Friendships	**16**	**7**
Job/Graduate or Professional School	**13**	**28**

Table 4. Abbreviated Frequency of Code Occurrence by Sex; for full comparison see Appendix B

Table 4 displays the frequencies of code occurrence for men and women in this study in order to examine potential similarities and differences between the sexes. As was previously noted, primary codes remained similar between the sexes, suggesting that perceptions of major factors of stress are similar between men and women. Overall, frequencies between the sexes are fairly similar for most coded and subcoded items; however, a few significant differences are evident. Women in the study listed items related to “Finances” at a higher frequency than men, with rates at 31% and 14%, respectively. This difference suggests that, overall, women in our study perceive finances to be a more prominent source of stress in their daily lives than men in our study. Women also listed items assigned the subcode of “Exams/Grades” with a higher frequency (21%) than men (7%). Conversely, men in our study listed items related to “Schoolwork” with a higher frequency than women, though to a lesser extent at 45% and 36%, respectively. Men in the study also listed items related to the subcode “Job Prospects/Graduate or Professional School” with a higher frequency than women, at 28% and 13%, respectively.

Codes	School	Relationships	Health and Wellness	The Future	Time Management
Subcodes	Schoolwork, Writing/ Research, Exams/Grades, Qualifying Exams	Romantic Relationships, Family, Friends, Parenting	Lack of Sleep, Mental Health, Diet and Exercise, Family Health, and Chronic Illness	Fear of Failure	Job Prospects/ Graduate or Professional School, Deadlines
Examples	"schoolwork (being behind)" "dissertation writing anxiety" "schoolwork/ worrying about grades (not enough hours in the day)" "school expectations" "school work accumulation" "grad school as a whole" "upcoming thesis deadline"	"maintaining long-distance relationship" "family obligations" "needs of husband" "friends and family to support/ worry about" "miss my family" "finding time with significant other" "love life"	"not enough sleep" "mental health conditions" "my health/wondering if I am healthy" "weight loss/fitness" "chronic fatigue" "my weight/diet/ exercise" "depression"	"worrying about the future and not being good enough" "future aspirations panning out" "what I am doing after I graduate" "being successful" "fear of failure"	"not enough time to complete tasks" "not having time to spend with friends and family" "finding enough time in the day to finish everything" "running late" "keeping up with deadlines"

Figure 11. Code chart providing subcodes and examples

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Inflammation as Characterized by C-reactive protein

As described in the "Methods" chapter, CRP samples were fit to a standardized curve based on eight samples of known concentrations run as duplicates for the ELISA assay. Figure 1, below, displays the standard curve to which the March assay was fit. The serial dilution should produce standards with known values of 0.0 ng/mL, 0.78 ng/mL, 1.56 ng/mL, 3.12 ng/mL, 6.25 ng/mL, 12.5 ng/mL, 25 ng/mL, and 50 ng/mL. Standard values for our assay are displayed below in blue, with orange representing the mean between the two duplicates. Analysis of the variance between the two duplicates, as shown below, displays our relative margin of error for each of the known standards.

Additionally, Table 5, below, displays the deviation for each standard and sample that were run as duplicates. In total, eight standards and ten samples were run as duplicates for the March assay. Average deviations for the standards and samples were 0.0718 mg/L and 0.0867 mg/L, respectively. Overall, average deviation for the duplicates was 0.080mg/L. A set of Quantikine ELISA Kit controls (Control Set 960) was utilized as an additional source of verification for the assay and results. These controls contained recombinant human cytokines at low, medium, and high concentrations within the following ranges: 1.31-3.82 ng/mL, 5.13-12.8 ng/mL, and 15.5-36.6 ng/mL (Quantikine DCPR00-QC70). Our readings for the controls were as follows: 2.75 and 3.00 ng/mL, 13.3 and 14.1 ng/mL, and 16.7-16.9 ng/mL.

	Single	Duplicate	Deviation	Average Deviation
Standards	0.002	0.002	0.000	
	0.090	0.067	0.011	
	0.137	0.160	0.011	
	0.308	0.348	0.020	
	0.528	0.693	0.082	
	1.22	1.32	0.047	
	2.37	2.60	0.116	
	5.25	4.68	0.287	0.072
Samples	0.468	0.471	0.001	
	3.57	3.09	0.236	
	0.026	0.018	0.004	
	0.069	0.065	0.002	
	0.183	0.193	0.005	
	2.35	2.87	0.260	
	0.009	0.053	0.022	
	0.573	0.860	0.143	
	2.80	3.16	0.180	
	0.239	0.214	0.012	0.087
				Overall
				0.080

Table 5 (above). Deviation for duplicates of standards and samples run during the March 2018 assay.

Distributions of CRP by sex are displayed in the histograms and box plots below. Overall, mean CRP levels were 1.14 ± 1.70 mg/L, which falls well within the normal range for a relatively young, Western population. Stratification by sex shows a significant difference of mean CRP between male and female participants (p=0.002). Mean CRP for men was 0.463 ± 0.636 mg/L, while mean CRP for women was 1.42 ± 1.91 mg/L. As is displayed in Table 2 and the figures below, CRP values for women throughout the distribution are generally higher than those for men, and the overall distribution is significantly broader within our sample. Again, the more limited sample for men may play a role in the lack of overall variation compared with women that is depicted in the CRP distribution.

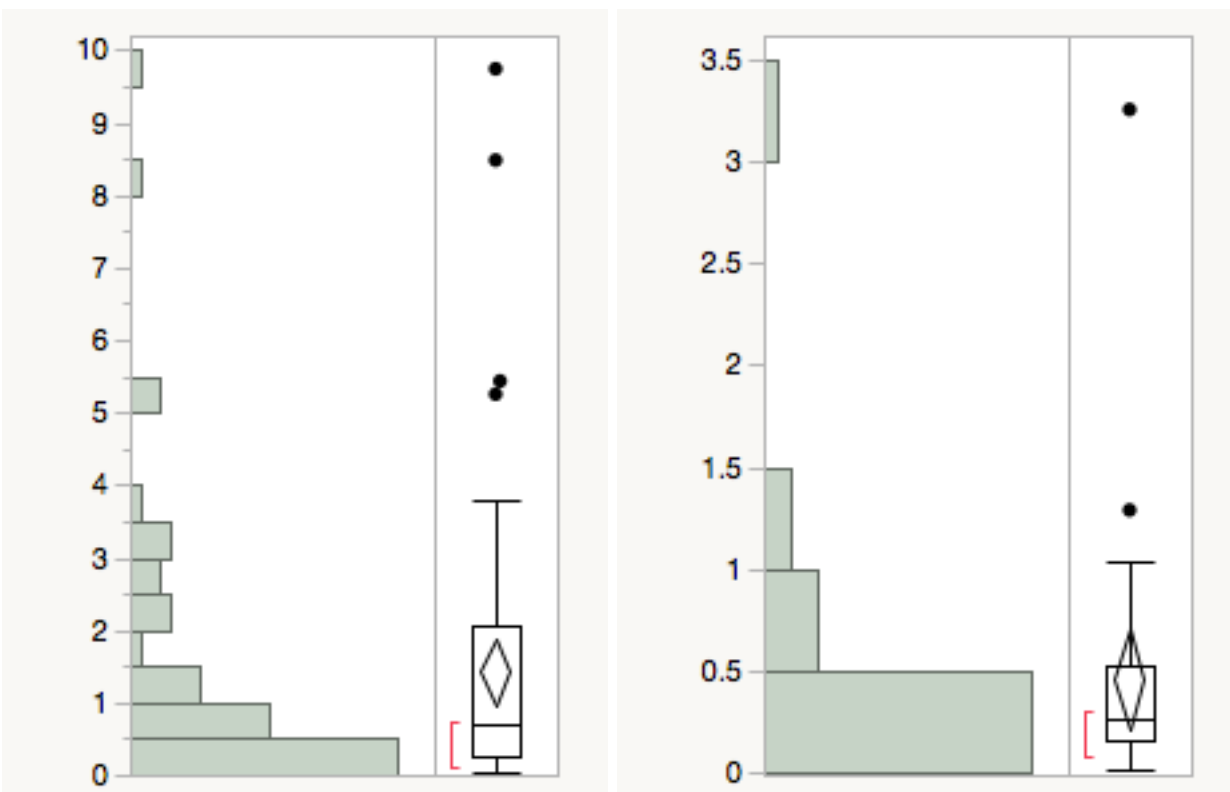

Figure 12. Female distribution of CRP (left) and male distribution of CRP (right)- (note vertical axes scaling differs between the sexes)

As is displayed in the box and whiskers plots and table above, distributions of CRP for men and women differed significantly (p=0.005). Both distributions are right skewed, as expected. CRP levels are log-transformed in regression analyses in order to normalize the distribution.

Descriptive variables and CRP

	Women (n=66) (71%)	**Men (n=27) (29%)**
Age	23.5 (3.6)	24.2 (5.4)
Current smoker	2 (3%)	1 (4%)
Ever smoked	7 (11%)	3 (11%)
Geometric mean CRP	0.656	0.265
CRP <1.0 mg/l	41 (62%)	24 (89%)
CRP between 1.0-3.0 mg/l	15 (23%)	2 (7%)
CRP > 3.0mg/l	10 (15%)	1 (4%)
Body mass index	24.5 (5.8)	23.0 (2.7%)
Overweight	10 (15%)	5 (19%)
Obese	8 (12%)	0 (0%)
PSS Global Score	18.4 (6.3)	17.6 (5.9%)
Oral Contraceptive Use	29 (44%)	

Table 6. Descriptive characteristics of study sample in relation to CRP

Descriptive statistics for key physiological and psychological parameters in relation to CRP are displayed in Table 6. The majority of participants (62% of women and 89% of men) had low levels of CRP, as was predicted given their age. Women in the study had higher frequencies of elevated CRP levels than men in the study. Fifteen percent of women and 4% of men were in the “high-risk” group based on clinical guidelines of CRP and cardiometabolic risk (Salazar et al.

2014). As noted earlier, women in the study also had a higher prevalence of overweight/obesity (27% for those included in the CRP sample), compared with men (19%). Women and men both reported an approximately equal prevalence of smoking regularly and having ever smoked. Smoking has been associated with CRP in several studies and thus was considered in this study. Oral contraceptives have also been associated with increased levels of CRP; though there were no significant differences in CRP levels found in our study for either smoking behavior or oral contraceptive use using a two-tailed T-test.

Table 6 displays the geometric mean CRP for women and men, which was calculated as the exponential of the mean log-transformed CRP values (Geomean=e^(mean(lnCRP)). Since the distributions of CRP are typically right-skewed, concentrations were transformed using the lnCRP for analyses in order to approximate normality.

Illness Reported	**CRP<1 mg/L (n=65)**	**CRP 1-3 mg/L (n=17)**	**CRP>3 mg/L (n=11)**	**Total Occurrence (n=93)**
Allergies	21 (32.3%)	4 (23.5%)	2 (18.2%)	27 (29.0%)
Asthma	3 (4.6%)	1 (5.9%)	0 (9.1%)	4 (4.3%)
Chronic Condition	6 (9.0%)	1 (5.9%)	2 (18.2%)	9 (9.7%)
Cold/Flu	4 (6.2%)	6 (35.0%)	3 (27.3%)	13 (14%)
Diarrhea	13 (22.0%)	2 (11.8%)	2 (18.2%)	17 (18%)
Fever	1 (1.5%)	0	1 (9.1%)	2 (2.2%)
Vomiting	1 (1.5%)	0	1 (9.1%)	2 (2.2%)

Table 7. Symptom history frequency chart stratified by CRP classification

Participants were asked to indicate if they had any chronic medical conditions, allergies, or asthma, and were also asked to indicate if they had experienced symptoms of chronic conditions, allergies, asthma, vomiting, fever, diarrhea, or cold and or flu, in the two weeks prior to data collection. This data was coded, and is displayed above in Table 7 as stratified by clinical classifications of CRP values. As the chart demonstrates, the most commonly reported illness was general allergies at 29%, followed by diarrhea at 18%, and cold and flu at 14%. Notably, three of the eleven individuals with elevated levels of CRP (above 3mg/L) reported symptoms of cold/flu within the past two weeks. CRP levels for these individuals were 5.03 mg/L, 9.74 mg/L, and 8.48 mg/L. Moreover, an additional six participants cited having cold and/or flu within the past two weeks and had CRP values within the more moderate 1mg/L-3mg/L range. Concentrations of CRP in relation to various illnesses and ailments are discussed in the CRP portion of the discussion chapter.

		Q1	**Q2**	**Q3**	**Q4**	**P-value**
Men						
	BMI	0.38	0.20	0.31	0.27	0.315
	PSS	0.15	0.34	0.70	0.38	0.285
Women						
	BMI	0.38	0.40	0.97	1.52	0.030
	PSS	0.45	0.76	1.11	0.48	0.568

Table 8. Least squares mean CRP values (mg/L) by quartile of BMI and PSS Global scores for male and female students. The P-value represents the difference in least square means in Q1 vs Q4.

In women, CRP levels were significantly higher with increasing quartiles of BMI, particularly when evaluated as clusters of Q1 and Q2 compared with Q3 and Q4 CRP values, but were not associated with increasing quartiles of PSS Global scores. The geometric mean values of CRP increased from 0.38 in the lowest quartile of BMI to 1.52 in the fourth quartile. Similarly, there is a relatively broad range between the geometric mean value of CRP in the lowest and third quartile of PSS for both men and women, though this trend deviates with the highest quartile. There is no association between BMI quartile and geometric mean of CRP for men in our study.

Perceived Stress and CRP

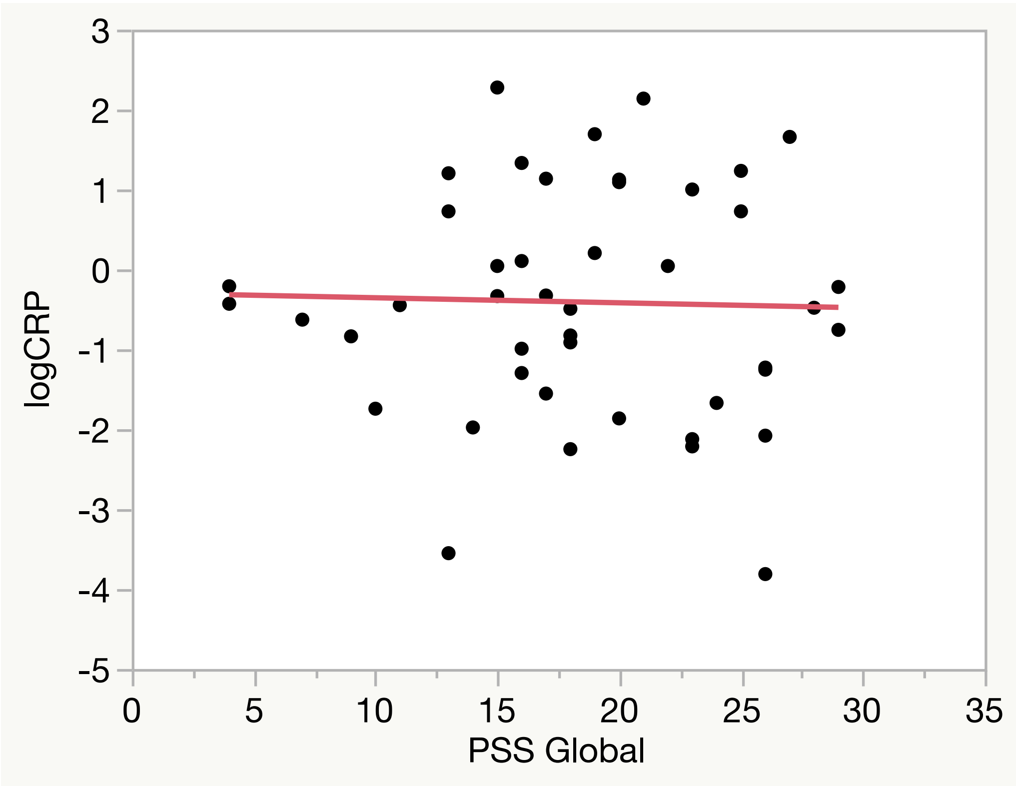

Figure 13. lnCRP against PSS Global Score for women; r=-0.028, p=0.854

Stratification by sex showed no significant associations between CRP and perceived stress in female participants (r=-0.028, p=0.854).

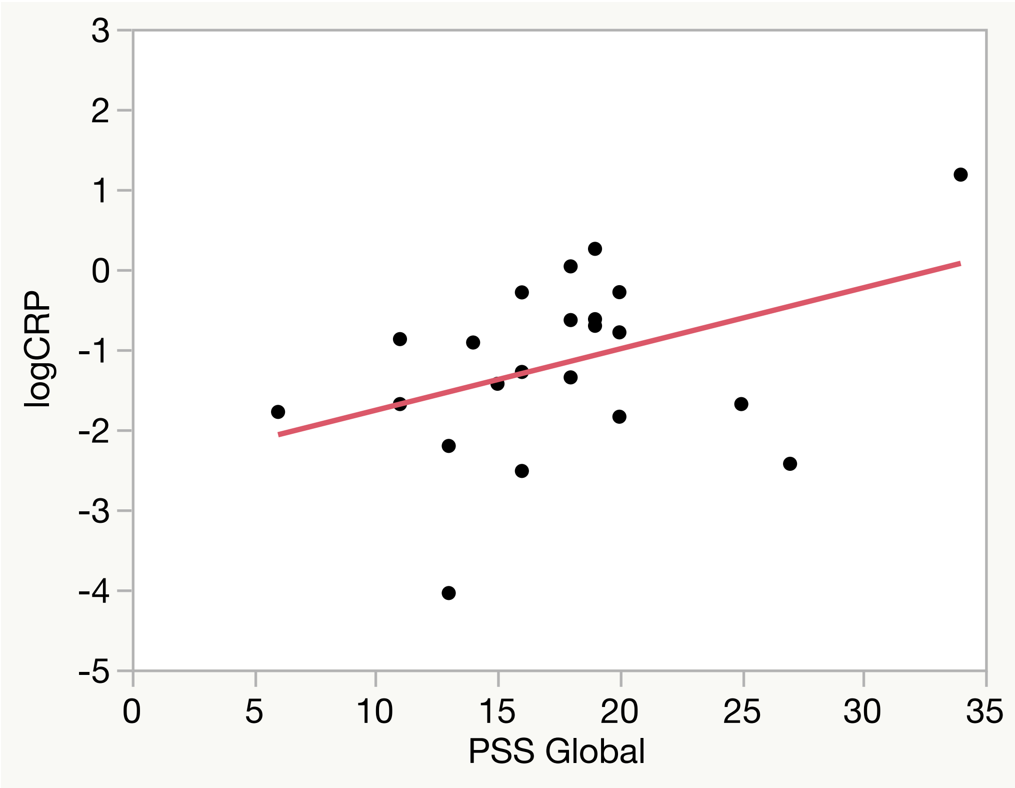

Figure 14. lnCRP against PSS Global Score for men; r=0.405, p=0.0613

Conversely, Figure 14, above, displays the scatterplot for lnCRP against PSS global score for men, and demonstrates a positive association between the two variables (r=0.405), though the relationship does not meet statistical significance at $p<0.05$ (p=0.0613).

BMI and CRP

This study hypothesized that CRP will be positively correlated with BMI such that individuals that fall within the CDC classifications for overweight/obese will have the highest levels of CRP overall.

	CRP<1 mg/L	CRP 1-3 mg/L	CRP >3 mg/L
Men			
N	24	2	1
BMI	23.25	21.8	20.4
Women			
N	41	15	10
BMI	23.6	23.6	29.43

Table 9. CRP classifications by clinical guidelines and corresponding average BMIs for male and female students

Table 9 shows the average BMI for men and women as grouped by CRP classification. Since most men in the study fell within the lowest category of CRP, analysis in terms of associated variables and CRP classification is limited. Regression analyses were conducted to examine potential associations between BMI and CRP and were also stratified by sex.

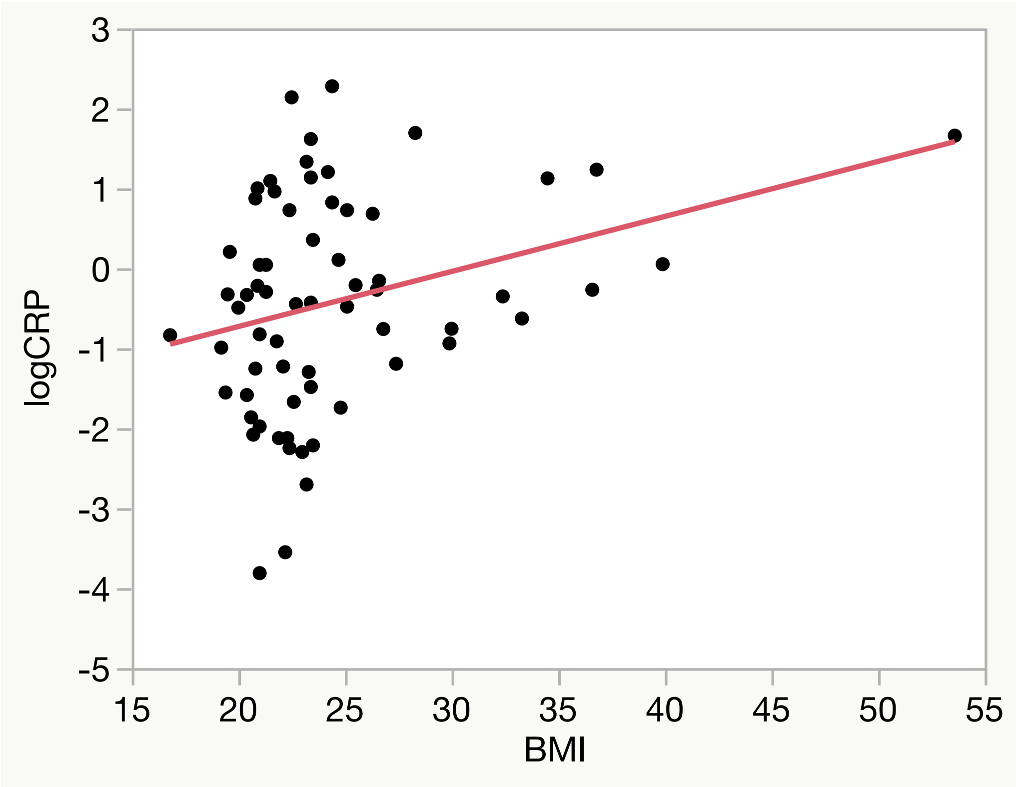

Figure 15. Scatterplot of lnCRP against BMI for women; r=0.298, p=0.0152

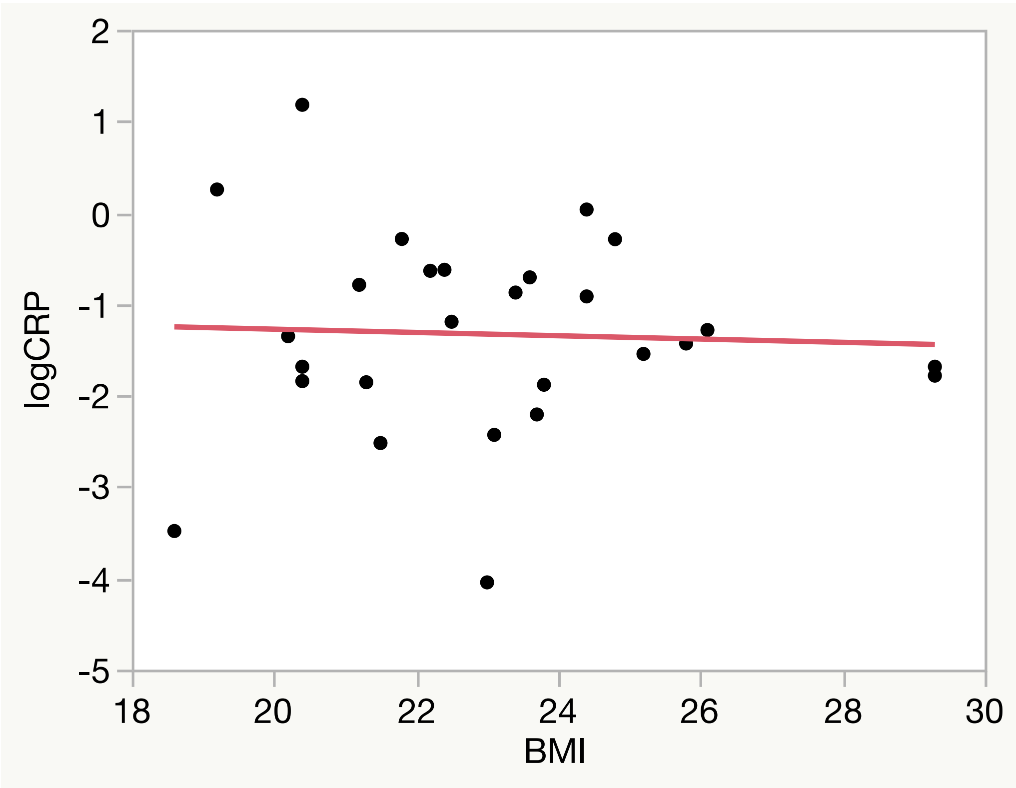

Figure 16. Scatterplot of lnCRP against BMI for men; r=-0.0433, p=0.830

As is displayed in Figure 16, a regression analysis of CRP against BMI does not find a statistically significant relationship between the two variables for men in our study.

Age and CRP

Though age was not a specific variable of focus for the hypotheses of this thesis, a considerable amount of research has demonstrated an association between age and CRP, though much of this research has focused on a broader age range, and generally older individuals. Table 7 stratifies participants into two age groups: 18-22 and 23-36, thought to best align with typical age as an undergraduate and as a graduate student, respectively. As is shown in the table, geometric means and medians are similar for each of the groups, with the exceptions of women ages 23-36 years old. Mean CRP levels for the younger cohort of our study are slightly higher than the older cohort, though a t-test between these groups did not find a statistically significant difference in means.

	Age Group				
Men		N	CRP	Geometric Mean	Median
	18-22	14	0.530	0.250	0.245
	23-36	13	0.281	0.281	0.259
Women					
	18-22	33	1.57	1.17	1.03
	23-36	33	1.31	0.572	1.52

Table 10. Mean and median CRP by age and sex stratification

Similarly, linear regressions were conducted to analyze the relationship and found no significant associations between age and CRP in either sex in our study sample (for men, r=0.009, p=0.966; for women, r=0.074, p=0.549.

Chapter 4
Discussion

Summary of Results

This thesis seeks to add to a limited literature on the role of stress in the embodiment of differential health and well-being by analyzing the associations between BMI, perceived stressors and levels of stress and CRP in students. In order to analyze these relationships, several hypotheses were proposed, and related findings are briefly discussed below, and at greater length later in this chapter.

H1a: was not supported in this study, as there were no significant associations between perceived stress and CRP levels. H1b: male and female students did not have significantly different levels of perceived stress, but there were differences between male and female students' sources of perceived stress, an interesting, but perhaps unsurprising finding. H1c: Data on factors of stress did support our hypothesis that reported stressors would vary in correlation with levels of perceived stress.

H2: while there was not a statistically significant association between BMI and CRP in male students, this relationship was evident in female students. Also in support of H2, women in the highest classification for CRP also had higher BMIs than those within the clinically normal range, though the sample size for both elevated CRP and high BMI was very limited.

H3: There was an unexpected statistically significant association between perceived stress and BMI in this study such that men who had a higher BMI had lower perceived stress. The negative correlation present in this study may result from the small sample of men, but should be explored in future studies on BMI and stress in young adults. Due to the limited sample size for high stress/high BMI individuals, the potential compounded effect of the two variables was not examined.

H4: This study did demonstrate the unique factors of stress that are commonly shared amongst most university students from our sample, and also demonstrated how many key factors of stress are unique to the educational setting in which students are immersed.

BMI and Overweight/Obesity Prevalence

In our sample, 20% of men and women fell into overweight/obese categories. NHANES data from 2013-2014 demonstrates that approximately one in three adults were considered to be overweight, and one in three adults were considered to be obese (NHANES). Sex differences in proportion of overweight to obese were also evident, with more men considered overweight, and more women considered obese (NHANES). Similarly, sex differences exist in our sample, with more female participants considered obese, but less that were considered overweight when compared with male participants. More limited male participation may contribute to the relative lack of variation within the male sample overall.

Differences in BMI distribution between young students and the general population are to be expected given age-distributions of BMI and other potential factors including access to recreational facilities and nutritional resources. Several studies have also demonstrated the difference between BMI distribution in the general population, as documented by NHANES, and distributions on college campuses. One such study, the American College Health Association's National College Health Assessment (NCHA) has collected survey data from undergraduate and graduate students annually for three years. The 2017 data set contains survey responses from 63,487 respondents from various institutions across the United States. BMI in the study was calculated from self-reported height and weight. Data from the NCHA (2017) found that the mean BMI for men was 24.68 +/- 5.09 and was 24.22 +/- 5.45 for female undergraduates.

Comparing this with our study, a T-test demonstrates that these means do not differ significantly ($p=0.993$, $p=0.691$, for men and women respectively). The NCHA also calculated the percentage of participants who fit the overweight/obese categories defined by WHO, and showed that 27.5% of men and 19.5% of women were overweight, and 11% of men and 11.8% of women were obese (NCHA). Proportions of overweight to obese within the sexes match overall trends of a higher prevalence of overweight in men, and a higher prevalence of obesity in women (NHANES, NCHA).

From our data set more individuals fall into the “healthy weight” category than anticipated given the results of the NCHA study. Limitations of our study including a small sample size, skewed sex ratio, and convenience sampling may also play a role in this difference. Since this sampling occurred at flu-vaccination clinics it should be considered that participants in this study may be more health-conscious relative to the general student population; it should also be noted that many of these clinics were held in buildings related to pre-health or public health fields, and were therefore more convenient for students within those fields. Few studies have considered the influence of psychosocial stress on BMI in college students.

Psychosocial Stressors and Perceptions of Stress

Though the Cohen PSS-10 is a frequently employed measure for perceived stress, few studies have considered it within the context of a college or university setting. A study conducted by Cohen and Janicki-Deverts provides normative data for individuals of various ages and demographics based on three national surveys administered in 1983, 2006, and 2009 (Cohen and Janicki-Deverts 2012). Due to the scale of their study, it is frequently used as a normative data set, and thus provides a means of comparison for studies such as this. Cohen and Janicki-Deverts demonstrate that perceived stress has risen overall since 1983, and also demonstrate that perceived stress varies between men and women, though not always to the extent of statistical significance (Cohen and Janicki-Deverts 2012). Since the most recent year for survey administration was 2009, this study highlights a few of those values as reference points, and are as follows:

Sex	n	M	SD
Men	968	15.5	7.4
Women	1032	16.1	7.6
Age			
Less than 25	223	16.8	6.9
25-34	433	17.5	7.3
Education			
Some college	784	16.0	7.5
Bachelor's degree	513	15.2	7.2
Advanced degree	231	14.7	7.1

*data adapted from Table 1 *Who's Stressed? Distributions of Psychological Stress in the United States in Probability Samples from 1983, 2006, and 2009- Cohen and Janicki-Deverts*

The data from this study demonstrates that men and women have average PSS-10 scores of 18.6 ±6.2 and 17.3 ± 5.9. which is not statistically different from the overall means derived in Cohen and Janicki-Devert's study, though it is slightly elevated (Cohen and Janicki-Deverts 2012). As is also evident, we failed to find support for Hypothesis 1b, which proposes that women will report higher average levels of perceived stress than men. The difference of means between the present study and normative data, though not statistically significant, does suggest a subtle difference between perceived stress in students and the general population. Our study is notable in that it focuses on college and university students; while Cohen and Janicki-Deverts demonstrate the negative association between education and perceived stress, few studies have considered the impact of receiving an education on perceived stress. Future studies on stress within a student context should consider the employment of a standard scale, such as the PSS-10, to provide a large-scale normative data set for students and to investigate potential intra- and intercollegiate variation of experience with stress.

Individuals immersed in a collegiate environment are likely to have a unique experience with stress when compared with a more general population, and this study aims to highlight the beginnings of research within that area of focus. As is highlighted within our study, older individuals tend to have lower perceptions of stress than younger individuals, even within a collegiate context. This finding may be indicative of related hypotheses: particular qualities of being younger than 25 induce more perceived stress than the qualities and experiences of older individuals and that within a collegiate context, younger individuals have higher perceptions of stress due to the particular stressors of an undergraduate experience and acclimation-related stress.

This study is one of the few to consider a multi-methods approach to stress research in a student population through the employment of the Cohen PSS-10 and free-listing methods. By combining these forms of data, researchers can begin to understand the variation not only in perceived stress, but also in stressors, providing a window into the complexities of stress variation. From the free-list data, five major themes emerged as factors of perceived stress: school, relationships, health and wellness, the future, and time management. Notably, there was variation in the ways in which individuals reported and characterized their experiences with these stressors. Specificity is variable within the free list data; most individuals concisely listed elements of experience, but others listed more complex and nuanced characterizations of their stressors. “School” emerged as the most prevalent and salient code of stress for participants.

The ways in which experience with “School” are described are variable, some of which are outlined in this section and Figure 12, with a more detailed analysis of code determination provided in Appendix B. Descriptions of “School” as a source of stress included more general responses such as “School”, “Schoolwork”, and “Exams”, but several individuals described this stressor in more emotionally-connoted and nuanced; for instance, participant responses included “schoolwork (being behind)”, “dissertation writing anxiety”, “schoolwork/ worrying about grades (not enough hours in the day)”, “school expectations”, and “school work accumulation”. These responses suggest that these individuals associate stress with particular aspects and qualities of the “school” experience. As is demonstrated, many of these participants describe schoolwork as linked with feelings of worry or anxiety. These emotions are often associated with perceived stress, and are some of the elements captured within the PSS-10 question items as well (Cohen, Kamarck, and Mermelstein 1983). The attribution of these emotions to school also suggests that not only are certain individuals daily stressed by school, but are stressed by the particular ways that experience makes them feel. Similarly, individuals often connote school-related stressors with elements of time, including deadlines and an explicit mention of “lack of time”. Better understanding the particular aspects of school-related stressors experienced by college and university students may provide academicians and policy-makers with ways to enhance the collegiate learning experience and overall health and well-being of students.

Following "School", descriptions of "Relationships" were prevalent sources of stress for college and university students. This too is anticipated, given the need to socially acclimate to a new environment as a student; moreover, social support has been negatively associated with levels of perceived stress, suggesting that relationships may act as a buffer for stress in a variety of contexts (Bovier, Chamot, and Perneger 2004). This study is unique in that it highlights that relationships themselves are a source of stress for students, with particular types of relationships emerging more prevalently than others. As is demonstrated in Figure 6 in the "Results" section, family emerged as the most frequent subcode, followed by friendships, and romantic relationships. These subcodes suggest that certain aspects of family relationships may be more prominent sources of stress than aspects of friendships or romantic relationships. Considering the collegiate experience, it may be hypothesized that students' lengthy separation from family and concern for their family may be sources of stress, which is evidenced by responses such as "miss my family", and "family safety". Other individuals attribute "conflict" and "obligations" as associated with relationships as a source of stress.

Within the "Health and Wellness" code, sleep emerged as the most frequent subcode, suggesting that students are particularly worried about the lack of sleep they are getting, likely due to its relationship with their overall health and wellbeing. Diet and exercise were also fairly prevalent subcodes, suggesting that students do have an awareness for the importance of nutrition, exercise, and sleep on their well-being, but also display concerns about their relationship with these items, which is reflected in responses such as "my health/wondering if I am healthy". As is expected, these university students show a keen awareness to the importance of good health, and express stress related to their relationship with particular facets of health and wellness. More rarely, individuals mention explicit experiences with chronic illness, including “chronic fatigue” and “upset stomach from food intolerance”. As an example of this, a participant reporting life-threatening health concerns fell within the high category of PSS, demonstrating the variability in severity and experience with particular stressors.

The future emerged as a uniformly frequent code, which may be expected given the liminal nature of the collegiate experience. Many participants connote the future with worry about achievement as success, including responses such as “worrying about the future and not being good enough” and “fear of failure”. Interestingly, fear of failure explicitly emerged with a frequency prevalent enough to provide it with a subcode. Again, this demonstrates the profound emotionally-connoted ways in which individuals view the future and worry about the trajectory of their perceived success.

Following the future, time management occurred with a relatively high frequency, suggesting the stress students associated with deadlines and an accumulating workload. More specifically, individuals note aspects of a lack of time, such as “not having time to spend with friends and family” and “not enough time to complete tasks”, which both demonstrate the overlap of time management and other codes such as relationships and schoolwork in some free-list responses.

A miscellaneous section code was also utilized to capture specific items that did not fall into recurring theme categories. Within the miscellaneous category the most recurring coded item was “commuting” (11 occurrences), followed by politics (7 occurrences), and chores and errands (5 occurrences).

This study hypothesized that reported factors of perceived stress would vary in correlation with levels of perceived stress, such that certain factors are present only in those within the highest category of perceived stress (Hypothesis 1c). This hypothesis was supported by evidence demonstrated in Figures 10 and 11 in the Results section on *Factors of Stress*. Uniquely, this demonstrates that particular qualities and aspects of experience are more likely to be associated with higher perceived stress than others. Differences were also evident between men and women in the study, though to a lesser extent than the differences demonstrated between PSS-categorized groups. In order to help students effectively target and cope with these stressors, it is imperative for researchers to first investigate them on a large-scale. By doing this, researchers have an opportunity to improve the overall health and well-being of students, which may improve the trajectory of their health and well-being throughout the rest of their lifecourse.

CRP and Inflammation in College Students

As hypothesized, students in this study had relatively low levels of CRP on average, with 85% of women and 96% of men with baseline levels of less than 3mg/L. Only five individuals, all female, had CRP levels over 5mg/L, three of whom reported having symptoms of cold and or flu in the past two weeks. Though this research does not focus on inflammation and illness, the brief symptom history analysis may provide data for future analyses on inflammation, inflammatory resolution, and self-reported experience with illness.

While a few hypotheses proposed in this thesis were not supported in the data, this study did demonstrate a significant association between BMI and CRP in college-aged women. This finding aligns with trends of BMI and CRP evident in older populations, and elucidates that young adult women tend to have more adipose tissue than men, and may have differential rates of overweight/obesity due to differential physiologies.

Despite the lack of association between perceived stress and inflammation in this particular study, evidence for associations between elevated CRP and self-rated health have been demonstrated utilizing data from the National Longitudinal Study of Adolescent Health (NSLAH) in 2014 (Shanahan et al. 2014). Interestingly, our findings on frequencies of low, elevated, and high CRP are lower than those collected from Shanahan and colleagues' study, suggesting that individuals within our study may have been healthier, which is also supported by the considerably lower average BMI in our sample (Shanahan et al. 2014. The NSLAH data study provides a framework of multiple survey methods as potential predictors of inflammation and physiological embodiment. Future studies may consider following this model of stress through the employment of additional surveys specific to the college experience, and should continue to analyze the multifactorial experiences of stress within a daily context.

This study is one of few to analyze CRP levels in a young, healthy adult population. By analyzing baseline CRP levels, this research not only illuminates differential baseline inflammation, but also provides a comparative framework for future research examining inflammation and inflammatory response in young adults. Moreover, this study adds to a body of literature that is beginning to understand how stress may be embodied and produce differential disease outcomes that are becoming increasingly pervasive globally, including metabolic syndrome and overweight and obesity more generally.

. Future studies may consider following this model of stress through the employment of additional surveys specific to the college experience, and should continue to analyze the multifactorial experiences of stress within a daily context. Similarly, studies considering the associations between adiposity and CRP may consider adding additional measures of adiposity to their study.

Conclusion

This study had several limitations that must be acknowledged, one of which is a relatively small male sample, and heavily skewed female sample. Bloodspots were collected only once, and therefore provided a cross-sectional reference sample to analyze baseline inflammation. A more longitudinal framework may be beneficial, particularly in studies for which inflammatory fluctuations are of interest. The implementation of other stress scales to supplement the Cohen PSS-10, as well as more interview-based qualitative data collection on how individuals conceptualize, characterize, and cope with their stress would have been beneficial to supplement free-list responses.

Studies such as this can be utilized to grasp an understanding of recurring sources of stress in the lives of students, as well as particular groupings of those themes. Future research should refine demographic categories and should consider collecting information on field of study, year, and employment to analyze more context behind differential sources and levels of perceived stress among students. While free-list data provides a window into prominent sources of stress, further work could include more ethnographic components such as interviews and focus groups in order to reveal the more nuanced complexities of stressors. Moreover, future research should gauge participants' perspectives on the severity and duration of stressors, as being able to differentiate between acute and chronic stressors and how intense or severe individuals perceive them to be will provide more insight into the unique stressors students face, and could be interesting for risk modeling.

Researchers might also analyze coping mechanisms and strategies employed by students to determine if certain coping mechanisms are more prevalent among individuals with lower stress in order to better understand which coping mechanisms students might be able to utilize.

The emphasis on the integration of methods traditionally employed in biological and cultural anthropologies will provide researchers with unique insight into the differential cognition, physiology, and behavior of individuals. Studies on university students additionally provide researchers with a chance to conduct comparative research across the nation and in international settings. By replicating studies such as this, with a few improvements, researchers can not only begin to understand differential stress and corresponding physiologies within a singular university setting, but also across student populations more broadly.

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Appendix A

Full Lab Protocol for ELISA Assay for hs-CRP Using Quantikine Kit

SAMPLE PREPARATION: DAY 1
Pre-activities

- Check kit expiration
- Put gloves on
- Set RD5P concentrate out to room temperature for later preparation of Calibrator Diluent
    - RD5P concentrate is at appropriate temperature when crystals/precipitate have dissolved
- Review forward pipetting technique:
    - depress to first stop, insert under surface, release, place in container on side of wall without touching, then depress to first stop, then depress to end

Bloodspot Samples: Labeling

- Lay out dust-free surface paper absorbent side down
- Bring out bloodspot samples from refrigerator
- Label bloodspot card ID numbers on small 2mL closing tubes and place in stand, consider using tape over the writing to ensure that it stays legible

Calibrator Diluent Preparation

- Process:
    - Combining 5mL of Calibrator Diluent RD5P Concentrate with 20mL DI H2O to make 25mL of Calibrator Diluent
- Add 20mL of DI H2O into 50mL beaker
- forward pipette 1mL*5 of Calibrator Diluent RD5P Concentrate into beaker
    - Note:
        - Use the 100-1000µL pipette
        - release on wall & check dial at 1mL
- Mix solution with pipette by injecting and releasing under surface to avoid bubbles
- Note: Sample preparation instructions by Quantikine suggests a 100 fold dilutions, but we are using the diluted Calibrator Diluent (above) to elute the blood spots to reconstructed whole blood (based on McDade's email instructions). This 25mL of diluted Calibrator Diluent will be enough to elute 80 blood spot samples and still have leftover to use tomorrow for the standards preparation.

Bloodspot Samples: Punching Bloodspots

- Select largest bloodspot from sample card, checking for complete absorption on the back
- Use 1/8 inch hole punch on center of bloodspot ensuring no white edging is taken
- Release the circle onto the dust-free paper
- Using the tweezers place circle into labeled 2mL tube checking for the corrected ID

- o Note: periodically check for debris on absorbent paper
- After tray is full place on counter

Bloodspot Samples: Elution

- Process:
  - o Eluding 3.2mm (1/8 inch) diameter bloodspots to 1.5 µL serum sample dilution (McDade's instructions) to specified requirements of the Quantikine CRP kit.
- Pick up 2mL tube containing the blood circle sample
- Pipette 250µL of Calibrator Diluent into one and cap
  - o Note:
    - Use forward pipetting & release on wall
    - Go slow because the pressure is high and you want to avoid it splashing out
- Vortex for 30 seconds
- Check circle is completely covered by solution
- Repeat for all samples
- Refrigerate overnight at 4˚C

Notes:

- DI H20 is deionized water
- When adding Assay Diluent to the plate, lean the pipette tip across and hit the side so it doesn't splash out

REAGENT PREPARATION & ASSAY: DAY 2

Pre-activities

- Bring kit to room temperature
  - o Put substrate in drawer
- Plan and fill out plate sheet with matching standards, duplicates, controls and samples
  - o 96 wells, using 73 unique samples
    - 16 standards: 8 standards run as duplicates
    - 2 controls: 2*1 (one will be used as a positive control, the other a negative control)
    - 5 duplicates: yielding total of 10, others are run singleton, if you can allocate more samples, do so
    - 68 single samples

Water Buffer Preparation

- Warm wash buffer to room temperature to dissolve crystals
- Invert wash buffer container to gently mix
- Add 20mL of wash buffer in 50mL graduated cylinder
- Add wash buffer to 600mL beaker
- Add 480mL of DI H20 to 500mL line
  - o Note:

        - Use DI H_2O water jug to pour near line
        - Then use squirt bottle til 500mL line
- Pour into new squirt bottle

Calibrator Diluent Preparation
- pipette 400µL of Calibrator Diluent RD5P Concentrate into 750mL glass tube
- pipette 1600µL of DI H20 in tube to make 2mL of Calibrator Diluent
- Mix with pipetting under surface- 1:5 (dilution)- this aligns with kit and prepared diluent from yesterday (5:20) 1:5 dilution; since we made more diluent solution yesterday, we will reuse

Standard Preparation
- Label 6 standard 2mL capped tubes
- Pipette 200µL of Calibrator Diluent into each tube
- Pipette 200µL of Standard concentrate into 25ng/mL tube and vortex for 1 min
- Continue dilution per kit instructions

Assay Procedures
- Change bench paper
- Notes:
    - Have someone track well filling
    - Fill by columns
    - Change out tip and slowly pipette to minimize bubbles
- Use kit instructions
- Add 50 µL of either standard, control, or sample to each well in accordance with designation on plate map
    - Note that for small amounts the last half stop can be discarded as the liquid has been expelled from the pipette tip and this can cause air bubbles to form in the wells; make sure to test using scale to ensure that pipette still expels 0.050g
- Cover with the adhesive strip provided with the kit and allow to incubate for two hours at room temperature
- After the incubation run a repeat of 4 aspirations and washes using the plate washer. Make sure that the plate washer has wash buffer loaded and connected
- After the last wash, remove any remaining wash buffer by aspiration or decanting, tap gently on paper towel to remove excess liquid
- Add 200 µL Human CRP conjugate to each well, cover with a new adhesive strip and allow to incubate for two hours at room temperature
- Repeat the aspiration and wash step as outlined above
- Mix the two bottles of Color Reagent (A and B) together to create Substrate Solution, use within fifteen minutes of mixing
    - Add 200 µL of Substrate Solution to each well and allow to incubate for 30minutes at room temperature; it is important to protect from light, so it is a good idea to re-insert into foil package
- Add 50 µL Stop Solution to each well. At this time there should be a color change in the well from blue to yellow, if well solution remains green try lightly tapping on plate or mixing gently with small pipette tip

- Determine optical density using microplate reader within 30minutes of this procedure
    - Note: wavelength is read at 450nm because that is in range for blue light; it is good to perform a correction if possible, 630nm works because it is not far from range of yellow light, 540nm and 570nm are recommended

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.056	0.055	0.051	0.182	0.428	0.441	0.21	0.206	0.562	0.304	0.41	2.866
	0.043	0.043	0.044	0.047	0.049	0.044	0.042	0.047	0.044	0.049	0.045	0.052
	0.013	0.012	0.007	0.135	0.379	0.397	0.168	0.159	0.518	0.255	0.365	2.814
	0.021	0.015	<0.000	0.692	2.137	2.249	0.879	0.828	3.03	1.384	2.05	34.316
B	0.206	0.171	0.52	0.559	0.374	0.388	1.096	2.179	3.312	0.832	0.321	2.42
	0.04	0.04	0.045	0.046	0.045	0.042	0.045	0.046	0.047	0.045	0.043	0.052
	0.166	0.131	0.475	0.513	0.329	0.346	1.051	2.133	3.265	0.787	0.278	2.368
	0.867	0.669	2.748	2.997	1.829	1.933	7.035	19.77	50.33	4.924	1.521	23.931
C	0.297	0.334	1.73	1.797	2.399	2.648	1.146	0.294	1.684	0.235	0.329	1.797
	0.044	0.043	0.057	0.06	0.052	0.053	0.048	0.039	0.05	0.045	0.044	0.049
	0.253	0.291	1.673	1.737	2.347	2.595	1.098	0.255	1.634	0.19	0.285	1.748
	1.372	1.599	13.338	14.123	23.529	28.73	7.441	1.384	12.874	1.005	1.563	14.261
D	0.566	0.63	1.984	1.99	0.072	0.146	0.27	0.422	0.751	1.031	1.264	2.746
	0.041	0.045	0.052	0.046	0.047	0.04	0.047	0.044	0.045	0.043	0.043	0.052
	0.525	0.585	1.932	1.944	0.025	0.106	0.223	0.378	0.706	0.988	1.221	2.694
	3.076	3.48	16.72	16.891	0.085	0.529	1.196	2.131	4.329	6.506	8.551	31.122
E	0.878	1.09	0.802	0.807	0.954	1.273	0.254	1.222	0.274	1.165	0.526	3.649
	0.047	0.052	0.048	0.049	0.062	0.047	0.046	0.05	0.042	0.044	0.053	0.066
	0.831	1.038	0.754	0.758	0.892	1.226	0.208	1.172	0.232	1.121	0.473	3.583
	5.258	6.925	4.679	4.709	5.731	8.597	1.109	8.1	1.249	7.643	2.735	>52.500
F	1.627	1.712	2.914	2.743	2.625	2.762	0.377	0.252	0.1	3.244	0.364	1.464
	0.048	0.053	0.053	0.056	0.062	0.049	0.044	0.047	0.038	0.054	0.048	0.042
	1.579	1.659	2.861	2.687	2.563	2.713	0.333	0.205	0.062	3.19	0.316	1.422

	12.237	13.17	35.666	30.946	27.999	31.606	1.853	1.092	0.287	47.105	1.75	10.529
G	2.411	2.531	0.102	0.089	0.462	0.416	2.991	0.689	0.462	1.115	0.498	0.54
	0.056	0.06	0.045	0.046	0.043	0.036	0.057	0.042	0.043	0.043	0.048	0.049
	2.355	2.471	0.057	0.043	0.419	0.38	2.934	0.647	0.419	1.072	0.45	0.491
	23.681	26.001	0.259	0.183	2.388	2.143	37.885	3.909	2.388	7.215	2.586	2.852
H	3.395	3.241	0.185	0.17	1.455	0.804	2.736	2.362	0.731	0.663	0.377	0.898
	0.057	0.059	0.05	0.043	0.052	0.05	0.056	0.055	0.047	0.045	0.044	0.057
	3.338	3.182	0.135	0.127	1.403	0.754	2.68	2.307	0.684	0.618	0.333	0.841
	>52.500	46.777	0.692	0.647	10.333	4.679	30.772	22.78	4.171	3.707	1.853	5.334

Figure 1. Plate matrix

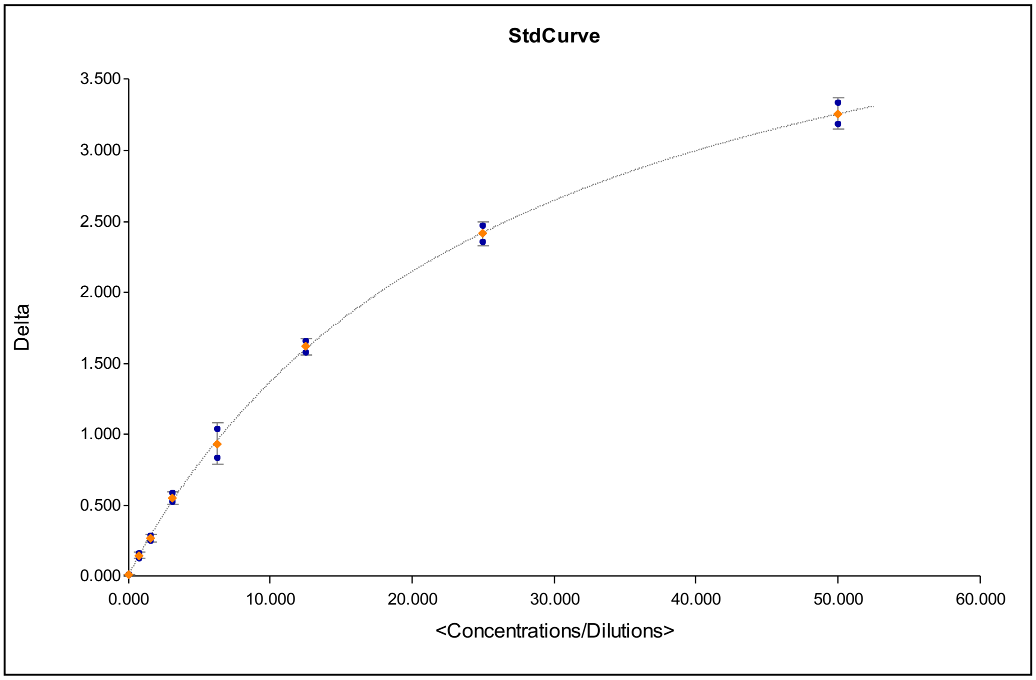

Figure 2 . Standard curve to which CRP samples (March assay) were fit in order to calculate concentration

Appendix B

Code System and Additional Qualitative Figures

1 Social Life	21
1.1 Living Situation	6
2 Occupation	30
2.1 Teaching	5
3 School	111
3.1 Qualifying Exams	4
3.2 Schoolwork	40
3.3 Writing/Researching	13
3.4 Exams/Grades	17
4 Relationships	73
4.1 Parenting	2
4.2 Friendships	13
4.3 Romantic Relationships	9
4.4 Family	30
5 The Future	50
5.1 Job/Graduate or Professional School	22
5.2 Fear of Failure	10
6 Finances	26
7 Extracurricular Commitments	26
8 Time Management	46
8.1 Deadlines	9
9 Health and Wellness	55
9.1 Mental Health	7
9.2 Family Health	5
9.3 Diet and Exercise	16
9.4 Lack of Sleep	17

9.5 Chronic Illness	4
10 Miscellaneous	40
10.1 Chores and Errands	5
10.2 Technology	2
10.3 Stock Market	1
10.4 Climate Change	1
10.5 Imposter Syndrome (Belonging)	1
10.6 Pets	2
10.7 Hungry	3
10.8 Step One	1
10.9 Visa/Acculturation	4
10.10 Wedding Planning	1
10.11 Commuting	11
10.12 Politics	7

Figure 1. Code System as Produced in MAXQDA

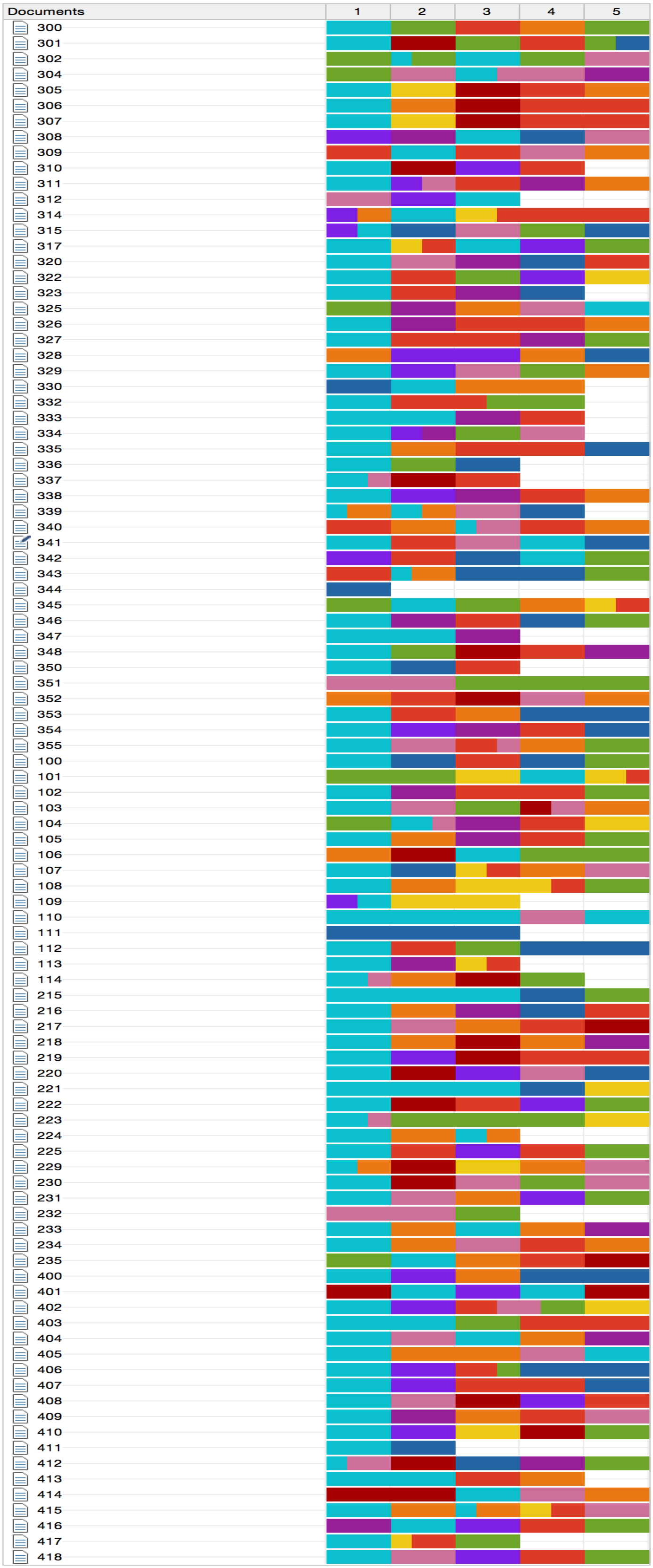
Documents
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Figure 2. Visual Representation of Salience, with coded items sorted in numerical list order; light blue:school; lime green: health and wellness; yellow: time management

	%Low to Mod PSS	**%High PSS**
School	92.94	100.00
Relationships	58.82	57.14
Health and Wellness	43.53	57.14
The Future	41.18	35.71
Time Management	38.82	35.71
Schoolwork	37.65	42.86
Miscellaneous	34.12	14.29
Family	32.94	14.29
Occupation	30.59	21.43
Finances	27.06	21.43
Extracurricular Commitments	22.35	35.71
Job/Graduate or Professional School	17.65	14.29
Lack of Sleep	17.65	14.29
Social Life	16.47	35.71
Exams/Grades	14.12	35.71
Writing/Researching	12.94	14.29
Commuting	12.94	0.00
Friendships	11.76	21.43
Diet	10.59	7.14
Fear of Failure	9.41	14.29
Deadlines	8.24	14.29
Exercise	8.24	7.14
Romantic Relationships	5.88	28.57
Politics	5.88	7.14
Family Health	5.88	0.00

Chores and Errands	5.88	0.00
Teaching	4.71	0.00
Qualifying Exams	4.71	0.00
Hungry	3.53	0.00
Living Situation	2.35	28.57
Mental Health	2.35	28.57
Parenting	2.35	0.00
Pets	2.35	0.00
Visa/Acculturation	2.35	0.00
Chronic Illness	1.18	7.14
Technology	1.18	7.14
Stock Market	1.18	0.00
Climate Change	1.18	0.00
Imposter Syndrome (Belonging)	1.18	0.00
Step One	1.18	0.00
Wedding Planning	1.18	0.00

Figure 3. Full Comparison of Codes for Low to Moderate and High Stress Participants

	Total % Women	**Total % Men**
School	95.7	89.7
Relationships	57.1	62.1
Health and Wellness	45.7	44.8
The Future	40.0	41.4
Time Management	38.6	37.9
Schoolwork	35.7	44.8
Finances	31.4	13.8
Miscellaneous	28.6	37.9
Occupation	28.6	31.0

Extracurricular Commitments	27.1	17.2
Family	27.1	37.9
Exams/Grades	21.4	6.9
Lack of Sleep	17.1	17.2
Social Life	17.1	24.1
Friendships	15.7	6.9
Job/Graduate or Professional School	12.9	27.6
Writing/Researching	12.9	13.8
Diet	11.4	6.9
Romantic Relationships	10.0	6.9
Commuting	8.6	17.2
Fear of Failure	8.6	13.8
Deadlines	7.1	13.8
Exercise	7.1	10.3
Chores and Errands	5.7	3.4
Living Situation	5.7	6.9
Mental Health	5.7	6.9
Politics	5.7	6.9
Teaching	5.7	0.0
Family Health	4.3	6.9
Chronic Illness	2.9	0.0
Hungry	2.9	3.4
Qualifying Exams	2.9	6.9
Climate Change	1.4	0.0
Imposter Syndrome (Belonging)	1.4	0.0
Pets	1.4	3.4
Step One	1.4	0.0
Technology	1.4	3.4
Visa/Acculturation	1.4	3.4
Wedding Planning	1.4	0.0

Parenting	0.0	6.9
Stock Market	0.0	3.4

Figure 4. Full Comparison of Codes for Women and Men