Cardiometabolic Disease Risk and College Students: Relationships Between Inflammation, Psychosocial Stress, and Diet

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Abstract

Diet is a factor that has been shown to mitigate the impacts of cardiometabolic disease (CMD) risk in adult populations while sustained levels of psychosocial stress has been shown to worsen the impacts of CMD. Inflammation is a possible pathway through which these lifestyle and behavioral factors impact CMD risk. This poses an alarming issue for populations that are predisposed to higher levels of sustained psychosocial stress – college students. This study seeks to define the most prominent factors interlinking diet, psychosocial stress, and inflammation as it pertains to CMD risk in college students. Data collection processes included the use of questionnaires, anthropometric measurements, and biomarkers of stress (cortisol) and inflammation (C-Reactive Protein). These methods were used to (1) examine the relationship between BMI, central adiposity, and CMD risk, (2) assess the effects of psychosocial stress on subsequent inflammation profiles, and (3) explore how dietary practices may underlie the quantitative data surrounding stress and CMD risk. Data were collected on 21 UNC Chapel Hill undergraduates. Findings suggest increased psychosocial stress can be regarded as a prominent factor in underlying CMD risk among college students as cortisol levels and CRP levels have positive linear relationship. In addition, a diet falling in line with the Western Diet can be associated with increased CMD risk based on inflammatory profiles generated from CRP levels and anthropometric data. These results support the combination of dietary and stress interventions for reducing inflammation and down-regulating the negative impacts of psychosocial stress on CMD risk in college students.

Chapter 1

Introduction

Obesity, along with many cardiometabolic diseases, has become a global epidemic. This epidemic impacts individuals of all ages, with 1 in 10 United States children under age 5 suffering from obesity (Messiah et al., 2019). Studies show that cases of childhood and adolescent obesity result in many cardiometabolic diseases (CMD) later in adult life, which can result in cardiometabolic disorders such as stroke, heart attack, diabetes, hypertension, and high cholesterol (Messiah et al., 2019). CMD is umbrella term used to discuss a syndrome consisting of these types cardiovascular disorders, which is the world's leading cause of death as of 2016 (Mozaffarian et al., 2016; Sidney et al., 2016). Because of the increasing potential for CMD risk to begin early in life, it is necessary to tackle this issue by gaining a greater understanding of the potential risk factors. This can be done, as my study suggests, by examining CMD risk among healthy college-aged students.

Millions of heart attacks, strokes, and cardiovascular events occur throughout the United States each year, and nearly half of these will occur in seemingly healthy individuals that show no traditional signs of cardiometabolic disease (CMD) (Ridker, 2003). CMD prevalence across the globe has instigated a shift throughout many disciplines in the health sciences, including medical anthropology, causing researchers to look into the underlying predispositions, lifestyle factors like psychosocial stress and diet, and interplaying health factors that contribute to CMD and CMD risk. These factors have been shown to modulate the levels of cytokines (serum proteins) produced during the inflammation process of the immune response, and as such, elevated inflammation rates are a common marker for CMD risk (Després, 2012). The individual relationships between psychosocial stress and diet in regards to CMD risk have been shown

through previous studies, but the interaction of these factors and their combined impact on CMD risk is virtually unknown.

Inflammation can be measured via the use of biomarkers, specifically C-Reactive Protein (CRP). CRP is a vital marker for CMD because its circulating levels are impacted by the amount of adipose tissue and general obesity which is a common precursor to CMD (Hotamisligil, 2006). During the inflammation response, macrophages detect injury and release a cascade of cytokines that results in the production of CRP near the site of injury or infection, therefore making it an excellent marker for nonspecific pro-inflammation (Del Giudice & Gangestad, 2018).

Just as CRP serves as a reliable, quantitative measure of systemic pro-inflammation, biomarkers for stress can be assessed to form a more coherent picture of the interplay of an individual's lifestyle factors and health risks. This study utilizes salivary cortisol as a biomarker of stress since cortisol can be used to assess the functioning of the hypothalamic-pituitary-adrenal (HPA) axis, which modulates the stress response (Elzinga et al., 2008). By analyzing the prevalence of these biomarkers, inflammation and stress profiles can be generated in regards to central adiposity to develop a predictive measure of CMD.

Through analysis of these common lifestyle factors – psychosocial stress and diet – I am addressing various gaps in the literature by focusing on how these factors modulate the inflammation process and subsequent CMD risk in otherwise healthy college-aged individuals. This study seeks to aid in developing a holistic understanding of CMD risk in college-age, healthy individuals by (1) examining the relationship between these lifestyle factors and CMD risk, (2) assessing the effects of psychosocial stress on subsequent inflammation profiles, and (3) exploring how dietary practices may underlie the quantitative data surrounding stress, inflammation, and CMD risk among college students. By focusing on the demographic of

college students, these findings will prove useful in public health programming and future recommendations used to reduce the growing instances of CMD in the United States through promoting early preventative measures. New preventive measures and subsequent interventions would benefit from further understanding of how lifestyle factors contribute to the progression of CMD, which begins to manifest from a young age.

Hypotheses

1a) College-aged students with higher salivary cortisol levels will have higher measures of CRP and therefore generate an increased inflammatory profile.

1b) In college-aged students, Cohen Perceived Stress scores and salivary cortisol levels will track together to represent a similar measure of stress.

2a) College-aged students with higher BMI's will present higher CRP levels as a measure of increased risk for CMD.

2b) College-aged student with increased waist circumference will present higher levels of CRP indicating a relationship between central adiposity and CMD risk.

3a) College-aged students will report diets that fall more in line with the "Western Diet," which will consist of higher processed and cured foods, as opposed to the "Mediterranean Diet."

Literature Review

Life Course Theory and Cardiometabolic Disease Risk

The life course theory is the concept that early life events influence the trajectory of an individual's life, impacting several biological variables that can often result in disease later in life (Brunner, 1997; Friedman et al., 2015; Gluckman et al., 2007). Because of this, it is vital to address the CMD epidemic at early ages and focus on preventative measures rather than treatment. To do this, researchers must obtain a greater understanding of how the interplay of

risk factors presents itself before the onset of CMD. Too often, research and subsequent intervention are focused on instances of CMD in the later portions of individual's lives, because this is when the risk for CMD is greatest and when symptoms begin to present (Godfrey et al., 2010). By focusing on later life instances of CMD, the concepts of life course theory are often undermined as key concepts and causal factors in the ultimate development of CMD.

The precursors contributing to CMD, specifically diabetes, hypertension, and atherosclerosis, have been shown to begin in adolescence (ages 12-19) (Berenson, 2009; Friedman et al., 2015; Johnson et al., 2009). Despite developing in adolescence, it is often difficult to establish direct causal relationships between instances of CMD and exposure to risks due to the long and drawn-out timeline of the diseases that makeup CMD (Berenson, 2009; Sidney et al., 2016). In a comprehensive study addressing the prevalence of risk factors for metabolic syndrome among adolescents (12-18), Johnson et al. (2009) found that 50% of the individuals in the study expressed at least one measurement outside the normal established parameters for cardiometabolic disease risk, and 8.6% expressed at least three risk factors constituting a diagnosis of metabolic syndrome. This is alarming given the age parameters of the study and how early these risk factors present themselves which can translate into a diagnosis of CMD.

The developmental trajectories established early in an individual's life have been proven to impact the ability of that individual to respond to changing life circumstances (Gluckman et al., 2007; Godfrey et al., 2010). Gluckman et al. (2007) has cited the period from conception to infancy as a key phase in which permanent changes in metabolic function and other physiological processes can occur. These changes are a result of environmental conditions, as well as genetic and epigenetic manifestations, that present themselves early in life. One such

early life course factor that has been associated with CMD is reduced fetal growth and poor nutrition early in life (Hales & Barker, 2001; Worthman & Kuzara, 2005). Lack of proper nutrition in infancy increases occurrences of insulin resistance and permanently modifies the way the body intakes, uses, and stores nutrients (Hales & Barker, 2001). This relationship has been termed the "thrifty phenotype hypothesis" as it results in an individual's metabolism attempting to store excess nutrients in an effort to remedy the reduced nutrition faced during fetal development and infancy (Hales & Barker, 2001). This often results in obesity and diabetes later in life when the nutrient deficits that were faced in early on are no longer issues in the later stages of life (Hales & Barker, 2001). In a separate meta-analysis conducted by Baumeister et al. (2016) which included 18 studies involving CRP as a marker of inflammation, there was a significant association between early childhood trauma and later life increases in CRP levels. This meta-analysis proposed a method for how early life trauma impacts later life disease via the inflammatory pathway and additional immune responses that involve regulation of the hypothalamic-pituitary-adrenal (HPA) axis which relates to stress. Studies like these highlight the instances in which CMD risk factors begin early in life and further emphasizes the need to examine CMD risk in younger individuals as it pertains to the interplay of inflammation and psychosocial stress.

Aside from biological deficits like reduced nutrition, sociological factors such as perceived safety, family structure, and overall adversities experienced in adolescence can contribute to CMD later in life (Suglia et al., 2018). A study by the American Heart Association stated that "adverse experiences in childhood and adolescence, defined as subjectively perceived threats to the safety or security of the child's bodily integrity, family, or social structures, are known to be associated with cardiometabolic outcomes over the life course into

adulthood" (Suglia et al., 2018). Though this study does not directly address factors associated with early life adversity, it is an important relationship to understand when attempting to justify why addressing CMD risk in college-aged students is an essential task. Though college students are past adolescence and their developmental phase is nearing an end, the modulation of the lifestyle factors addressed in this study – diet and stress – can serve as potential interventions based on the principles of life course theory when addressing the CMD epidemic. This is crucial to instigating further channels of research that address CMD risk early in populations that may be prone to these conditions later due to the various sociological, biological, and epigenetic factors faced early in life.

Inflammation as a Marker of Cardiometabolic Disease

The inflammatory response is a normal and necessary part of proper immune function, but an increased rate of inflammation is a precursor for CMD (Ridker et al., 2000). Though inflammation has been linked to instances of CMD and CMD risk, the rate of inflammation in healthy young adults is not yet well understood (Choudhury & Leyva, 1999; Ridker et al., 2000). To understand the severity of CMD and its predispositions, researchers utilize the biomarker C-reactive protein (CRP), as it is a blood protein produced during the inflammation process (Ridker et al., 2000). Throughout the inflammation process, CRP is vital for wound healing and fighting bacteria to prevent infections and ensure overall survival (Ridker, 2003). Higher blood concentrations of CRP are indicative of increased rates of inflammation and subsequent instances of CMD (Mendall et al., 1996). CRP levels less than 1 mg/L are considered low while levels falling within the 1-3 mg/L are considered normal. CRP levels greater than 3 mg/L are considered high risk for CMD, and levels above 10 mg/L are likely due to persistent inflammation due to infection (Salazar et al., 2014).

Several studies have shown the relationship between increased CRP levels and higher risk for heart attack, stroke, and arterial disease (Ridker, 2003). Evidence from a study conducted by Scherer et al. (2017) found that higher blood CRP levels were associated with a greater incidence of major adverse cardiovascular events. This study included "conventional" risk factors, such as diet and exercise, as well as accounting for inflammation via measures of CRP. Ultimately, results showed that increased levels of inflammation amplifies CMD risk even when receiving other treatments for factors outside of inflammation.

One of the key ways in which overproduction of CRP results in CMD is through its impacts on the vascular system (Libby et al., 2002). The way in which the inflammation process functions to protect our bodies also actively impacts the manifestation of arteriosclerosis and other CMD related vascular diseases, which can be seen in Figure 1. (Libby et al., 2002). Inflammation plays an intricate role in the manifestation of CMD related diseases via the biological

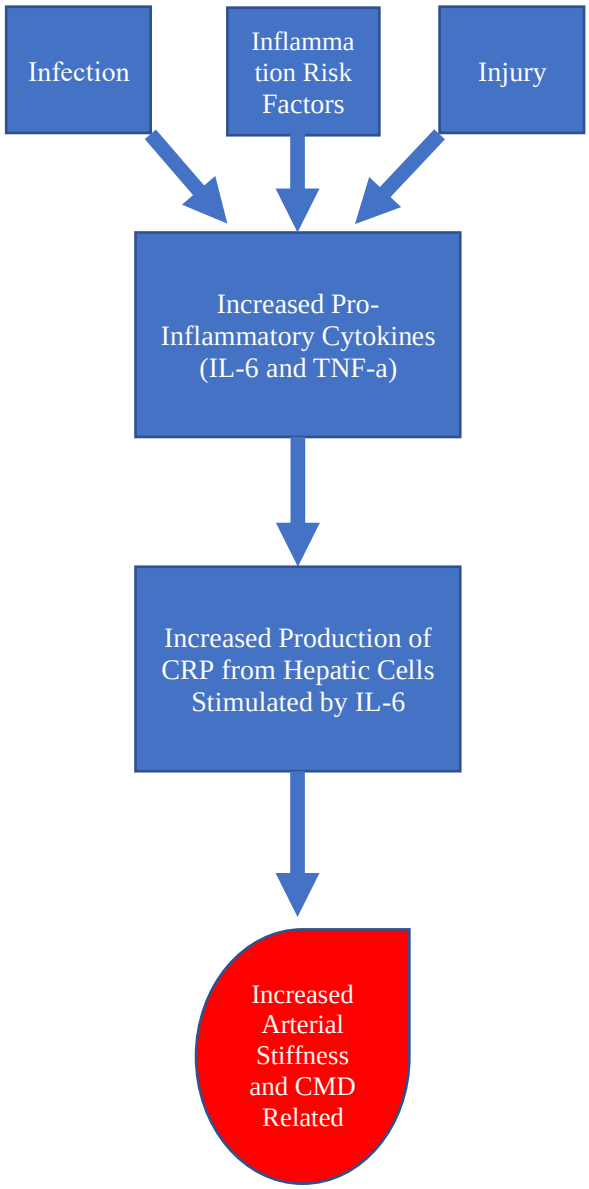

Figure 1. Inflammatory Process. IL-6 (Interleukin-6) and TNF (Tumor Neurosis Factor – alpha) stimulate CRP (C-Reactive Protein) production which results in increased arterial stiffness and other CMD related diseases after entering blood circulation.

intermediates and cytokines produced in the process. Some cytokines stimulate the release of others in a cascade manner, such as Interleukin-6 (IL-6 and Tumor Neurosis Factor – alpha (TNF-a), which stimulate increased production of CRP from the liver (Brevetti et al., 2010; Libby et al., 2002). These cytokines and intermediates cause changes in the endothelial tissue lining the arteries, which impacts blood flow, blood pressure, and heart function, that can be exacerbated by other lifestyle factors like smoking, exercise, and diet (Brevetti et al., 2010; Mozaffarian et al., 2016). A longitudinal study conducted by Berg et al. (2015) began by measuring baseline levels of CRP in 2003, and years later in 2008/2009 measured the instances of arterial blood flood in the same cohort. They found that elevated levels of CRP, and in some cases baseline levels, led to increased instances of diseases related to CMD, such as increased arterial stiffness.

The rate of CRP production varies between individuals based on a variety of factors including genetics diets, and habits like smoking or drug use (Ridker, 2003). Understanding how this variability in inflammation arises between individuals and how other lifestyle factors like diet, physical activity, and stress can impact inflammation is an important step in lowering the epidemic of CMD and CMD risk. A quote from Ridker, 2003 states "…older tests for CRP, which are adequate for monitoring severe inflammatory conditions, do not have the ability to measure levels accurately within the range needed for cardiac risk detection." To address this issue, many medical professionals and researchers utilize high-sensitivity CRP tests to measure blood CRP levels that are more in line with overall CMD risk as opposed to extreme cases of CMD (Ridker, 2003). In this study, we will be analyzing CRP via high-sensitivity CRP tests to detect CRP levels in the realm of CMD risk in otherwise healthy college-aged individuals.

Diet, Adiposity, and Cardiometabolic Disease Risk

Several studies have shown that there is a strong positive correlation between central adiposity as a measure of obesity, CMD risk factors, and rates of morbidity and mortality among adolescents (Adair et al., 2014; Song et al., 2017). In regards to body mass index (BMI), individuals with a BMI of 18.5 to 25 are considered to be normal weight. Individuals with a BMI of 25.0 to 30 are considered overweight, and a BMI of 30.0 or higher is considered obese (Harrington et al., 2013). Several factors can impact BMI, such as genetics, diet, and physical activity levels.

Additional studies have shown that nutrition therapy can successfully treat and even prevent instances of diabetes, stating "clinical trials also provide evidence for the effectiveness of nutrition therapy in the prevention of diabetes" (Franz et al., 2014). When it comes to diet as a potential lifestyle factor impacting CMD and CMD risk, there is much debate about the specific nutrients, vitamins, and minerals that have direct correlations with CMD (Funtikova et al., 2015). However, analysis by Franz et al. (2014) found that large amounts of sugar-sweetened beverages greatly increased the risk for CMD. Additionally, they found that "The two interventions resulting in the largest amount of weight loss at one year were the Mediterranean-style eating pattern (-6.2 kg) in persons with newly diagnosed diabetes" (Franz et al., 2014). Additional studies have shown supporting evidence that adhering to the Mediterranean diet greatly improves health and reduces the instances of CMD throughout life (Sofi et al., 2008). This strongly suggests that changing factors in diet can decrease risk and even reverse the effects of CMD to some degree when coupled with altering additional lifestyle factors.

Many studies involving dietary analysis of adults in regards to CMD have yielded similar results when conducted in children. However, far fewer dietary studies have been conducted in

children and adolescents in regards to CMD and CMD risk (Funtikova et al., 2015). Most of these dietary studies focused on the rates of consumption of fast food, dairy, and meat with little focus on particular nutrients and specific dietary components (Funtikova et al., 2015). Funtikova et al., 2015 points out that "a special gap exists in studies of a priori dietary patterns - very little research has been done on vegetarian diets, the Mediterranean diet, and dietary indexes" (Funtikova et al., 2015). Further analysis of dietary patterns and classification of dietary types in young adults and adolescents is needed to fill this gap in the literature as this study seeks to do.

Fletcher et al, 2015 states that "the variability of dietary variables between studies suggests further work is needed to understand the role of dietary intake when examining these associations in youth" (Fletcher et al., 2015). This further exposes a gap in the literature that emphasizes a need to study dietary intake in younger individuals. Most studies of dietary intake focus on specific nutrients, but this can be ineffective in determining health outcomes because people do not consume individual nutrients. Nutrient interactions play a vital role in overall health outcomes, which is why taking a holistic approach to examining dietary intake could be crucial to understanding diet as it related to CMD risk.

Psychosocial Stress and Cardiometabolic Disease Risk

The hypothalamic-pituitary-adrenal (HPA) axis is the primary controller of the stress response (Cohen et al., 2007). The HPA axis mediates the immune system and the stress response through the release of cortisol and its function in a negative feedback system as seen in Figure 2. (Cohen et al., 2007). The HPA axis functions by the release of corticotropin-releasing hormone (CRH) from the hypothalamus which then stimulates the anterior pituitary to produce and release adrenocorticotropic hormone (ACTH). ACTH targets the adrenal cortex of the adrenal glands, stimulating them to produce cortisol – the body's stress hormone. Initially, the stress response is a necessary reaction that is vital in the short term by heightening arousal, improving reflexes, and improving attention. However, studies have shown that if this response goes on too long, it can be detrimental to immune function (Chrousos, 1995; Cohen et al., 2007). Additionally, a prolonged stress response can result in the increased release of glucocorticoids (cortisol) from the HPA axis, which suppresses CHR through negative feedback

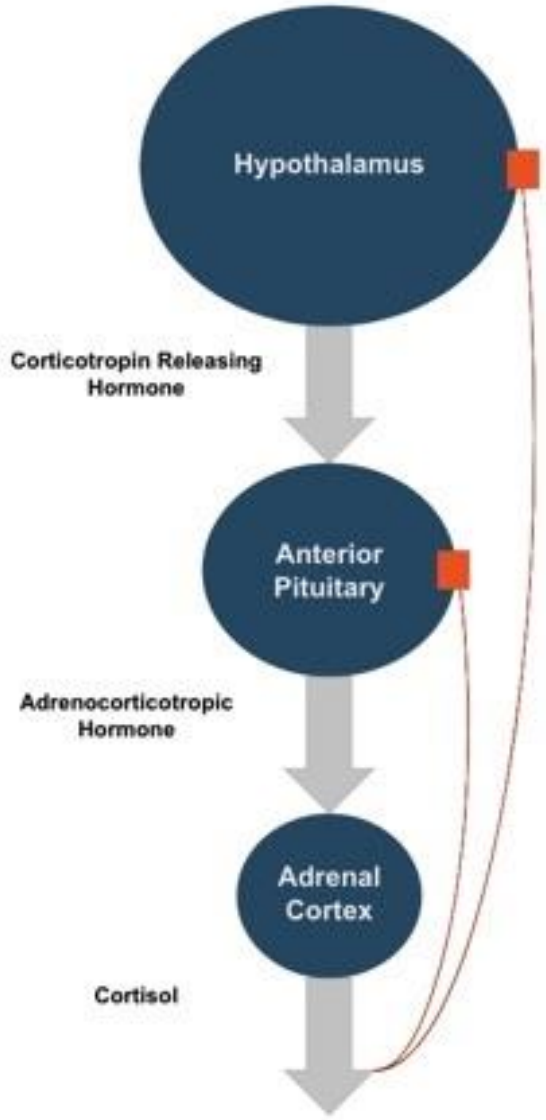

Figure 2. Hypothalamic-Pituitary-Adrenal (HPA) Axis Pathway. Cortisol is the key factor which is both a product of the HPA axis as well as its principle regulator. Mis-regulation of this pathway results in an improper stress response that can have broader reaching implications.

inhibition. Glucocorticoids play a role in the circulation of leukocytes in the bloodstream and suppress the production of cytokines and other mediators of inflammation. This relationship between glucocorticoids and inflammation in the body is similar to the way increased physical activity can reduce cytokine function (Chrousos, 1995). Because of this relationship, improper regulation of the HPA axis due to chronic stress can greatly impact how the stress response works in conjunction with the body's immune function and how an individual's CMD risk can be elevated.

The stress response often targets factors involved in the fear and reward center, the sleep-wake cycle, and the central nervous system, and as such, these are the areas that are often impacted most greatly by stress disorders (Chrousos, 2009; Cohen et al., 2007). Stress disorders are often not due to short-term stress, but rather long-term stress which can feed into a vicious cycle in which stress creates more stress, furthering CMD risk. Many long-term stress disorders impact the body in a greater context, such as impeding growth and development, suppressing the immune system, and inhibiting proper sleep. Because of this relationship between chronic stress and overall health, assessing an individual's level of stress has become an increasingly important health measure. As a way of measuring stress, doctors and researchers use salivary cortisol as biomarker of stress, which if collected over time, can be analyzed to generate an accurate stress profile (Elzinga et al., 2008).

Summary

There is a lack of data in the field of biological anthropology, and in the field of cardiovascular health in general, concerning the prevalence of inflammation and subsequent CMD risk in younger populations. In order to help fill this gap, this study seeks to examine

inflammation rates in college-age individuals in conjunction with outside factors such as a stress and diet. This study combines quantitative biomarker data with qualitative measures of perceived stress and self-reported dietary intake to uncover general relationships that may reveal trends in CMD risk in college-aged individuals (Figure 3).

Chapter 2

Methods

Participant Recruitment

Data for this project was collected from Spring of 2018 through Fall 2019 in conjunction with an Undergraduate Research Consultant Team (URCT) funded by UNC's Office of Undergraduate Research (OUR). Participants were recruited via list-serve email and flyers and participation was limited to UNC undergrads, graduate, and professional students who were between the ages of 18 and 24. Further stipulations excluded anyone who may have been pregnant or breast feeding, diagnosed with any form of CMD, or part of a UNC sports team. In total, 21 individuals participated in the study. Of the 21 participants included in this study, 10 were compensated with payment of one $25 Target gift card after completion of the study. Students consented to participation after a lab member described the study in-depth and read through the consent form IRB Study # 18-3156 (appendix D). Their signature was attained and they were allowed to keep the informative portion of the consent packet. Participation was voluntary and all participants could drop out at any time without penalty.

Anthropometry, Diet, and Biomarker Data Collection

Anthropometric measurements were collected using standard techniques. Height was measured in centimeters. Weight and BMI were taken using an electric scale after inputting the appropriate values for height, age, and sex. Skin folds were measured using Baseline Medical

Skinfold calipers and were taken in triplicate to obtain average values. Skin folds were measured at the biceps, triceps, subscapular region, and suprailiac region. Arm and waist circumferences were measured in addition to blood pressure.

Saliva samples containing salivary cortisol were self-collected by participants 5 times a day on 3 separate days over a 7-day period for a total of 15 samples collected. Participants were instructed to passively drool into 15 ml vials provided immediately after waking, 30 minutes after waking, before eating lunch, in the evening, and immediately prior to going to sleep. They were instructed to:

a) Avoid foods with high sugar or acidity, or high caffeine content, immediately before sample collection, since they may interfere with the cortisol measurement.
b) Document consumption of alcohol, caffeine, nicotine, and over-the-counter medications within the prior 12 hours.
c) Document vigorous physical activity.
d) Do not eat a major meal within 60 minutes of sample collection.

Participants were provided a portable cooler to use in storing the saliva samples collected during the day, but were asked to place them into their personal freezer at the end of each collection day.

Dried blood spots (DBS) used for CRP analysis were collected from every participant at their initial and final lab visits for a total of two blood spots per participant. DBS were collected via a non-invasive finger prick onto Whatman No. 903 protein filter paper from the participant's non-dominant hand. After collection, DBS were allowed to air dry and were then stored in a -20℃ until analysis.

Dietary data was collected using the National Cancer Institute's Automated Self-Administered 24-hour Dietary Assessment Tool. Participants were given an autogenerated username and password and asked to logon to the website: https://asa24.nci.nih.gov, where they completed the 24-hour dietary recall listing all food and beverage consumed in the past 24 hours in addition to information regarding food procurement. Specific details amount amounts of food eaten were also included in the 24 hour recall questionnaire.

CRP Assay

Assays were conducted using an enzyme-linked immunosorbent assay technique with a R&D systems human C-reactive protein Quantikine ELISA kit. They were carried out in the Human Biology Laboratory at the University of North Carolina at Chapel Hill. DBS samples were collected for analysis using a 1/8" hole punch of the sample. A sample free of debris was chosen from the center of the DBS absorbent paper. After the DBS was punched out, they were placed into test tubes along with elution buffer. After soaking, samples were placed into a standard household refrigerator and incubated overnight, stored at approximately 38° F.

Following overnight incubation, the samples were removed from the refrigerator and allowed to set while the plate was prepared. 50 μL of either standard, control, or sample were added to the wells of a clear-bottom 96 well plate. Standards were prepared via serial dilution following the instruction of the ELISA kit. After preparation, the plate contained eight standards and two Quantikine controls run as duplicates for low and high concentrations of cytokines. There were 21 samples, each consisting of two separate dry blood samples collected 7 days apart. 18 of the samples were run in duplicate. The wells of the plate were designed with antibodies to bind CRP already existing in the well-bottoms. This allows the CRP to remain bound after four rounds of plate washing. An additional antibody was added after washing that

bound to the complex CRP-antibody remaining in the well-bottom, which instigated a color change in the assay from blue to yellow. Once this color change occurred fully after 30 minutes, the plate was analyzed to assess optical density given that the intensity of the yellow color is directly proportional to the concentration of CRP in the sample. To measure absorbance and optical densities, we utilized a Biotek ELx800 absorbance microplate at 450nm and 630nm. Samples were corrected by a factor of 1.6mg/L because DBS CRP levels are higher than serum levels. The standards in this assay were used to generate a "standard curve" of known CRP concentrations from the which the sample concentrations can be compared. CRP standards for comparison, low (<1 mg/L), moderate (1 to 3 mg/L) and elevated inflammation (3 to 10mg/L), are taken from clinical practice. For a full lab protocol, see Appendix A.

Cortisol Assay

Cortisol assays were conducted using an enzyme-linked immunosorbent assay technique with a R&D systems salivary cortisol ELISA kit. They were carried out in the Human Biology Laboratory at the University of North Carolina at Chapel Hill. All saliva samples were centrifuged at 50000 RPMs for 15 minutes prior to sample preparation to move all unwanted materials to the bottom of the tube. Only the supernate was used for this assay. Each Sample required a 5 fold dilution that was done by taking 50 µL of sample + 200 µL of Calibrator Diluent RD5-43.

All reagents and standards were prepared following the ELISA kit assay guide and 150 µL of Calibrator Diluent RD5-43 into the non-specific binding (NSB) wells while 100 µL of Calibrator Diluent RD5-43 to the zero standard (B_0) wells. After this, 100 µL of standard or sample was added to the appropriate wells of a clear bottom 96 well plate. After adding the

appropriate conjugates and antibody solutions the plate incubated for 2 hours on a plate rotator. Following incubation, the plate was washed with wash buffer 4 times. The substrate solution was added following washing and allowed to incubate for an additional 30 minutes in a dark place away from light. After 30 minutes, the stop solution was added to each well and a color change from blue to yellow was observed. The optical density of each well was determined following the addition of stop solution using a Biotek ELx800 absorbance microplate set to 450 nm. For full lab protocol see appendix B.

Surveys and Questionnaires

The 10-item Cohen Perceived Stress Scale (PSS-10) was used to assess perceived stress of participants and was self-administered outside of the lab (Cohen et al., 1983). Four out of the ten items of the PSS-10 are worded in a positive manner while the additional six items are worded in a negative manner. Each response item was valued on a five-point scale (0 = never to 4 = very often). Total scores are tabulated by converting positive items' scores to negative values 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. To view the full survey and scoring procedure see appendix C.

Data Analysis

Cortisol and CRP were analyzed using RStudio 2019 version 1.2.5033 for Macintosh HD as well as excel 2019 version 16.35. The Cohen PSS, BMI, and self-reported factors were tabulated as independent variables in relation to CRP rates and cortisol levels in all participants. To calculate awakening response from cortisol data, the slope between the first two data points from cortisol collection was calculated. This was from the first saliva sample collected after waking to the 30 minutes post waking. Diurnal slope was calculated from the peak of the cortisol

levels to bedtime collection. Area under the overall cortisol curve (AUC) was calculated to represent and overall amount of cortisol produced by each individual.

Chapter 3

Results

Summary Data

Table 1 below shows summary data collected on all participants prior to beginning the biomarker collection process. Values for waist circumference were not able to be collected on four out of the 21 participants used in the study. Arm circumference was not collected on only one participant. Biological sex is reported in this summary table, but was considered as factor when examining the relationships between measurements as the majority of this sample is female.

Participant Anthropometrics

Participant Identification	Height (cm)	Weight (kg)	Waist Circumference (cm)	Arm Circumference (cm)	Blood Pressure	BMI Kg/m^2	Biological Sex (M/Fm)
101	163.3	84.8	96.2	33.4	97/60	33.1	Female
102	178.4	67.2	78.0	27.6	101/63	22.5	Male
103	183.5	90.1	91.7	30.1	105/72	26.9	Female
104	173.0	76.6	92.5	32.6	120/81	26.4	Male
105	166.0	60.1	77.0	-	103/65	22.7	Female
106	174.6	69.0	75.0	27.8	125/77	23.8	Female
107	183.6	77.4	79.0	28.3	106/62	23.1	Female
108	165.7	57.3	68.0	25.8	99/68	21.7	Female

109	162.9	60.6	72.0	24.9	110/27	23.7	Female
111	172.2	105.1	40.7	39.9	119/78	35.4	Male
120	162.0	44.2	-	23.9	98/68	16.7	Female
121	152.0	53.1	48.0	14.0	98/66	23.0	Female
122	160.2	68.1	75.2	20.8	108/74	25.9	Female
123	167.0	76.9	-	30.0	137/74	27.6	Male
124	172.5	51.2	-	29.0	102/70	17.2	Female
125	170.0	68.3	-	25.0	112/79	23.6	Female

Table 1. Summary data of participant anthropometrics and vitals measurements.

Table 2 shows the various BMI categories our participants fall in in relation to being considered underweight, normal weight, overweight, or obese. We had participants in all BMI ranges and weight classifications with the majority falling into the normal or underweight categories.

BMI Breakdown

Classification	**Range**	**Number of Participants Within Given Range**
Underweight	<18.5	2
Normal Weight	18.5–24.9	8
Overweight	25–29.9	4
Obese	>30	2

Table 2. Summary data of BMI index values compared to recorded participant BMI values.

Perceived Stress and Salivary Cortisol

Table 3 outlines summary data from completion of the Cohen perceived stress test by 21 participants. The median, mean, and standard deviation for Cohen PSS are reported with the mean score being 18.8 and a standard deviation of 5.5. Cohen PSS scores of 13 are considered average stress levels. Scores of around 20 points are considered high stress groups and scores of 20 or more are considered very high stress.

Cohen Perceived Stress

N	**21**
Median	**20.0**
Mean	**18.8**
Standard Deviation	**5.5**

Table 3. Cohen PSS Summary Data.

Figure 1 displays the relationship between perceived stress as reported by the Cohen Perceived Stress test and BMI. A higher BMI is generally associated with a higher perceived stress score as shown by this weak linear relationship with a correlation coefficient (r) of 0.541.

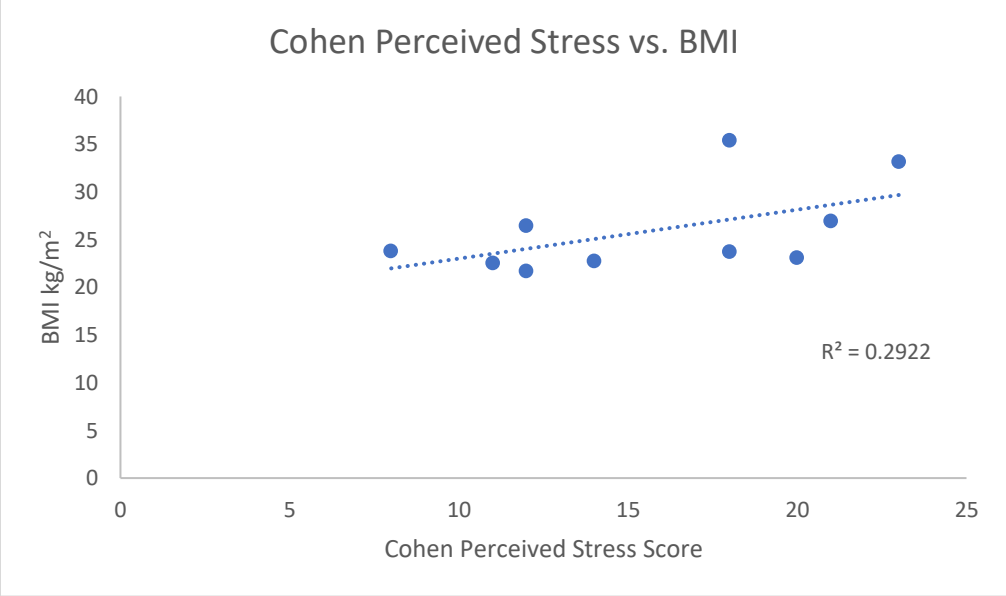

Figure 1. BMI tracking with Cohen perceived stress scores

Figure 2 displays the average daily cortisol fluctuation throughout the day. Sample were collected 5 times per day for 3 nonconsecutive days over a 7 period. The cortisol samples collected at corresponding times were averaged over the 3 collection days to give an average daily value of cortisol fluctuation. On the X-axis, time point one represents sample collection immediately after waking. Time point 2 represents sample collection 30 minutes after waking. Time point 3 represents collection mid-day collection. Time point 4 represents evening sample collection and time point represents sample collection 30 minutes prior to bed.

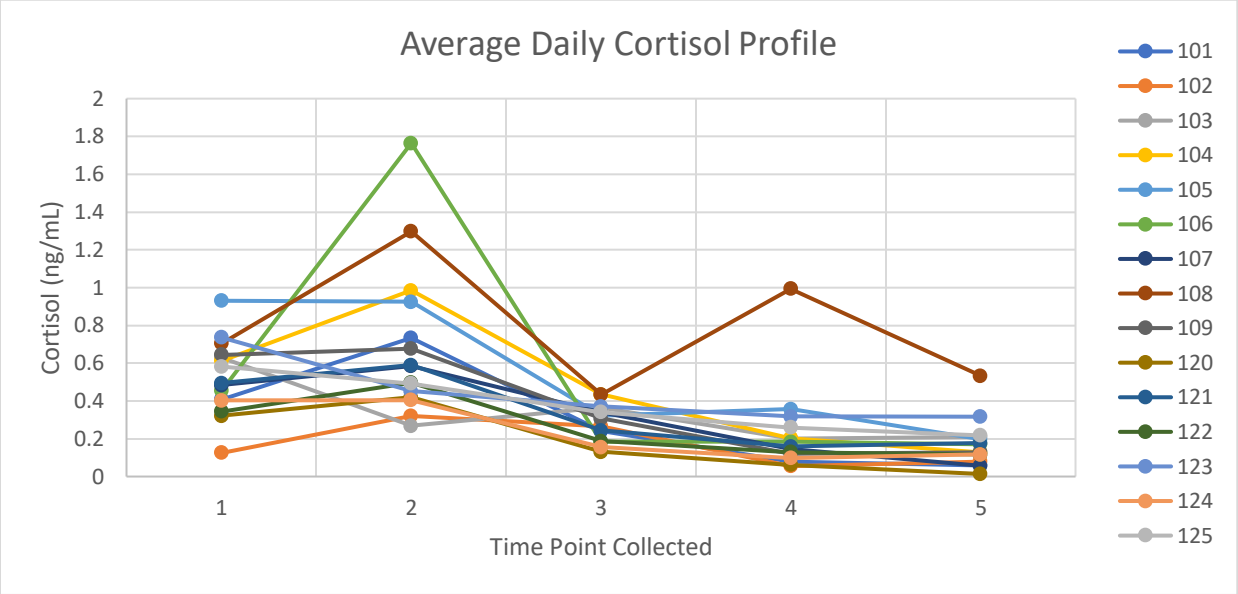

Figure 2. Daily Cortisol Fluctuation.

Table 4 displays cortisol summary data from calculated from Figure 1. Awakening response is the slope of the cortisol curve from the time of awakening to 30 minutes prior to waking. This slope should be a steady increase in typical cortisol curves for healthy individuals and renders a positive awakening response value. Area under the curve (AUC) of the cortisol curve represents the total amount of cortisol fluctuating throughout the day and can be

considered a direct measure of stress. The average value across our participants for area under the curve was 3.644 ng/L with a standard deviation of 1.754.

Cortisol Summary Data

Participant Number	**Awakening Response (ng/L)**	**Diurnal Response (ng/L)**	**Area Under the Curve (AUC) (ng/L)**
101	0.325	-0.186	2.859
102	0.195	-0.028	1.795
103	-0.362	0.122	3.210
104	0.373	-0.201	4.595
105	-0.006	-0.110	4.334
106	1.311	-0.418	5.418
107	0.102	-0.138	2.684
108	0.594	0.101	8.693
109	0.034	-0.097	3.448
120	0.099	-0.127	1.704
121	0.097	-0.020	3.203
122	0.153	-0.048	2.481
123	-0.284	0.166	4.311
124	0.001	-0.018	2.1855
125	-0.089	0.053	3.744
Averages	0.169	-0.063	3.644
Std. Dev.	0.396	0.148	1.754

Table 4. Summary Data of cortisol measurements.

Figure 3 below explains where the different measurements of the summary data in Table 4 are calculated from in the slopes shown in Figure 1. Overall AUC can be measured by dividing the curve into separate trapezoids and using the formula area of a trapezoid for to calculate individual areas. Once calculated, summing the individual areas provides a total AUC. The overall slope should be a negative value which is reported as the diurnal response in Table 4 above. Diurnal response is the slope of the curve from the peak of cortisol levels to bedtime.

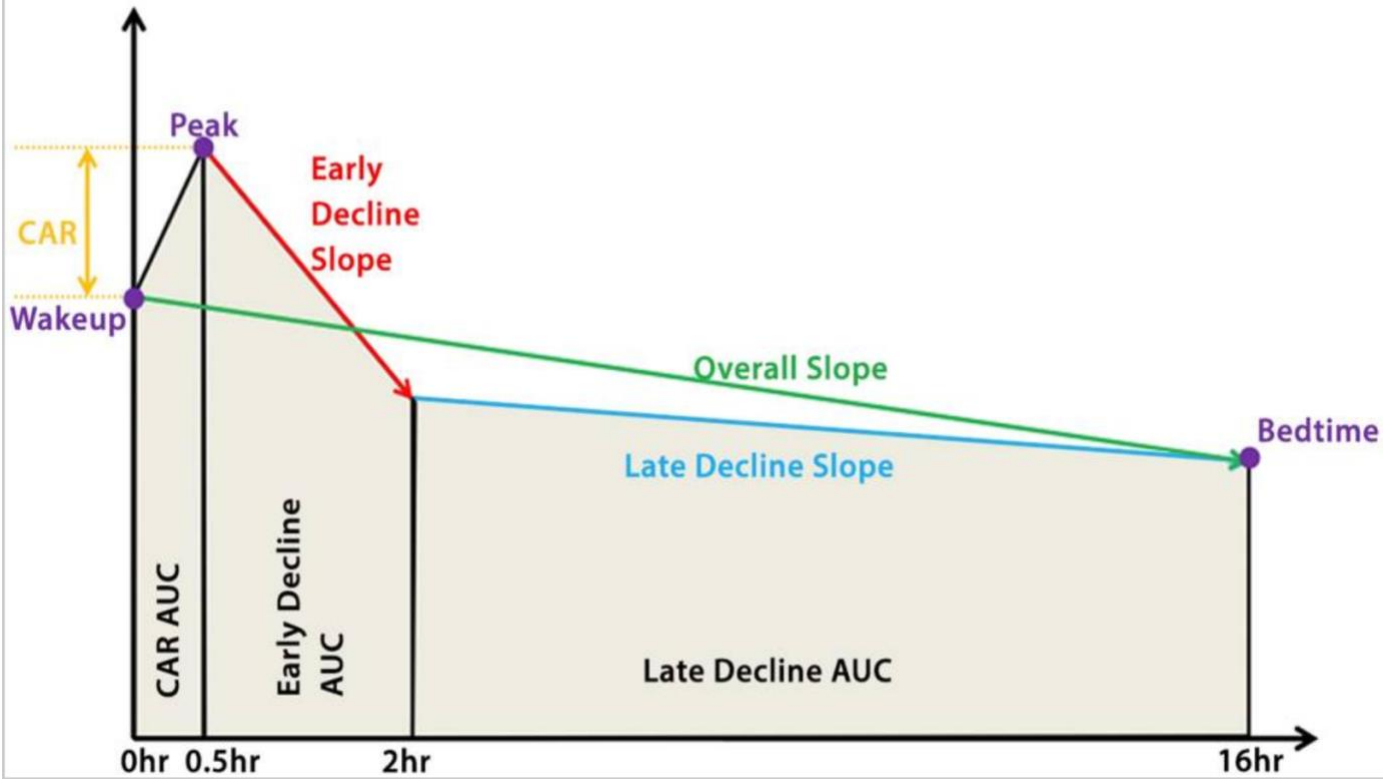

Figure 3. Explanation of cortisol summary data calculations adapted from (He et al., 2015).

Figure 4 shows both variables that served as a measure of stress in this study. A negative trend is apparent between Cohen PSS and salivary cortisol levels such that as Cohen PSS scores increases, CRP levels decrease. The correlation coefficient (r) here was -0.697.

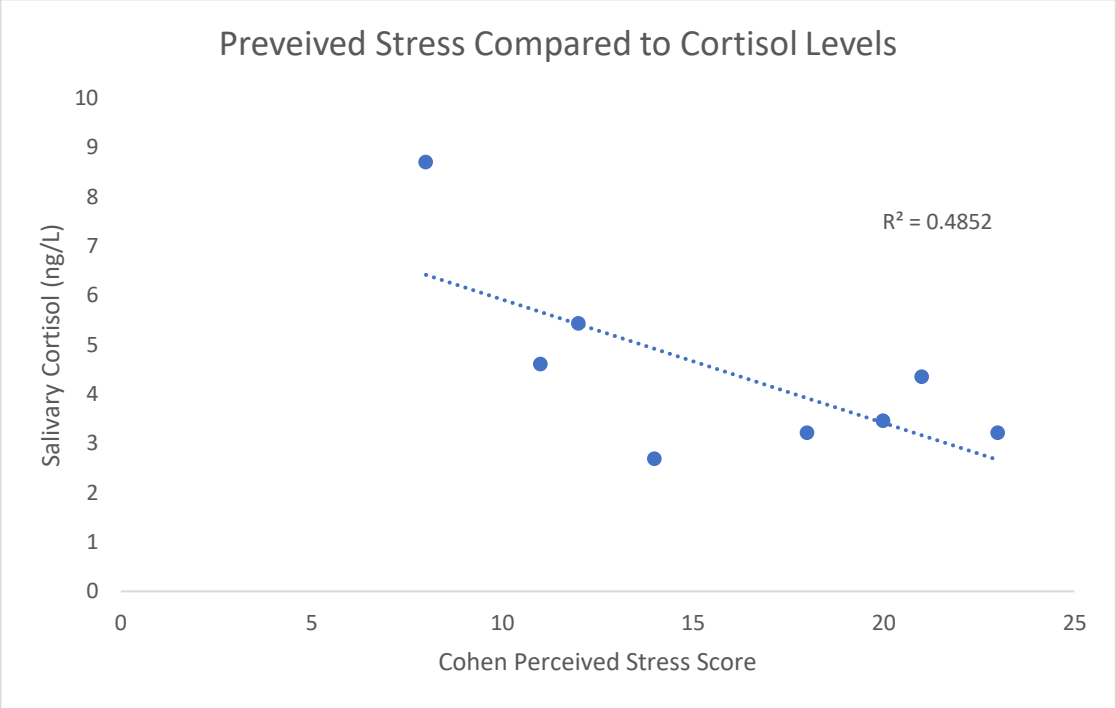

Figure 4. Cohen perceived stress vs. salivary cortisol levels.

CRP as a Marker of Inflammation in Relation to Other Factors

CRP levels shown below in Figure 5 categorize the number of participants that fall into ranges of CRP levels with the majority of participants falling in the low to mid-inflammation range of CRP. CRP standards for comparison are low (<1 mg/L), moderate (1 to 3 mg/L) and elevated inflammation (3 to 10mg/L). As seen in Table 5, an average CRP level from two combined visits was found to be 4.22 with a standard deviation of 3.83 which falls in the elevated inflammation range.

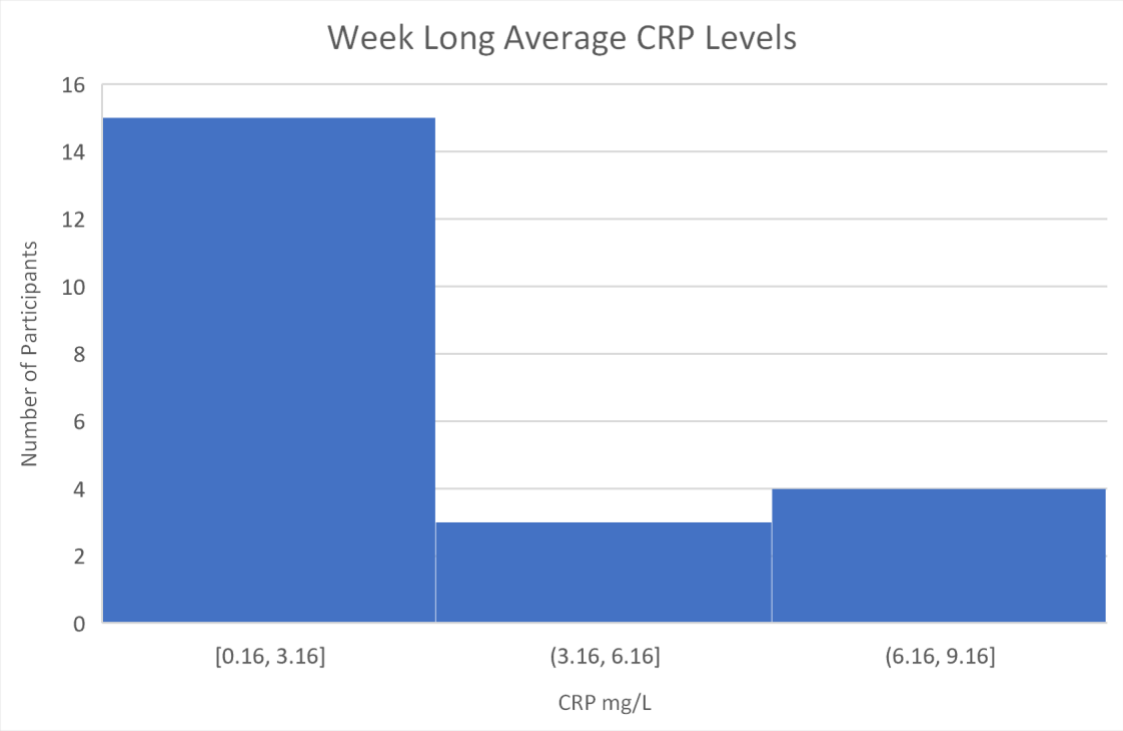

Figure 5. Categorized average CRP levels.

CRP Breakdown

	Average at Visit 1	**Average at Visit 2**	**Week-long Average**
CRP (mg/L)	2.94	2.33	2.64
Std. Dev.	3.43	2.24	2.39

Table 5. Average CRP values form DBS collected at first visit and visit 2, spaced 7 days apart.

Figure 6a shows the relationship between overall CRP levels as they pertain to cortisol levels. With the exception of approximately 3 participants, cortisol and CRP levels track together, increasing and decreasing following similar trends. Figure 6a shows a positive linear correlation between CRP and Cortisol with a correlation coefficient (r) of 0.514. Figure 6b shows the individual CRP and salivary cortisol levels of each participant.

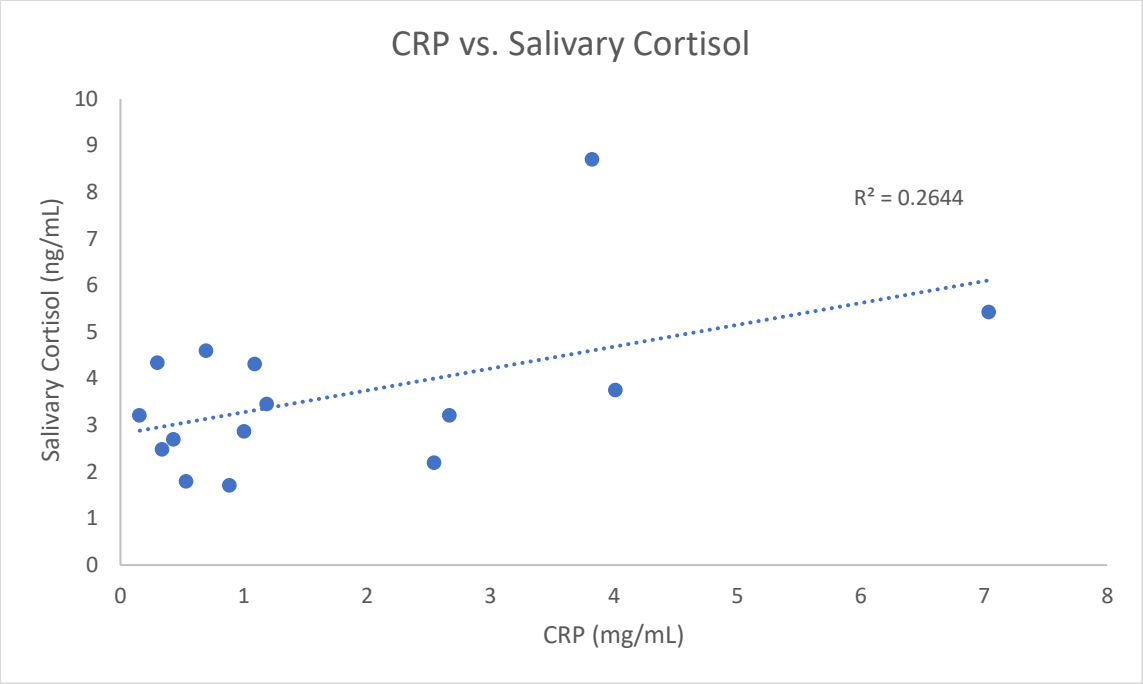

Figure 6a. CRP levels vs. AUC salivary cortisol levels.

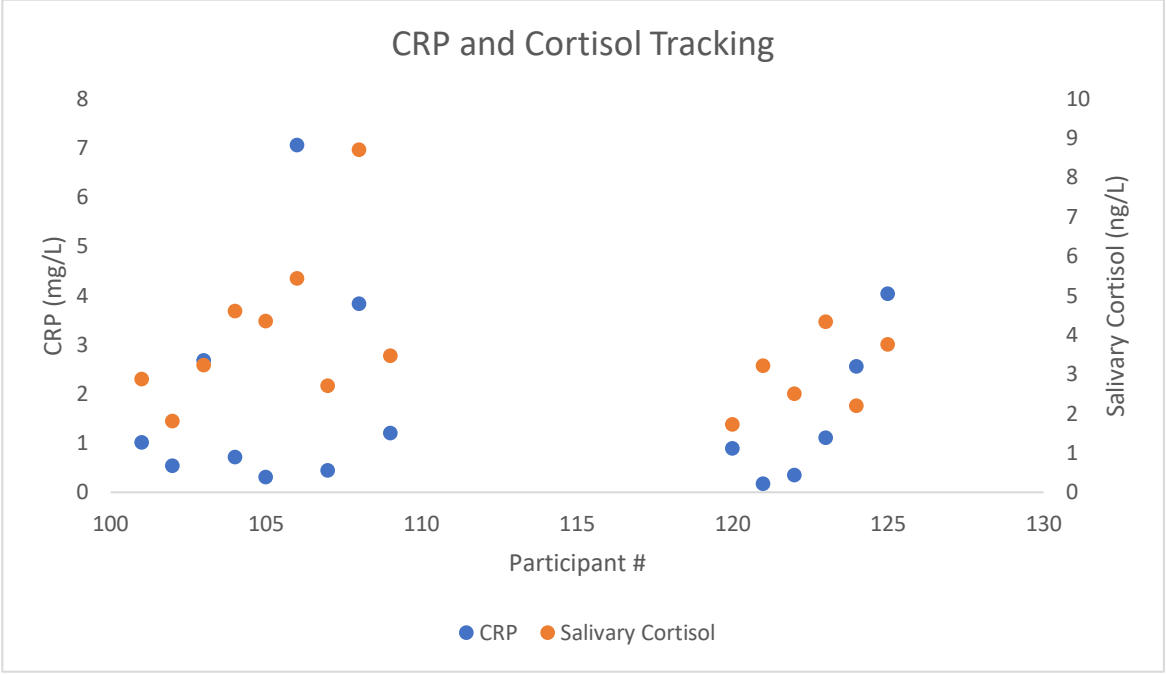

Figure 6b. Individual's CRP levels tracking with AUC cortisol levels

Figure 7 shows the CRP level of each individual participant as it relates to the waist circumference of each participant for which a measurement was taken. With a correlation

coefficient of 0.069, there is essentially no linear relationship here indicating no relationship exists between CRP levels as a waist circumference for this sample. Figure 8 shows CRP level of each individual participant as it relates to the BMI of each individual participant for which a measurement was taken. There a no real linear relationship between these variables with a very weak correlation coefficient of 0.145. A single CRP sample >10mg/L was excluded from figure 8, as it is indicative of current infection.

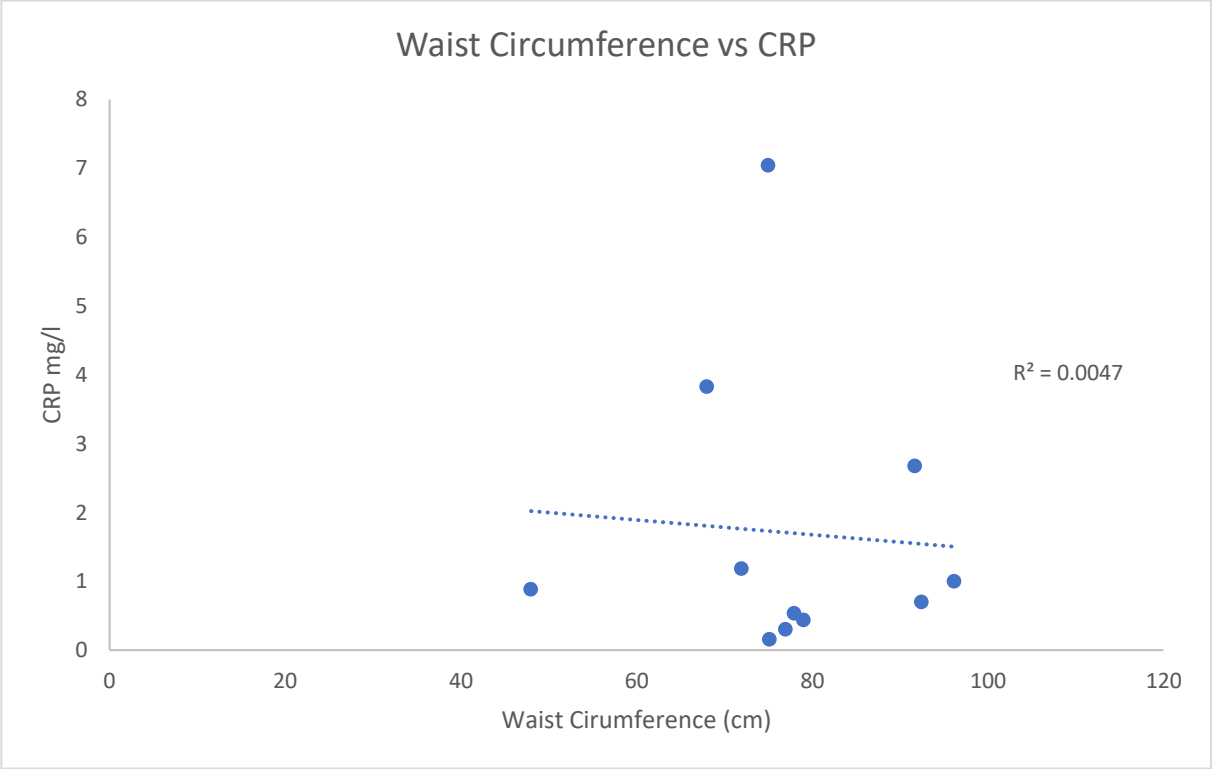

Figure 7. Participant breakdown of waist Circumference vs. CRP levels.

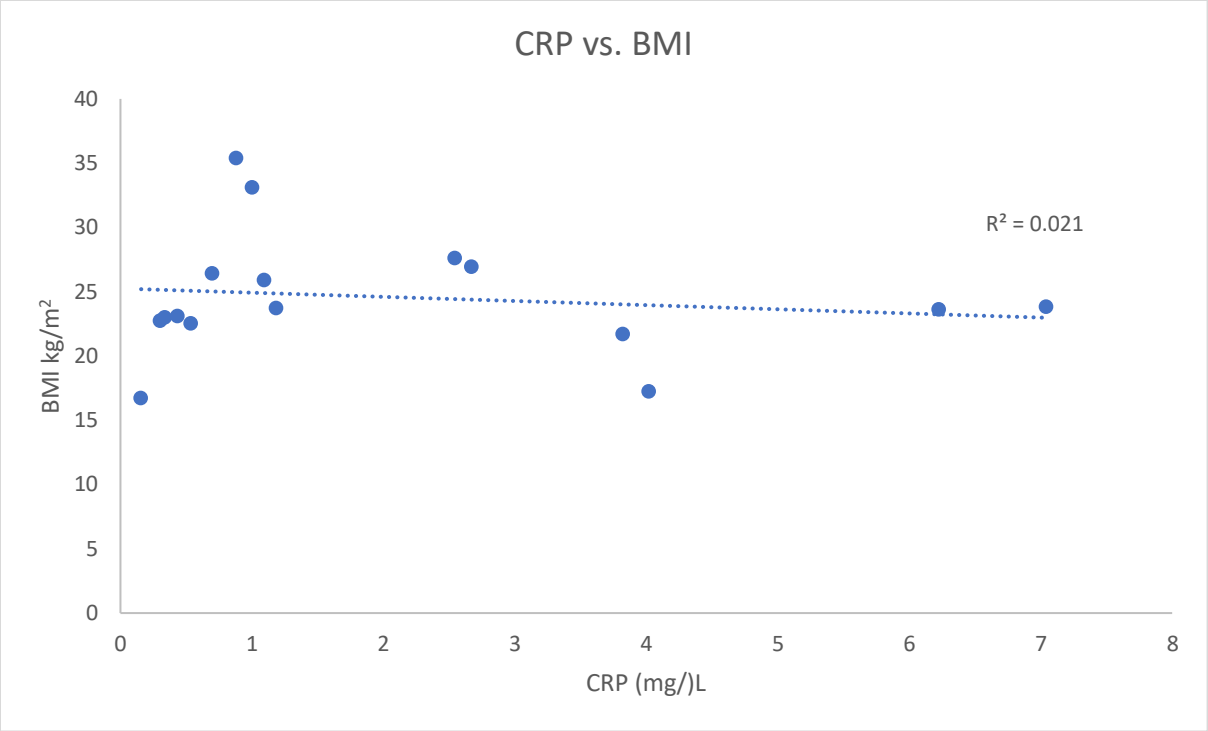

Figure 8. Participant breakdown of CRP levels vs. BMI

Trends in Dietary Practices Among College Students

Figure 9 shows the amounts of food consumed in grams of various food categories over a 24 hour period by our sample of college students. The amounts of food consumed were based on a 2000 calorie diet and the foods and amounts were self-reported via the National Cancer Institute's ASA24 Automated 24-hour Dietary Assessment Tool. The majority of the foods consumed by college-aged individuals in this sample fell into the category of carbohydrates, followed by sugars.

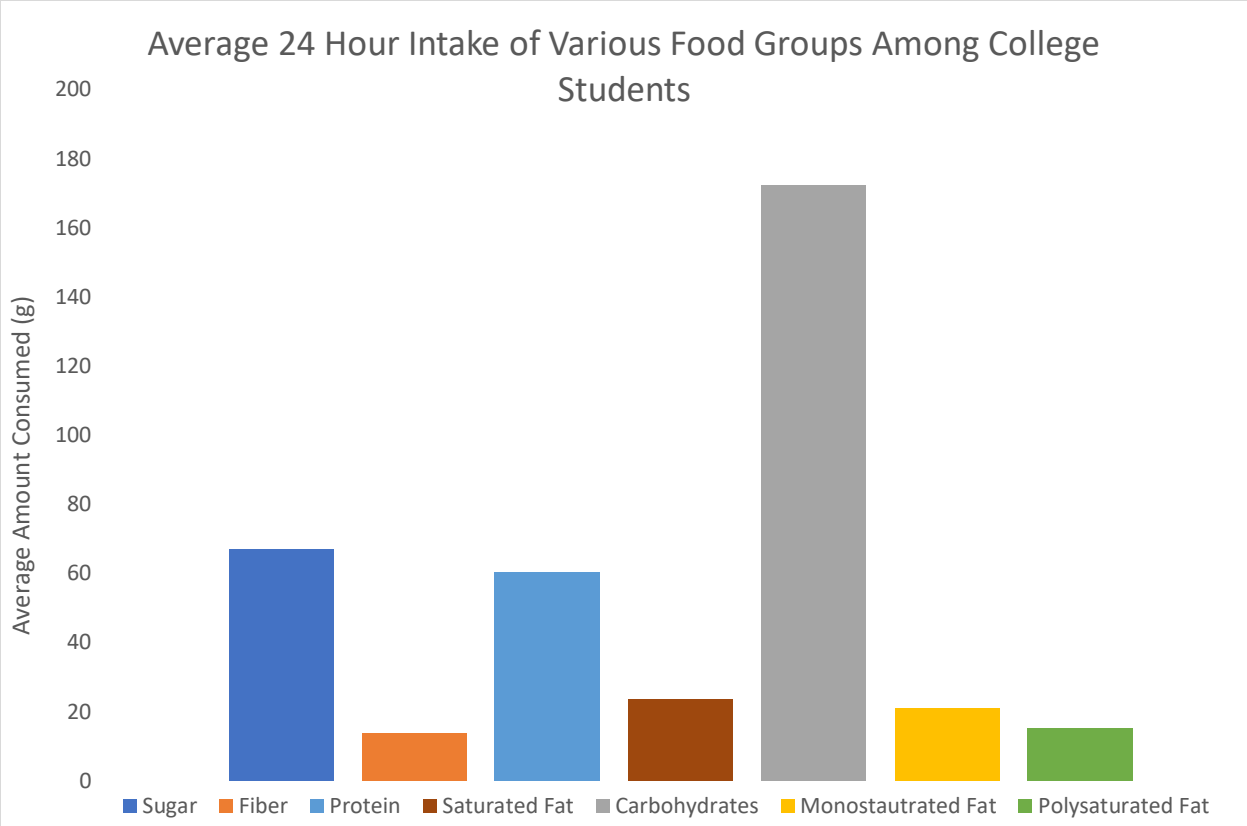

Figure 9. Breakdown of food groups and amounts of consumption self-reported by college students in a 24 hour recall. Data is based on a 2000 calorie diet.

Figure 10 shows the amounts of specific nutrients and minerals consumed in a 24 hour period by our sample of college students. Of the various minerals and nutrients reported, potassium was most consumed by college-ages, followed by calcium. A relatively low amount of iron was consumed by this sample of college-aged individuals.

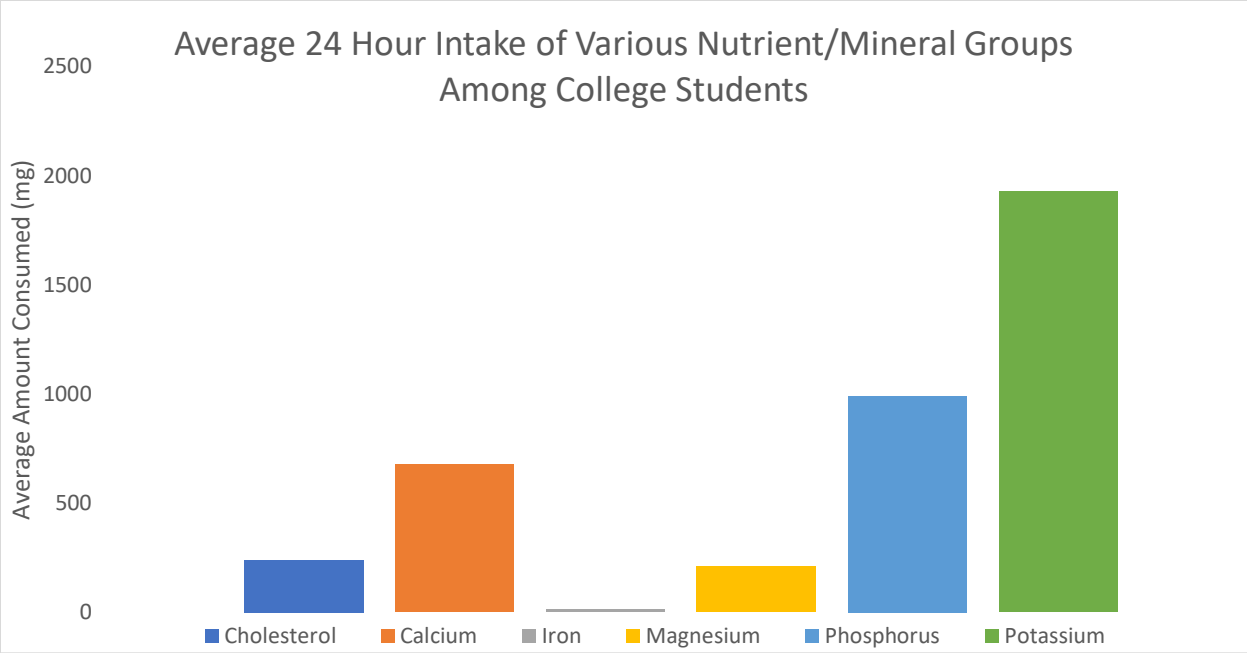

Figure 10. Breakdown of average amounts of nutrients and minerals self-reported by college students in a 24 hour recall. Data is based on a 2000 calorie diet.

Figure 11 shows a breakdown of the sources of protein reported by college-aged students in this study. Of the 10 various sources of protein submitted as having been eaten in the last 24 hours at the time of reporting, poultry was consumed most frequently followed by cured meat which is high in sodium. Eggs and seafood low in n-3 fatty acids were consumed in moderate amounts as well.

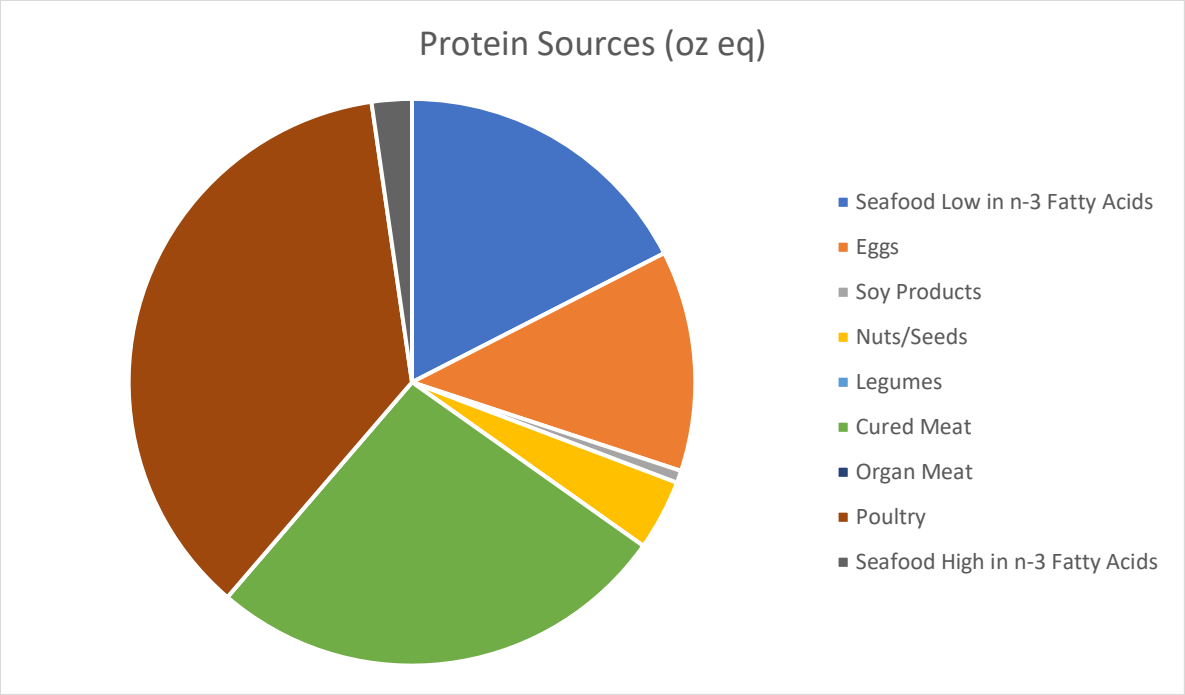

Figure 11. Breakdown of protein consumption self-reported by college students in a 24 hour recall. Data is based on a 2000 calorie diet.

Figure 12 is an image adapted from (Ministry of Health and Welfare Supreme Scientific Health Council of Greece, 1999) that displays suggested portions of various food groups including red meats, fish, poultry, fruits, vegetables, and grains on the basis of monthly, weekly and daily servings in correspondence with the Mediterranean diet. This diagram also includes dietary suggestions to reduce sodium intake and increase consumption of water.

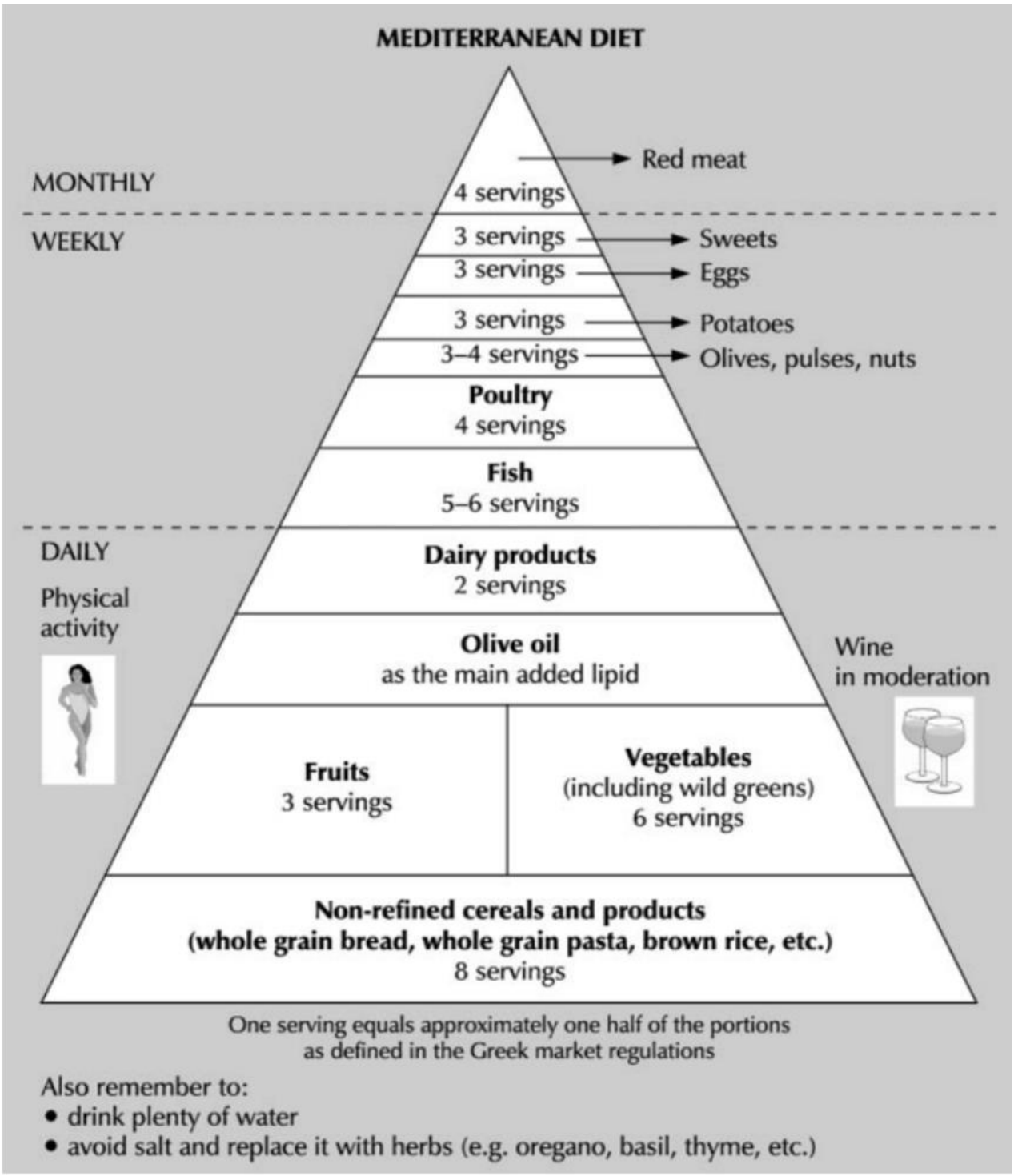

Figure 12. Breakdown of the Mediterranean diet adapted from (Ministry of Health and Welfare Supreme Scientific Health Council of Greece, 1999)

Chapter 4

Discussion

Summary of Data and Hypotheses

This thesis attempts to address a lack of data concerning the cardiovascular health and overall CMD risk in younger populations that traditionally don't show signs of CMD. To do this, both qualitative and quantitative variables were measured and analyzed to help form holistic inflammatory, stress, and dietary profiles for this college-aged sample of 21 students. Though this sample is relatively small, it can be extrapolated to represent overall trends in college-aged individuals until larger-scale studies are carried out on this topic and particularly with this demographic.

Hypothesis 1a was supported as the individuals that presented with higher rates of serum CRP levels also presented with higher rates of salivary cortisol comparatively among the sample group. There was correlation coefficient of 0.514 between these two variables indicating their relationship. This linear association indicates the potential for treatment alleviating factors involved in stress or inflammation could help to mitigate the other. Hypothesis 1b was not supported in that Cohen Perceived Stress scores and salivary cortisol levels had a negative linear relationship with a correlation coefficient of -0.697. Students who scored higher on the Cohen PSS reported lower levels of salivary cortisol. This relationship was surprising and helps to solidify the understanding that self-reported stress and salivary cortisol as a marker of stress are impacted by different lifestyle factors and could be measuring different aspects of stress.

Hypothesis 2a was not supported based on the data provided in this study showing that students with higher BMI's did not present a linear relationship when compared to CRP. This relationship showing that as BMI increased CRP levels did not change, had a correlation

coefficient of 0.145, indicating very little correlation between BMI and inflammation rates. Hypothesis 2b was not supported in that an association between waist circumference and CRP did not exist. These data together had a very week correlation coefficient of 0.069.

Hypothesis 3a was supported given that college-aged students tended to report diets that consisted of high intakes of carbohydrates and processed foods which falls in line with parameters dictating the Western style diet. Students also reported an increased intake of red meat compared to the Mediterranean diet.

Salivary Cortisol and Perceived Stress in Relation to Inflammation

For this study, total AUC of the cortisol graph was used as a measure of total cortisol fluctuation over the collection period (7days). Higher AUC has been associated with increased risk of diabetes in other studies, but the average AUC reported for this sample was 3.644 ng/L with a standard deviation of 1.754, which falls within normal cortisol ranges. However, when looking at some individual data, interesting curves presented for almost one third of the 15 person sample used to generate the cortisol curves. Four individuals out of the 15 person sample presented a positive diurnal response which is uncharacteristic of a typical cortisol curve. Generally, cortisol levels are high upon waking, increase within the first 30 minutes of waking, and steadily decrease throughout the day. For the individuals here, presenting a positive diurnal response indicates that their initial cortisol levels were lower upon waking and increased or remained steady throughout the day. In addition to this, a negative awakening response was seen for three of the four individuals that presented a positive diurnal response, showing that upon waking their cortisol level decreased within the first 30 minutes. Trends such as this could indicate a disruption in the HPA axis that controls the stress response and cortisol release via a

negative feedback system. Potential disruptions in the HPA axis could present as lower cortisol levels in general, while ultimately representing chronic stress. This is an important factor to note as we further explore the trends between stress and inflammation.

As hypothesized, CRP and overall salivary cortisol exposure mapped onto one another in a similar pattern for almost every individual in which both biomarkers were collected. With the exception of 3 individuals, as cortisol exposure increased, CRP did as well. This data had a relatively weak r value of 0.514 indicating a weak linear relationship between the variables. This weak linear relationship may be attributed to the small sample size and the potential for HPA axis disruption in a few participants as noted from the diurnal response of the salivary cortisol curve. Future studies involving larger samples would shed further light on this trend. Additional information regarding illness and infection would have supplemented the total cortisol exposure, awakening response, and diurnal response data to possibly explain any inconsistencies or unexpected cortisol levels.

Cohen PSS did not track as expected when compared with measured levels of salivary cortisol. Students who tested higher on the Cohen PSS tended to have lower total cortisol exposures. This negative linear relationship presented with a relatively high r value of -0.697. This indicated that these measures of stress assess different aspects of the stress response as it pertains to the lives of students. The Cohen PSS is targeted at understanding an individual's self-reported psychosocial stress levels while salivary cortisol is a biological response to stress. Both of these measured have their potential for bias, however these biases are reflected in very different ways. With the Cohen PSS, there can be high variability in answers based on time of day, setting in which the test was administered, and a multitude of other factors. By only taking this this survey once, circumstances could have changed in an individual's life that may have

elevated cortisol levels, which was not accounted for at the time of administering the Cohen PSS. Salivary cortisol samples were collected over a week long period to reduce bias. I suggest that in replication of this study, the Cohen PSS be administered more than once to average the scores in way that reduces these aforementioned biases and could potentially allow for clearer positive relationships between perceived stress and cortisol levels.

Central Adiposity and BMI in relation to Inflammation

Several studies have shown that there is a strong positive correlation between central adiposity as a measure of obesity, BMI, and CMD risk factors. This study utilized both waist circumference and BMI as measures of central adiposity and obesity respectively. However, the data collected in this study did not support our hypotheses, as we hypothesized that both measures of central adiposity and BMI would have strong positive correlations with CRP levels. There is no relationship reported between waist circumference as a measure of central adiposity and CRP as seen in this data due the nonexistent linear relationship exhibited via an r value of 0.069. A weak linear relationship was found between the BMI and CRP, yielding an r value of 0.145. We had participants in all BMI ranges, from underweight to obese, with the majority falling into the normal or underweight categories. A CRP value of 11.27 mg/L was excluded from analysis as this indicated a possible infection and skewed the data. Despite this exclusion, these weak or nonexistent relationships do not fully support the past literature examining CRP as it relates it central adiposity and BMI, but the limitations of our study could be an important factor in producing these trends. A larger sample size would likely have allowed for a stronger linear relationship between BMI and CRP as seen in previous literature.

Studies conducted by (Song et al., 2017) showed strong relationships between changes in BMI and waist circumference as they related to clustering of metabolic disease. In this study they controlled for genetic factors and drew conclusions that their findings were due to external environmental factors that could be aspects like diet or stress. Because outside factors have been shown to impact CMD risk via changes in BMI and central adiposity, it is likely that the small sample size used in this thesis is a limiting factor preventing this study from reaching similar conclusions. Dietary data examined later in a later section of this discussion supports the idea that college-aged students are at increased risk for CMD based on their eating practices and serves as an example of one potential environmental factor that directly contributes to central adiposity and BMI. Further studies should incorporate larger sample sizes of CRP, BMI, and waist circumference measurements to draw more accurate conclusions and comparisons to existing literature.

Dietary Trends in College-aged Individuals

There is a multitude of literature supporting the implementation of the Mediterranean Diet to help mitigate CMD risk. The bulk of this literature tends to focus on reversing symptoms of CMD once they have already presented at later life stages. Rarely are these studies investigating the existing dietary practices of non-symptomatic individuals and attempting to classify their eating habits in regards to the Mediterranean the Western Diet. This study attempts to classify the eating habits of college-age individuals as they pertain to the Mediterranean Diet and Western Diet.

The Mediterranean Diet is high in fresh fruits, vegetables, olive oil, dairy, and non-refined grains, allotting for multiple daily servings of each. Much of the Mediterranean Diet's

sources of protein come from 5-6 weekly servings of fish, 3-4 weekly servings of poultry, and 2-3 weekly servings of eggs. The Mediterranean Diet rarely relies on red meat or processed foods for sustenance unlike the Western Diet, which includes many refined grains, animal fats, sugars, and processed or cured meats that are high in sodium. The Western Diet is also relatively low in legumes and fresh fruits and vegetables (Bonaccio et al., 2012; Ministry of Health and Welfare Supreme Scientific Health Council of Greece, 1999).

I hypothesized that the small subset of college-aged students examined in this study would exhibit a diet more in line with the Western Diet. This hypothesis was supported by the data collected through a self-reported 24-hour dietary recall. During this recall, students were asked to report all foods, beverages, and supplements consumed in the las 24-hours from the time of initiating the assessment. In addition to reporting specific foods, beverages, and supplements, they were also asked to report the amounts of each consumed and where they procured the food.

The highest food groups consumed per gram based on a 2000 calorie diet in self-reported data were carbohydrates, sugars, and proteins. Of the protein sources eaten, the majority came from poultry or cured meat which has a high sodium content. Most students reported eating multiple servings of single items as meals rather than diversifying their plate. This too, is a characteristic of the Western Diet and results in lack of nutrients and can contribute to factors like BMI and central adiposity at later life stages. The majority of students reported purchasing food on campus or eating in dining halls which prevents many of them from buying fresh fruits and vegetables, or non-refined grains. Social and environmental contexts like this are important to consider when examining factors such as diet, which is greatly impacted by an individual's location and socioeconomic status. Many studies have shown that the quality of diet eaten by an

individual is directly correlated with socioeconomic status (Bonaccio et al., 2012). This emphasizes the disadvantage people of lower socioeconomic status face when it comes to eating well and avoiding the diseases that accompany cardiometabolic syndrome, many of which can be prevented to some extent via diet.

The data collected in conjunction with this portion of the study were less impacted by the small sample size than previous sections, however, future endeavors should seek to collect data from more individuals. In addition to this, conducting ethnographic interviews to determine what factors go into to student's food purchasing habits could help shed more light on the information provided in the section about why students dietary habits tend to align with the Western Diet. Further information about socioeconomic status could have an interesting interplay with location to have and overall increased risk on CMD risk in college-aged individuals.

Chapter 5

Conclusion

The findings in this study provide important data on a demographic that is seldom studied in the context of inflammation and CMD. However, there are several limitations that must be addressed to better conceptualize this data. One such limitation is the relatively small sample of 21 college-aged individuals. Some smaller subsets of this 21 person sample were used for variables like self-reported diet, perceived stress, and CRP levels. A larger sample would have captured more holistic data and provided better insight into the trends discussed throughout this thesis. The use of subsets of the overall sample for some variables led to a lack of corresponding information per individual in the sample. Due to funding limitations and participants withdrawing from the study, the CRP and cortisol data was not able to be run for every participant potentially skewing the trends seen. In addition to this, not all the participants

successfully completed the 24-hour dietary recall further lowering the sample size for this parameter of the study

A second limitation involves sex differences between BMI and perceptions of stress. Once again, due to the relatively small sample size the majority of this sample were female, and biological sex was not controlled for when dealing with this data. Differences in sex could have skewed the data and ultimately impacted the overall trends seen here. Future studies should seek to incorporate a more even distribution of biological sex as I would be interested to see how these relationships shift, if at all, based on this factor.

Future research can build off this study, using the relationships presented here a basis of study for the demographic of college-aged individuals and how inflammation can begin to manifest at these young ages. The relationships between stress and inflammation presented here provide insight into deeper rooted issues that can be further explored through analysis of the types of stress college students undergo and ways of possible mitigation. Studies such as this can offer guidance for the introduction of policy that impacts the health and well-being of students on college campuses across the country. The dietary analysis provided here serves to begin a conversation about the types of foods college students eat and why that may be so. Future research should investigate the way students procure their food through the use of ethnographic interviews. Interviews like this would further supplement this data and help in understanding why students may choose to stick with a diet more in line with the Western Diet and uncover whether healthier options may or may not be present. It is my hope that researchers will replicate studies like the one provided here and in making minor improvements, help add literature to the field of biological anthropology addressing stress and diet as it pertains to CMD risk in younger populations.

Current Contexts

During these unprecedented times of global pandemic and a national quarantine, many of the factors discussed throughout this thesis are of considerable interest as the lives of all college students have changed dramatically. A lack of daily activity in conjunction with potential dietary changes are likely to have an impact on central adiposity and BMI in the coming months, which despite the results reported in this study, are likely to impact CMD risk. In addition to this, the unparalleled levels of stress experienced by college students in this time of uncertainty are likely to have increased impacts on inflammation levels adding to the increasing risk of CMD as well. It is important, now more than ever, that future biological and anthropological research endeavors investigate the risks associated with CMD in younger populations before they manifest in later life stages.

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Appendix A: Elisa CRP Assay Protocol

SAMPLE PREPARATION

Serum samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 900 µL of Calibrator Diluent RD5P.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent – Add 20 mL of calibrator diluent RD5P to 80 mL of distilled water to prepare 100 mL of calibrator diluent RD5P diluted 1:5.

Human CRP Standard (Serial dilution)– use polypropylene tubes. Pipette 200 µL of calibrator diluent RD5P into each of 6 tubes. Add 200 µL of the human CRP standard to the 25 ng/mL tube and continue the dilution through a serial dilution across 6 tubes. Mix each tube well before continuing the dilution. The Human CRP standard (50 ng/mL) serves as the high standard. Calibrator diluent RD5P (0 ng/mL) serves as the zero standard.

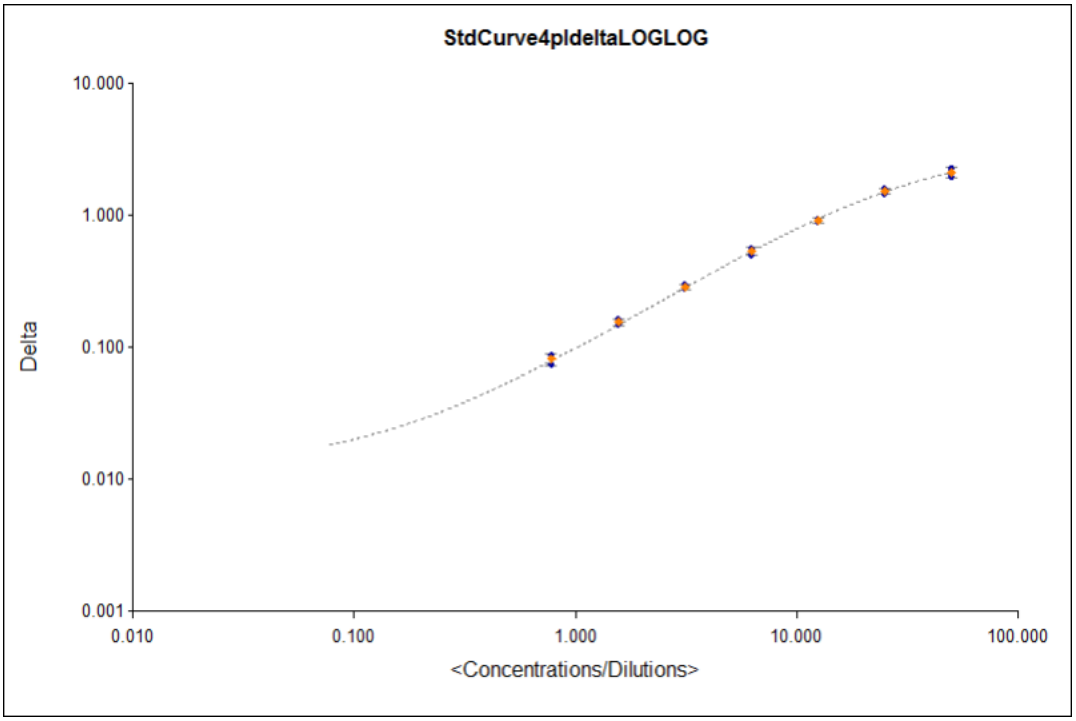
ASSAY PROCEDURE

1. Prepare all reagents, samples and standards,
2. Add 100µL of assay diluent RD1F to each well
3. Add 50µL standard or sample to each well. Incubate 2 hours at 37 °C,
4. Aspirate and wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. four times and add 200µL of prepared CRP conjugate to each well.
6. Incubate 2 hours at 37 °C,
7. Repeat the wash steps from step 4
8. Add 200µL of substrate solution to each well. Incubate 30 minutes at 37 °C protected from light.
9. Add 50µL of stop solution to each well and the color should change from blue to yellow.
10. Read at 450nm immediately to determine the optical density.

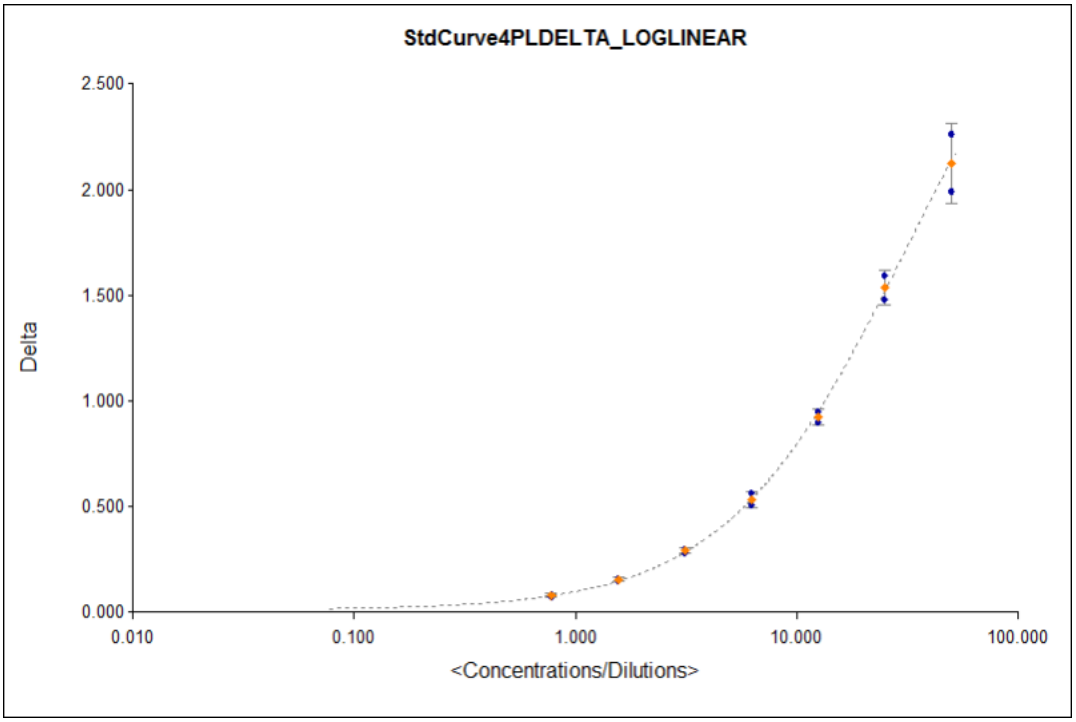
CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample then subtract the average NSB optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a logarithmic x-axis and draw the best fit curve through the points on the graph. The standard curves for the CRP assay run for this thesis are shown below



CRP Standard Curve Log-Log



CRP Standard Curve Log-Linear

Appendix B: Elisa Cortisol Assay Protocol

SAMPLE PREPARATION

Saliva samples require a 5-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5-43.

SAMPLE PRETREATMENT

1. Add 200 µL of serum or plasma and 200 µL Pretreatment E to a microcentrifuge tube. The tubes will have a precipitate. Mix well.
2. Incubate for 15 minutes at room temperature.
3. Centrifuge at ≥ 12,000 x g for 4 minutes.
4. Carefully remove and retain the supernate.
5. Add 100 µL of Pretreatment F to 200 µL of the retained supernate. Mix well.
6. The pretreatment step results in a dilution factor of 3. Pretreated serum or plasma samples require an additional 20-fold dilution in Calibrator Diluent RD5-43. The concentration read off the standard curve must be multiplied by the final dilution factor, 60.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Note: *Cortisol is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Cortisol Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Cortisol Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. \

Pipette 900 µL of Calibrator Diluent RD5-43 into the 10 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the 100 ng/mL standard stock to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5-43 serves as the zero standard (B_0) (0 ng/mL). **Use diluted standards within 60 minutes of preparation.**

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

Note: *Cortisol is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 150 µL of Calibrator Diluent RD5-43 into the non-specific binding (NSB) wells.
4. Add 100 µL of Calibrator Diluent RD5-43 to the zero standard (B_0) wells.
5. Add 100 µL of standard or sample* to the appropriate wells. A plate layout is provided to record standards and samples assayed.
6. Add 50 µL of Cortisol Conjugate to all wells. Wells will now be red in color.
7. Add 50 µL of the Primary Antibody Solution to each well **(excluding the NSB wells).** Cover with the adhesive strip provided. All wells will now be violet in color except the NSB wells, which will be red in color.
8. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.

9. Aspirate each well and wash for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
10. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Protect from light.
11. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the well is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample then subtract the average NSB optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on a linear y-axis against the concentration on a logarithmic x-axis and draw the best fit curve through the points on the graph. Do not include the B_0 in the standard curve.

If desired, % B/B_0 can be calculated by dividing the corrected O.D. for each standard or sample by the corrected B_0 O.D. and multiplying by 100. Calculate the concentration of Cortisol corresponding to the mean absorbance from the standard curve. Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Appendix C: Cohen Perceived Stress Survey

The following questions ask about your feelings and thoughts during THE PAST MONTH. In each question, you will be asked HOW OFTEN you felt or thought a certain way. Although some of the questions are similar, there are small differences between them and you should treat each one as a separate question. The best approach is to answer fairly quickly. That is, don t try to count up the exact number of times you felt a particular way, but tell me the answer that in general seems the best.

For each statement, please tell me if you have had these thoughts or feelings: never, almost never, sometimes, fairly often, or very often. (Read all answer choices each time)

	Never	Almost Never	Sometimes	Fairly Often	Very Often
B.1. In the past month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
B.2. In the past month, how often have you felt unable to control the important things in your life?	0	1	2	3	4
B.3. In the past month, how often have you felt nervous or stressed?	0	1	2	3	4
B.4. In the past month, how often have you felt confident about your ability to handle personal problems?	0	1	2	3	4
B.5. In the past month, how often have you felt that things were going your way?	0	1	2	3	4
B.6. In the past month, how often have you found that you could not cope with all the things you had to do?	0	1	2	3	4
B.7. In the past month, how often have you been able to control irritations in your life?	0	1	2	3	4
B.8. In the past month, how often have you felt that you were on top of things?	0	1	2	3	4
B.9. In the past month, how often have you been angry because of things that happened that were outside of your control?	0	1	2	3	4
B.10. In the past month, how often have you felt that difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

Perceived Stress Scale Scoring

Each item is rated on a 5-point scale ranging from never (0) to almost always (4). Positively worded items are reverse scored, and the ratings are summed, with higher scores indicating more perceived stress.

PSS-10 scores are obtained by reversing the scores on the four positive items: For example, 0=4, 1=3, 2=2, etc. and then summing across all 10 items.
Items 4, 5, 7, and 8 are the positively stated items.

Scores around 13 are considered average. In our own research, we have found that high stress groups usually have a stress score of around 20 points. Scores of 20 or higher are considered high stress, and if you are in this range, you might consider learning new stress reduction techniques as well as increasing your exercise to at least three times a week. High psychological stress is associated with high blood pressure, higher BMI, larger waist to hip ratio, shorter telomere length, higher cortisol levels, suppressed immune function, decreased sleep, and increased alcohol consumption. These are all important risk factors for cardiovascular disease.

Appendix D: Consent Form

University of North Carolina at Chapel Hill Consent to Participate in a Research Study Adult Participants

Consent Form Version Date: 11/27/18
IRB Study # 18-3156
Title of Study: Impact of Psychosocial Stress and Physical Activity on Inflammatory Profiles in College Students
Principal Investigator: Mark Sorensen
Principal Investigator Department: Anthropology
Principal Investigator Phone number: (919) 962-3280
Principal Investigator Email Address: msorensen@unc.edu

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary.
You may choose not to participate, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The purpose of this study is to investigate the impact of psychosocial stress on inflammatory profiles of traditional age college students and how this relationship is potentially modified by physical activity. The goals of this study are 1) to identify and map the most prominent cultural domains of stress amongst college students, 2) to understand how these chronic stressors impact inflammatory profiles, 3) to understand the inflammatory response of college students to acute stress, and 4) to illustrate the role of physical activity in moderating against the negative impacts of psychosocial stress on inflammation. Semi-structed interviews, free listing, pile sorts, and risk mapping will be used to identify the cultural domains of stress experienced by college students. Accelerometry and questionnaires will be used to quantify physical activity. Biomarker collection will be used to measure both cortisol and inflammatory profiles for each participant.

Are there any reasons you should not be in this study?

You should not be in this study if you fit any of the following exclusion criteria:

1. You are not currently enrolled at the University of North Carolina at Chapel Hill in an undergraduate, graduate, or professional program.
2. You are a UNC student athlete.
3. You are not between the ages of 18 and 25 years.
4. You have been previously diagnosed with heart disease.
5. You are diabetic or suffer from any metabolic disorders.
6. You are pregnant or breastfeeding.

How many people will take part in this study?

There will be approximately 70 people in this research study.

How long will your part in this study last?

Your participation in this study requires the following time commitment: 1. Recruitment: 5-10 minutes

2. Collection of Anthropometrics: 10-15 minutes 3. Initial Meeting: 20-30 minutes
4. Final Meeting: 60 minutes
7. Results Distribution (Optional): 10 minutes

In addition, all biological samples will be kept until the completion of the study at which time they will be destroyed.

What will happen if you take part in the study?

I. Recruitment

A total of 70 college students (ages 18-22) at UNC Chapel Hill will be recruited in person and through flyers, class announcements, and listserv emails. Recruitment will focus on insuring that the study sample is comprised of roughly an equivalent number of men and women.

II. Ethnographic Stress Assessment

All 70 UNC undergraduate students will visit the lab and complete a variety of activities and semi- structured interviews to assess the prevalence and severity specific psychosocial stress in their lives. This data will be used to identify the major cultural domains of stress in the lives of UNC college students. This information will be used to create a specific survey for UNC students that assesses the prevalence and severity of each domain.

a). Free Listing: Participants will be asked to list the factors that cause them psychosocial stress and to rank them by frequency and severity. The interviewer will ask open ended questions to ensure a comprehensive list is created without suggesting factors or introducing any sort of bias.

b). Pile Sorts: The participants will be given a series of index cards that contain words that college students most associate with stress. They will be given a second set of cards containing various emotions and feelings. The participants will be asked to sort the cards by the words that belong together through pair association.

c). Focus Groups: Two focus groups comprising of 10 individuals will be administered. During these groups, one research will ask open ended questions regarding the cultural domains that cause the most stress amongst college students. All focus groups will be recorded and transcribed. Audio data will be saved on a secure server in the Human Biology Laboratory. All transcriptions will be stored in a locked drawer in the Human Biology Laboratory.

III. Biometric Data Collection

A subset of 20 students will complete the biometric data collection protocol. The data collection process will take a total of 7 days. Saliva samples will be collected five times per day for three days to measure salivary cortisol. These days will not be consecutive and will be scattered across the seven day collection period. The Actigraph wristband will be worn without removal for the entire 7 days. Actigraphy will permit detailed analysis of physical activity patterns.

They will visit the lab 2 times: (1) dried blood spot collection, anthropometric measurements, instruction on saliva collection, and to receive all equipment (saliva collection equipment and Actigraph). (2) Trier Social Stress Test, dried blood spot collection, surveys (health, stress, and physical activity) and to turn in saliva samples and all equipment.

Data Collection Timeline

Day 1: Participants will meet with a research team member in the Human Biology Lab on the first day of the data collection period. They will each be provided with necessary equipment: Garmin heart rate monitor, Actigraph, cooler, mini tubes, and saliva collection straws, for the duration of the study. Research team members will explain the Actigraph and Garmin heart rate monitor to participants. Participants will also undergo training for collecting, storing, and transporting saliva samples. Participants will be given a paper copy of these protocols/directions for collecting, storing, and transporting saliva samples. A finger stick blood spot sample will be collected from each participant for analysis of C-Reaction Protein, Interleukin 6, and adiponectin, and TNF-alpha concentrations. Up to 5 drops of blood will be collected onto commercially available filter paper (Whatman 903 Protein Saver Card, GE Healthcare Life Sciences, Pittsburgh PA) using a sterile disposable microlancet. Anthropometric measures (stature, weight, and triceps, biceps, subscapular, suprailiac skinfolds) and systolic and diastolic blood pressure will also be taken at this visit.

Days 2-7: Saliva samples for measurement of salivary cortisol will be collected 5 times per day for 3 non- consecutive days. Collection times will be in the morning immediately upon waking; 30 minutes after waking; before eating lunch; evening; and immediately prior to going to sleep, using the passive drool technique (Salimetrics, State College, PA). Subjects will collect up to 5 mL of saliva into a sample collection tube using the Salimetrics saliva collection straw for each collection. Sample collection tubes will be frozen until the last (7th) day of the data collection period, when they will be moved to coolers and transported to the lab for analysis. Participants will receive a text message to remind them to collect their saliva sample each day. They will also be asked to send a picture of their collected sample to the assigned research assistant to ensure quality of the sample.

Days 1-7: The Garmin heart monitor chest strap will be worn without removal for 7 consecutive days. Each participant will link biofeedback data from device to their cell phones 2 times per day, in the morning and evening.

Day 7: At the end of the 7 day period, participants will return to the lab for a final debriefing and other data collection procedures. Participants will turn in their Garmin heart rate monitor, Actigraph, and bring their saliva samples. They will complete a series of questionnaires, including the SF36 general health survey, the 7-day recall IPAQ which assess physical activity over the last 7 days, and the UNC college stress evaluation. The UNC college stress evaluation is created using the data collected during the initial ethnographic assessments.

During the second visit, the principal investigators and research assistants will administer the Trier Social Stress Test to each participant. Testing the impact of acute psychosocial stress requires inducing a stress response in each participant. The Trier Social Stress Test (TSST) is recommended due to its application ease and validated ability to stimulate the HPA axis. The TSST involves a 30 minutes anticipation period and a 10 minute testing period. First the participant will wait for 30 minutes before being taken to the exam room. Once in the exam room, the participant will ask to stand before 3 seated individuals. The principal investigator will tell the individual that are taking over the role of “job applicant” and the seated individuals

represents the selection committee. They are asked to prepare for 3 minutes and then speak for 5 minutes about why they are the best person for the job. At the end of 5 minutes, the participant will be asked to serially subtract 13 from 2011. The participant will be asked to restart every time they do a subtraction incorrectly. The test will conclude after 5 minutes. The participants will be debriefed regarding the examinations purposes by the researchers who administered the test. The participants will wear a heart rate monitor during the duration of the TSST to test changes in heart rate during each portion of the test. Following the test, salivary samples and dried blood spots will be collected at the 10 minute mark.

IV. Data Distribution

If they choose, participants will return to the lab to have a private meeting with the research team, where their results will be discussed in private.

Data Analysis

Data Analysis: For statistical analysis, Pearson correlations will be used to examine associations among continuous outcome variables (e.g., C-reactive protein, Interleukin-6, Adiponectin, activity level, stress, and body composition, independent samples t-tests will be used to test for mean differences and longitudinal change will be measured using analysis of covariance and general linear models using Stata.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. The benefits to you from being in this study may be the understanding of relationships between lifestyle factors, acute phase proteins and other cytokines, and established cardiovascular risk markers in hopes of expanding our knowledge of pre-disease pathways involved in the development of cardiovascular disease. This research may contribute to recommendations about how to modify one's lifestyle factors to prevent the onset and severity of cardiovascular disease beginning at a young age.

The results of this study will provide the participants results on how their psychosocial stress and physical activity levels impact their acute inflammation. This information will allow participants to see how their lifestyle factors are contributing to their overall health and disease risk.

What are the possible risks or discomforts involved from being in this study?

There may be uncommon or previously unknown risks. You should report any problems to the researcher. Although rare, speaking about psychosocial stress may cause some emotional distress or embarrassment. Therefore, these questions will be asked with compassion and understanding. All results will be kept secure by following strict security guidelines in order to minimize any risk for breach of confidentiality.

In addition, you may experience initial discomfort in the few minutes following the two Dried Blood Spot sample collections. Steps will be taken to minimize this discomfort and to ensure no

risk or infection or alteration of daily activities following the puncture and dried blood spot collection. First, your finger will be sanitized with an alcohol wipe to clean the area and then covered with a bandage after collection is completed to ensure no risk of infection. Second, you will be monitored for sudden dizziness or discomfort beyond what is common from having a finger punctured. If dizziness or elevated discomfort occurs, you will be provided snacks and monitored after the collection of the dried blood spot to ensure your safety.

Lastly, measurement of blood pressure, glucose and lipids may indicate that you are at risk for certain cardiometabolic diseases. In such cases, research team members will print out a copy of the panel reading for you and refer you to an appropriate health care professional.

What if we learn about new findings or information during the study?

You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

How will information about you be protected?

In order to protect your privacy, you will be given a pseudonym in the form of an Identification Number. The Identification Number will be used consistently across all correspondences and measures for data collection. This includes all questionnaires, Garmin Vivo Fit wristband and Garmin heart monitor data, saliva sample collection tubes and salivary cortisol data, anthropometric measurements, diastolic and systolic blood pressure readings, and glucose lipid panels. All electronic data will be stored on a password protected project site on UNC servers (Sakai), and all physically documented data will be stored in a locked file cabinet in the Human Biology Lab. Only research team members will be able to access electronic data by logging in to the project sakai site on the UNC server. Only the co-principal investigators will have a key to unlock the Human Biology Lab. The principal investigator will have the key to unlock the file cabinet in the lab. Other co-principal investigators will have to check the key out from them in order to access physical data.

Participants will not be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety.

Participants must agree to not reveal anything they learn from interviews or focus groups.

By signing this informed consent document, you agree that some of the information generated by participating in this study and/or a copy of the consent form may be included in your medical record and that this information may be viewed by other physicians or caregivers who provide healthcare services to you. This will allow the doctors caring for you to know what study medications or tests you may be receiving as a part of the study and know how to take care of

you if you have other health problems or needs during the study. Additionally, the information may be shared with your medical insurance plan if the research services provided are billed to your insurance.

What will happen if you are injured by this research?

All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The University of North Carolina at Chapel Hill has not set aside funds to pay you for any such reactions or injuries, or for the related medical care. You do not give up any of your legal rights by signing this form.

What if you want to stop before your part in the study is complete?

You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

Will you receive anything for being in this study?

You will be receiving $20 for taking part in this study. Any payment provided for participation in this study may be subject to applicable tax withholding obligations

Will it cost you anything to be in this study?

It will not cost you anything to be in this study.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

_____
_____
_____
_____ _____

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions about the study (including payments), complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

What if you have questions about your rights as a research participant?

All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Participant's Agreement:
I have read the information provided above. I have asked all the questions I have at this time. I

voluntarily agree to participate in this research study.

_____ _____
Signature of Research Participant Date

_____ Printed Name of Research Participant

_____ _____
Signature of Research Team Member Obtaining Consent Date

_____ Printed Name of Research Team Member Obtaining Consent

Appendix E: ASA24 24-hour Dietary Recall Code Sheet

Field Name	Description	Data Type	Length
RECALLRECID	Unique intake day ID	Character	38
USERNAME	Study abbreviation plus researcher provided ID	Character	30
USERID	Unique user ID	Character	38
RECALLNO	Recall number.	Numeric	2
RECALLATTEMPT	Sequence number for attempt within recall	Numeric	2
RECALLSTATUS	The final status of this recall	Numeric	1
INTAKESTARTDATETIME	Date and time of the start of the 24 hour period for which intake is being reported	Date	22
INTAKEENDDATETIME	Date and time of the end of the 24 hour period for which intake is being reported	Date	22
REPORTINGDATE	The date that the last data were reported within the reporting period. Reporting period is the time within which respondents are allowed to report their intake.	Date	8
LANG	Language used for recall.	Numeric	1
NUMFOODS	Total number of FLTs included in this recall	Numeric	3
NUMCODES	Total number of Food Codes included in this recall	Numeric	3
AMTUSUAL	Respondent's assessment of amount of food consumed on intake day	Numeric	1
KCAL	Energy (kcal)	Numeric	12.6
PROT	Protein (g)	Numeric	12.6
TFAT	Total Fat (g)	Numeric	12.6
CARB	Carbohydrate (g)	Numeric	12.6
MOIS	Water (g)	Numeric	12.6
ALC	Alcohol (g)	Numeric	12.6
CAFF	Caffeine (mg)	Numeric	12.6
THEO	Theobromine (mg)	Numeric	12.6
SUGR	Sugars, total (g)	Numeric	12.6
FIBE	Fiber, total dietary (g)	Numeric	12.6
CALC	Calcium (mg)	Numeric	12.6
IRON	Iron (mg)	Numeric	12.6
MAGN	Magnesium (mg)	Numeric	12.6
PHOS	Phosphorus (mg)	Numeric	12.6
POTA	Potassium (mg)	Numeric	12.6
SODI	Sodium (mg)	Numeric	12.6
ZINC	Zinc (mg)	Numeric	12.6

COPP	Copper (mg)	Numeric	12.6
SELE	Selenium (mcg)	Numeric	12.6
VC	Vitamin C (mg)	Numeric	12.6
VB1	Thiamin (mg)	Numeric	12.6
VB2	Riboflavin (mg)	Numeric	12.6
NIAC	Niacin (mg)	Numeric	12.6
VB6	Vitamin B-6 (mg)	Numeric	12.6
FOLA	Folate, total (mcg)	Numeric	12.6
FA	Folic acid (mcg)	Numeric	12.6
FF	Folate, food (mcg)	Numeric	12.6
FDFE	Folate, DFE (mcg_DFE)	Numeric	12.6
VB12	Vitamin B-12 (mcg)	Numeric	12.6
VARA	Vitamin A, RAE (mcg_RAE)	Numeric	12.6
RET	Retinol (mcg)	Numeric	12.6
BCAR	Carotene, beta (mcg)	Numeric	12.6
ACAR	Carotene, alpha (mcg)	Numeric	12.6
CRYP	Cryptoxanthin, beta (mcg)	Numeric	12.6
LYCO	Lycopene (mcg)	Numeric	12.6
LZ	Lutein + zeaxanthin (mcg)	Numeric	12.6
ATOC	Vitamin E, alpha-tocopherol (mg)	Numeric	12.6
VK	Vitamin K, phylloquinone (mcg)	Numeric	12.6
CHOLE	Cholesterol (mg)	Numeric	12.6
SFAT	Fatty acids, total saturated (g)	Numeric	12.6
S040	4:0, Butanoic acid (g)	Numeric	12.6
S060	6:0, Hexanoic acid (g)	Numeric	12.6
S080	8:0, Octanoic acid (g)	Numeric	12.6
S100	10:0, Decanoic acid (g)	Numeric	12.6
S120	12:0, Dodecanoic acid (g)	Numeric	12.6
S140	14:0, Tetradecanoic acid (g)	Numeric	12.6
S160	16:0, Hexadecanoic acid (g)	Numeric	12.6
S180	18:0, Octadecanoic acid (g)	Numeric	12.6
MFAT	Fatty acids, total monounsaturated (g)	Numeric	12.6
M161	16:1, Hexadecenoic acid, undifferentiated(g)	Numeric	12.6
M181	18:1, Octadecenoic acid, undifferentiated (g)	Numeric	12.6
M201	20:1, Eicosenoic acid, undifferentiated (g)	Numeric	12.6
M221	22:1, Docosenoic acid, undifferentiated (g)	Numeric	12.6
PFAT	Fatty acids, total polyunsaturated (g)	Numeric	12.6
P182	18:2, Octadecadienoic acid (g)	Numeric	12.6
P183	18:3, Octadecatrienoic acid (g)	Numeric	12.6
P184	18:4, Octadecatetraenoic acid (g)	Numeric	12.6
P204	20:4, Eicosatetraenoic acid (g)	Numeric	12.6
P205	20:5 n-3, Eicosapentaenoic acid [EPA] (g)	Numeric	12.6
P225	22:5 n-3, Docosapentaenoic acid [DPA] (g)	Numeric	12.6
P226	22:6 n-3, Docosahexaenoic acid [DHA] (g)	Numeric	12.6

VITD	Vitamin D (D2 + D3) (mcg)	Numeric	12.6
CHOLN	Choline, total (mg)	Numeric	12.6
VITE_ADD	Added Vitamin E (mg)	Numeric	12.6
B12_ADD	Added Vitamin B-12 (mcg)	Numeric	12.6
F_TOTAL	Total intact fruits (whole or cut) and fruit juices (cup eq.)	Numeric	12.6
F_CITMLB	Intact fruits (whole or cut) of citrus, melons, and berries (cup eq.)	Numeric	12.6
F_OTHER	Intact fruits (whole or cut); excluding citrus, melons, and berries (cup eq.)	Numeric	12.6
F_JUICE	Fruit juices, citrus and non-citrus (cup eq.)	Numeric	12.6
V_TOTAL	Total dark green, red and orange, starchy, and other vegetables; excludes legumes (cup eq.)	Numeric	12.6
V_DRKGR	D ark green vegetables (cup eq.)	Numeric	12.6
V_REDOR_TOTAL	Total red and orange vegetables (tomatoes and tomato products + other red and orange vegetables)(cup eq.)	Numeric	12.6
V_REDOR_TOMATO	Tomatoes and tomato products (cup eq.)	Numeric	12.6
V_REDOR_OTHER	Other red and orange vegetables, excluding tomatoes and tomato products (cup eq.)	Numeric	12.6
V_STARCHY_TOTAL	Total starchy vegetables (white potatoes + other starchy vegetables) (cup eq.)	Numeric	12.6
V_STARCHY_POTATO	White potatoes (cup eq.)	Numeric	12.6
V_STARCHY_OTHER	Other starchy vegetables, excluding white potatoes (cup eq.)	Numeric	12.6
V_OTHER	Other vegetables not in the vegetable components listed above (cup eq.)	Numeric	12.6
V_LEGUMES	Beans and peas (legumes) computed as vegetables (cup eq.)	Numeric	12.6
G_TOTAL	Total whole and refined grains (oz. eq.)	Numeric	12.6
G_WHOLE	Grains defined as whole grains and contain the entire grain kernel ? the bran, germ, and endosperm (oz. eq.)	Numeric	12.6
G_REFINED	Refined grains that do not contain all of the components of the entire grain kernel (oz. eq.)	Numeric	12.6
PF_TOTAL	Total meat, poultry, organ meat, cured meat, seafood, eggs, soy, and nuts and seeds; excludes legumes (oz. eq.)	Numeric	12.6
PF_MPS_TOTAL	Total of meat, poultry, seafood, organ meat, and cured meat (oz. eq.)	Numeric	12.6
PF_MEAT	Beef, veal, pork, lamb, and game meat; excludes organ meat and cured meat (oz. eq.)	Numeric	12.6
PF_CUREDMEAT	Frankfurters, sausages, corned beef, and luncheon meat that are made from beef, pork, or poultry (oz. eq.)	Numeric	12.6

PF_ORGAN	Organ meat from beef, veal, pork, lamb, game, and poultry (oz. eq.)	Numeric	12.6
PF_POULT	Chicken, turkey, Cornish hens, duck, goose, quail, and pheasant (game birds); excludes organ meat and cured meat (oz. eq.)	Numeric	12.6
PF_SEAFD_HI	Seafood (finfish, shellfish, and other seafood) high in n-3 fatty acids (oz. eq.)	Numeric	12.6
PF_SEAFD_LOW	Seafood (finfish, shellfish, and other seafood) low in n-3 fatty acids (oz. eq.)	Numeric	12.6
PF_EGGS	Eggs (chicken, duck, goose, quail) and egg substitutes (oz. eq.)	Numeric	12.6
PF_SOY	Soy products, excluding calcium fortified soy milk and immature soybeans (oz. eq.)	Numeric	12.6
PF_NUTSDS	Peanuts, tree nuts, and seeds; excludes coconut (oz. eq.)	Numeric	12.6
PF_LEGUMES	Beans and Peas (legumes) computed as protein foods (oz. eq.)	Numeric	12.6
D_TOTAL	Total milk, yogurt, cheese, whey. For some foods, the total dairy values could be higher than sum of D_MILK, D_YOGURT, and D_CHEESE because Misc dairy component composed of whey which is not included in FPED as separate variable. (cup eq.)	Numeric	12.6
D_MILK	Fluid milk, buttermilk, evaporated milk, dry milk, and calcium fortified soy milk (cup eq.)	Numeric	12.6
D_YOGURT	Yogurt (cup eq.)	Numeric	12.6
D_CHEESE	Cheeses (cup eq.)	Numeric	12.6
OILS	Fats naturally present in nuts, seeds, seafood; unhydrogenated vegetable oils, except palm oil, palm kernel oil, coconut oils; fat in avocado and olives above allowable amount; 50% of fat present in stick/tub margarines, margarine spreads (grams)	Numeric	12.6
SOLID_FATS	Fats naturally present in meat, poultry, eggs, dairy (lard, tallow, butter); hydrogenated/partially hydrogenated oils; shortening, palm, palm kernel, coconut oils; coconut meat, cocoa butter; 50% of fat in stick/tub margarines, margarine spreads (grams)	Numeric	12.6
ADD_SUGARS	Foods defined as added sugars (tsp. eq.)	Numeric	12.6
A_DRINKS	Alcoholic beverages and alcohol (ethanol) added to foods after cooking (no. of drinks)	Numeric	12.6

DATACOMP	This is an indicator which shows if the portion and/or nutrient data was complete or missing for any food/beverage in the recall (refer to INFMYPHEI file to locate the individual foods/beverages with missing data).	Numeric	1