LINKING SELF-PERCEPTION OF STRESSFUL EXPERIENCES WITH BLOOD PRESSURE AND SALIVARY CORTISOL LEVELS IN UNDERGRADUATE COLLEGE STUDENTS

By

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Anthropology

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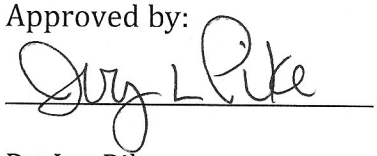

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ABSTRACT

A large body of research suggests self-perception of stressful experiences is not always a good predictor of stress biomarkers. On this front, anthropologists have an opportunity to disentangle the interactions between individual perceptions of stress and the stress response. To better understand these interactions we chose a sampling frame that allows individual participants to self-identify as high, medium, and low stress responders. We chose to conduct this research in an undergraduate student community for two reasons: 1) final exams serve as a similarly timed stressor, 2) given the perceived stress associated with student work loads, recruitment should be easier in an undergraduate community. With two data collection points, we recruited and sampled thirty-two students. Stress biomarker data include blood pressure and salivary cortisol, analyzed using Salimetrics high sensitivity salivary cortisol enzyme immunoassay kits. A short questionnaire was used to indicate an individuals' perception of the role of stress in their lives. Our interview data suggest an awareness of highly variable responses to stress. By comparing the interview data to stress biomarkers across self-designated categories of stress reactions we plan to link variation in perception, reactivity, and biomarkers to develop a more nuanced understanding of the stress response and its physiological outcomes.

Statement of Roles and Responsibilities

This project was developed and designed by KS Wiley and ME Silva. Both students completed the survey instruments created for this study and designed the interview protocol. Interviews and collections were done by both Wiley and Silva. Both students performed assays for cortisol. Data was complied largely by KS Wiley with assistance from ME Silva. Professor of Anthropology Dr. Ivy Pike performed statistical analysis. The abstract was written by KS Wiley and ME Silva.

The thesis write up was completed entirely by KS Wiley. Any interpretations of the data are his alone.

INTRODUCTION

Objectives

The objective of this study was to examine the impact of subjective individual perception of stress and stress level on biomarker levels in a sample of undergraduate college students. Participants were asked to categorize their self-perceived stress level by identifying with one of three categories of stress load: constantly, periodically, and rarely stressed. Biomarkers and other indicators of stress were collected at two points, during finals week and mid-semester before midterms. These points were meant to simulate periods of high and low psychosocial stress respectively. Blood pressure, saliva for salivary cortisol assay, and the Cohen Perceived Stress Scale were measured and analyzed in relation to self-selected stress category.

Overarching Hypotheses

We hypothesized that the self-identified stress categories will significantly predict biomarkers. That is to say that participants who self-selected the constantly stressed category will have higher stress biomarker levels at mid-semester, a period of low stress, than those that identified as periodically or rarely stressed. It is also hypothesized that individuals who identified as constantly stress will have similar biomarker levels during finals, a period of high

stress, than those who identified as periodically or rarely stressed due to a blunted stress response due to chronic activation of the hypothalamic-pituitary-adrenal axis. Finally, we hypothesize that the Cohen Perceived Stress Scale should serve as a predictor of the stress biomarkers.

Physiological Mechanisms of Stress

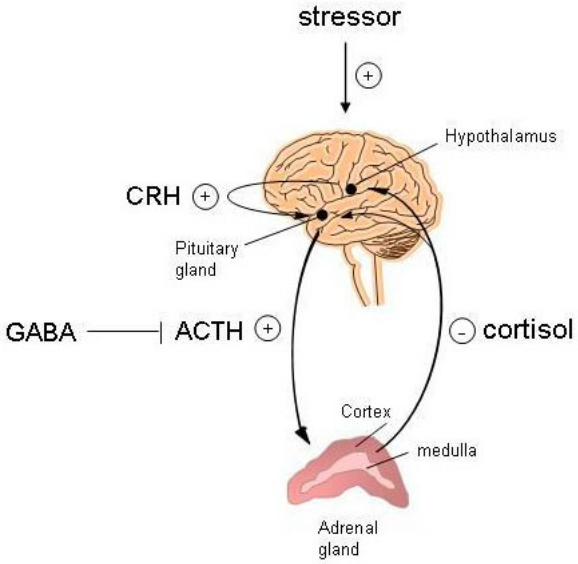
The concept of stress was first described by Seyle (1950) as a nonspecific response to different types of stressors and functions as a system to help the body reach and maintain homeostasis. Stressors are defined as any stimulus that activates the stress response (Wiley, 2009). Physiology can be altered in response to many different types of stressors including physical and psychosocial stress. More recently the concept of stress has been refined to include concepts of allostasis, allostatic load and allostatic overload (McEwen and Wingfield, 2003).

Allostasis has been used to acknowledge the range of physiological 'normal' variation by physiological system and environment, supporting homeostasis by expanding the boundaries of normal function in response to environmental or social change (McEwen and Wingfield, 2003). Allostatic load has been described as the costs of maintaining a stress response result during which allostatic overload can occur and may produce negative fitness or health outcomes (McEwen and Wingfield, 2003).

The stress response is an extremely complex process that involves activation of many physiological systems and behavioral response. Immediate activation of the stress response occurs in the central nervous system. This system is referred to as the autonomic nervous system, a largely involuntary system that has two components: the sympathetic nervous system, which is activated during a stress response, and the parasympathetic nervous system, which is suppressed during stress (Wiley, 2009). The activation of the nervous system is an immediate response to stress that allows an organism to respond immediately. However, this response is difficult and costly to maintain for long periods of time (Sapolsky, 2009).

A long term and sustained response to stress occurs in the endocrine system and is controlled via release and secretion of various hormones including the glucocorticoids (For a generalized diagram see figure 1). The hypothalamic-pituitary-axis (HPA) is a major component of the hormonal stress response. The activation of the HPA occurs when corticotropin releasing factor is secreted from the hypothalamus after the perception of stress. This results in secretion of adrenocorticopin hormone, which signals the adrenal glands to release glucocorticoids (Sapolsky, 1999). The glucocorticoid, cortisol, is a steroid hormone released by the adrenal glands that has an important role in this response. Cortisol, as well as other glucocorticoids, is involved in energy mobilization in order to sustain a short-term activation of the stress response and after the cessation of exposure to a stressor and is involved in renewing energy stores used up in this process (Wiley, 2009).

Figure 1: Diagram of the HPA Axis



(Heulens and Kooy, 2011)

Stress and Health

The stress response and exposure to glucocorticoids and other stress hormones is necessary for insuring survival in response to an immediate and life threatening stressors. However, long-term exposure to glucocorticoids is toxic and can have deleterious effects on health and fitness. Cortisol has receptors and binding sites on many organs and tissues throughout the body and long-term secretion of cortisol may inhibit function in these targets (Wiley, 2009).

While all mammals must balance the physiological costs and consequences of activation of the stress response, humans and other primates present a unique example of the consequences of a long-term response. In contrast to humans and primates, other mammals only face immediate challenges to survival and thus fitness and are not constantly subject to a constant battery of social stressors (Sapolsky, 2004). Thus suppression of organ

function is only over a short-term period with longer-term alterations rarely occuring.

Dominance hierarchies have a significant role in the lives of all herd animals in determining which individuals are stressed and which are not. However, primates are unique because they are constantly subject to an array of social stressors. The development of social hierarchies created a novel situation in which primate individuals were not only subject to the fitness costs of low rank in a hierarchy but also a battery of social stressors related to social rank. Effects of lack of predictability, control and outlets for frustration of social stress are felt differentially across the social hierarchy and determine differences in health status and outcomes (Sapolsky, 2005). While dominance hierarchies are not as directly observable in humans, there are certainly proxies, such as socioeconomic status, that can function as markers of status and place in a hierarchy (Sapolsky, 2004).

The mobilization of the stress response is energetically expensive, and constant activation can have major physiological costs. In females, for non-human primates and humans, prolonged exposure to stress can disrupt reproductive function. Excess glucocorticoids and other hormones circulate between the hypothalamic-pituitary-adrenal axis, ovaries, and the uterus resulting in declines in fertility due to lower levels of reproductive hormones, later onset of puberty, high miscarriage rates, later onset of puberty, and longer interbirth intervals (Sapolsky, 2004).

Chronic activation of the stress response impacts immune function and can greatly alter its physiological function if exposure is prolonged. The field of psychoneuroimmunology has elucidated important pathways through which psychosocial experience can modulate immune function. Studies have shown that stress mediates processes such as susceptibility to the common cold and other health outcomes mediated by immune function as well as variability in response to vaccination have been associated with stress (Cohen et al. 1991, Glaser et al. 1992). Immunosuppression by lower levels of circulating lymphocytes is a potential mechanism through which stress impacts immune function (Sapolsky, 2004).

The effects of stress on the cardiovascular function are well known and particularly detrimental to health. The activation of the sympathetic nervous system increases heart rate and blood pressure and can increase the risk of blood vessel damage (Sapolsky, 2004). When such damage occurs, lipids and cholesterol flood the damaged area resulting in plague formation. Stress causes aggregation of circulating platelets, which may worsen plaque formation (Sapolsky, 2004).

Stress has been identified as a major mechanism through which the social determinants of health and inequality become embodied, producing differential health outcomes across the socioeconomic gradient and other social factors such as race. Differential exposure to stress and stressors, both physical and social, as well as differences in access resources and coping strategies have been recognized as pathways through which stress impacts health and creates

social patterns in health disparities in race (Dressler et al. 2005; Gravlee, 2009; Kuzawa, 2009). Stress has also been cited as one of the mechanisms through which income inequality and subjective social status operates to produce social gradients in health (Marmot, 2005; Wilkinson and Pickett, 2006).

MATERIALS AND METHODS

Sample Characteristics

Participants in this study were undergraduate students from the University of Arizona, ranging from sophomores to seniors, recruited in the form of a convenience sample. Freshmen were excluded from the sample due to the unique stress-inducing challenges of the first year of university study and adapting to university life. Colleagues of the investigators were asked if they would like to participate. Recruitment emails were also sent through School of Anthropology undergraduate listserv requesting for participation in the study. The principle investigators made announcements requesting participation in classes that they were enrolled in or were a preceptor for. Due to the relatively small sample of interested students, it was not possible to randomly select to reduce bias. A gender bias did occur in the sample as more females signed up to participate than males. For characteristics of the sample see figure 1.

Table 1:

Sample Characteristics	
	n
Total	27
F	24
M	3

Sample size by sex

Convenience samples are often critiqued as a potential source of bias. However the sample used in this study incorporated students across selected year of study, of both genders and subject of study. We believe that this reduces the potential for bias while realizing that it is difficult to capture the range of students and experiences at the university level in such a small sample size.

Saliva Samples

Saliva samples were collected between 8:00 am and 11:30 am. Participants were asked not to eat or drink anything but water on the morning of the collection and not to drink alcohol within twenty-four hours. Collected samples were stored at 4°C within two hours of collection and then frozen and stored at -20°C until time of assay. Saliva was collected using the Salimetrics oral swab. The swab was placed under the tongue and left for two minutes. Swabs were then stored in Salimetrics cryovials and labeled to correspond to participants survey data. Saliva collection and storage was performed in accordance with the recommendations of Salimetrics.

In order to capture periods of high and low stress, samples were collected from participants on two different days. For the majority of participants, the first collection occurred during finals week in order to capture a period of high stress. The next collection occurred during the following semester several weeks after the start of classes but before midterms. This collection was timed to occur during a period of relatively low stress. Due to a small sample size in the first cohort, a second cohort was established to boost numbers. The first collection for this second cohort occurred at the same time as the second collection for the first cohort, capturing a period of low stress. In order to reduce potential bias, the second collection for the second cohort occurred during finals week, thus capturing a period of relatively high stress.

Salivary Cortisol

Cortisol levels were analyzed using a high sensitivity salivary cortisol enzyme immunoassay kit purchased from Salimetrics. Kits were stored at 4°C until use. Saliva samples were defrosted and reagents warmed to room temperature before the assays were run. Cortisol levels were measured by analyzing optical density on a standard plate reader at 450 nm after binding salivary cortisol to antibodies linked to horseradish peroxidase bound to a ninety-six well plate. Bound cortisol is measured from the reaction of peroxidase and a tetramethylbenzidine after the reaction is stopped with sulfuric acid. The amount

of cortisol is inversely proportional to the amount of cortisol peroxidase present. (Salimetrics, LLC, 2011).

Blood Pressure

Blood pressure was measured using a manual sphygmomanometer and a stethoscope. Participants' blood pressure was collected twice, at the beginning and end of each interview. Collecting blood pressure twice was meant to reduce white coat syndrome as well as any initial nervousness on the part of participants.

Self-perceptions of Stress

Several survey instruments were used to gather data about how participants perceived stress in their lives. The Cohen Perceived Stress Scale was used along with a short open-ended survey measure written by the investigators of the study.

A ten-item version of the Cohen Perceived Stress scale (Cohen et al. 1983) was used to measure the degree to which participants regarded their lives as stressful. The Cohen PSS was distributed during each of participants meetings to determine how periods of lower and higher stress altered the degree to which participants viewed their lives as stressful. The PSS has been validated

in many samples, including college students, as a valid and reliable measure of appraisal of stress (Cohen et al. 1983).

A short open-ended survey was developed by the investigators to allow participants to better explain their definition of stress as well as the role of stress in their lives. While the answers did not often adhere to the technical definitions of 'stress' and 'stressor,' it allowed participants to explain the ways stress was present and managed in their lives. Participants were also asked, "How do people perceive the attitudes [and work] of people who are stressed" and "How do people perceive the attitudes [and work] of people who are not stressed" to determine whether they viewed it as a positive or negative. A three-item scale was developed in which participants were asked to self-identify with the group that best described their self-perceived stress level, with the options of Episodically Stressed, Periodically Stressed, and Chronically Stressed. For a distribution of the sample by self-selected stress category see table 2.

Table: 2

Perceived Stress Scale Score by Stress Category

Stress Category	N	Mean PSS High Stress	Mean PSS Low Stress
1	4	20	17.25
2	17	18.0625	21.0909
3	6	11.6	12.2

Stress category by size and mean PSS score

In addition to the Cohen Perceived Stress Scale and the short opened-ended survey, participants were also asked to list and rank the five most stressful parts of their lives. This allowed for analysis based on the type of stressor in relation to the other collected data. Stressors listed by participants ranged from specific to general and were grouped into broad categories such as school, social relationships and work.

RESULTS

Perceived Stress Scale Scores

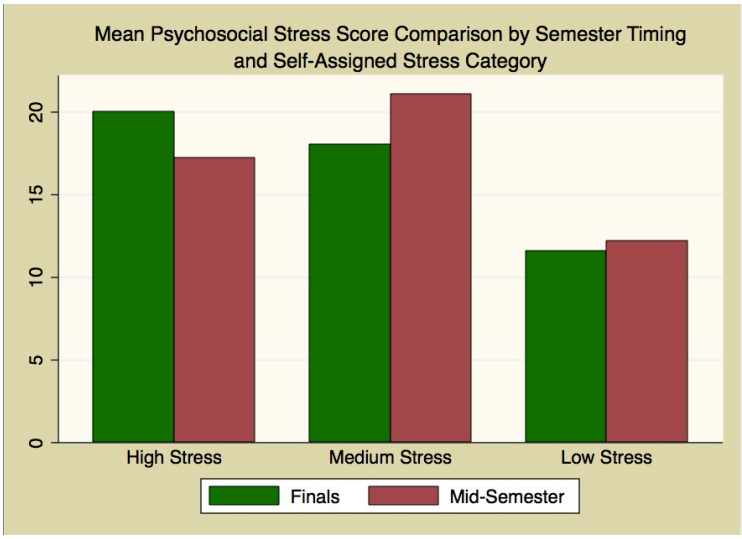
Using a trend analysis, Perceived Stress Scale varied significantly by stress category during finals week (Graph 1). However, PSS scores did not significantly vary at the mid-semester measurement by category using either parametric or nonparametric statistics (Graph 1). However, a paired t test showed statistically significant differences between PSS score by stress category between the finals and mid-semester measure (Table 3). These findings should be viewed with caution as the degrees of freedom are high.

Table 3: Paired T-Test

```
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
    pss1 |      17    16.94118    1.650469    6.805059    13.44234    20.44001
    pss2 |      17    19.76471    1.663911    6.860479    16.23737    23.29204
---------+--------------------------------------------------------------------
    diff |      17   -2.823529    1.560626    6.434626   -6.131909     .48485
------------------------------------------------------------------------------
    mean(diff) = mean(pss1 - pss2)                                t =  -1.8092
Ho: mean(diff) = 0                         degrees of freedom =       16

 Ha: mean(diff) < 0         Ha: mean(diff) != 0         Ha: mean(diff) > 0
 Pr(T < t) = 0.0446         Pr(|T| > |t|) = 0.0892      Pr(T > t) = 0.9554
```

Graph 1:



	PSS Finals	PSS Mid-semester
z	-1.98	-0.85
Prob > |z|	0.048	0.395

Salivary Cortisol Concentration

Salivary cortisol comparisons can be seen in Graph 2. No statistically significant differences were found between finals and mid-semester for the mean of all cortisol concentrations when using a paired T-test (Table 4). While analysis of variance tests (ANOVA) do not suggest differences between mean cortisol concentrations across stress categories, for finals or mid-semester, nonparametric tests of trend analysis suggest a difference (Prob>|z|=0.038). However, Kruskal-Wallis tests do not show significance for these measures (Table 5).

Graph 2:

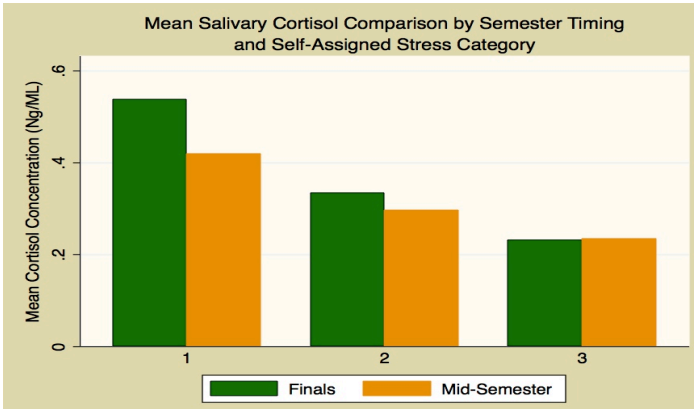


Table 4: Paired t-Test between Finals and Mid-semester Cortisol concentration

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
cort1	16	.33425	.0439892	.1759566	.2404893	.4280107
cort2	16	.2881875	.047271	.1890838	.1874318	.3889432
diff	16	.0460625	.0457875	.18315	-.0515313	.1436563

mean(diff) = mean(cort1 - cort2) t = 1.0060
Ho: mean(diff) = 0 degrees of freedom = 15

Ha: mean(diff) < 0 Pr(T < t) = 0.8348
Ha: mean(diff) != 0 Pr(|T| > |t|) = 0.3304
Ha: mean(diff) > 0 Pr(T > t) = 0.1652

Table 6:

Kruskal-Wallis Test
Cortisol Finals

stress~t	Obs	Rank Sum
1	3	55.00
2	16	206.00
3	5	39.00

chi-squared = 4.296 with 2 d.f.
probability = 0.1167

chi-squared with ties = 4.298 with 2 d.f.
probability = 0.1166

Kruskal-Wallis Test
Cortisol Mid-semester

stress~t	Obs	Rank Sum
1	4	44.00
2	11	109.50
3	4	36.50

chi-squared = 0.224 with 2 d.f.
probability = 0.8942

chi-squared with ties = 0.224 with 2 d.f.
probability = 0.8940

Blood Pressure

Comparisons of systolic blood pressure are displayed in Graph 3. Analysis using Kruskal-Wallis (Table 7) and Paired t-Tests (Table 8) revealed no significant differences for systolic blood pressure between stress categories or between finals and mid-semester collections.

Graph 3:

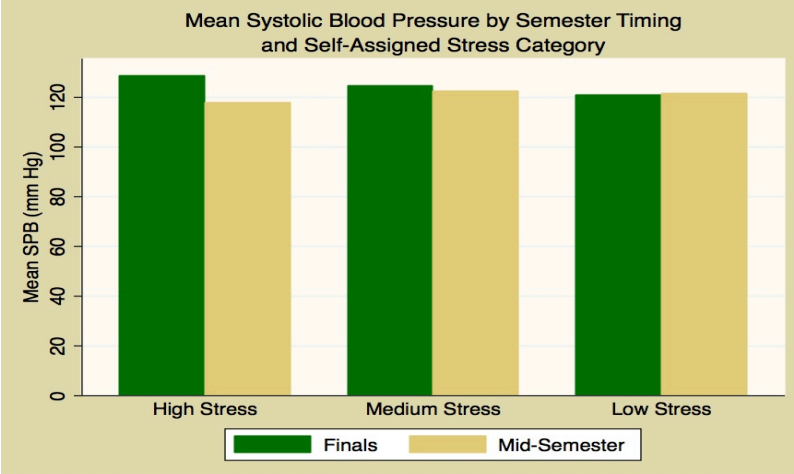


Table 7:

Kruskal-Wallis
Systolic BP-Finals

stress~t	Obs	Rank Sum
1	3	49.00
2	16	202.50
3	5	48.50

chi-squared = 1.673 with 2 d.f.
probability = 0.4331

chi-squared with ties = 1.686 with 2 d.f.
probability = 0.4304

Kruskal-Wallis
Systolic Blood Pressure Mid-semester

mstress2	Obs	Rank Sum
1	11	93.00
2	2	25.00
4	3	18.00

chi-squared = 2.240 with 2 d.f.
probability = 0.3263

chi-squared with ties = 2.263 with 2 d.f.
probability = 0.3225

Table 8: Paired T-Test for Systolic Blood Pressure

```
------------------------------------------------------------------------------
Variable |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
systol1 |      17    125.2353    2.033776    8.385474    120.9239    129.5467
systol2 |      17    122.5294     1.33977    5.524012    119.6892    125.3696
---------+--------------------------------------------------------------------
    diff |      17    2.705882     1.82258    7.514691   -1.157815     6.56958
------------------------------------------------------------------------------
    mean(diff) = mean(systol1 - systol2)                      t =   1.4846
    Ho: mean(diff) = 0                     degrees of freedom =       16

 Ha: mean(diff) < 0           Ha: mean(diff) != 0           Ha: mean(diff) > 0
 Pr(T < t) = 0.9215         Pr(|T| > |t|) = 0.1571          Pr(T > t) = 0.0785
```

Trends in diastolic blood pressure can be seen in Graph 4. A Paired T-Test revealed significant differences between means for finals and mid-semester diastolic blood pressure (Table 9).

Graph 4:

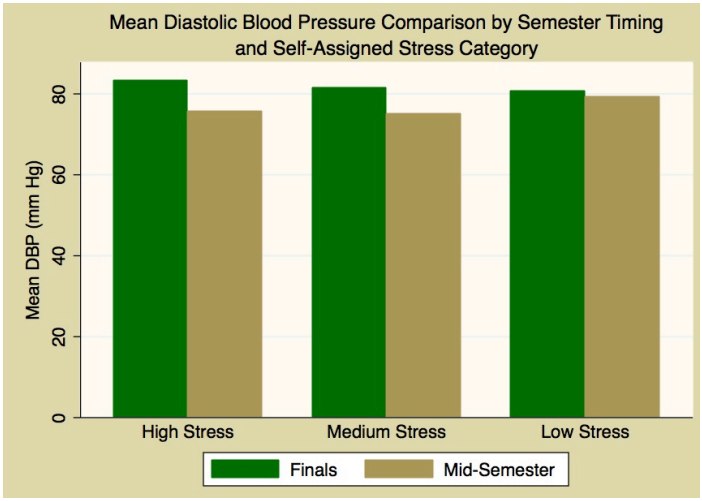


Table 9: Paired T-Test Diastolic Blood Pressure

Variable	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
diastol1	17	82.17647	1.157569	4.772778	79.72253	84.63041
diastol2	17	77.05882	1.370569	5.651002	74.15335	79.9643
diff	17	5.117647	1.651386	6.808839	1.616865	8.618429

mean(diff) = mean(diastol1 - diastol2) t = 3.0990
Ho: mean(diff) = 0 degrees of freedom = 16

Ha: mean(diff) < 0 Ha: mean(diff) != 0 Ha: mean(diff) > 0
Pr(T < t) = 0.9966 Pr(|T| > |t|) = 0.0069 Pr(T > t) = 0.0034

Kruskal-Wallis tests did not reveal any statistically significant results between diastolic blood pressure and stress categories (statistics omitted). Diastolic blood pressure for finals and mid-semester was covaried with PSS score and no significant results were found (Table 10).

Table 10: Trend Analysis of Diastolic Blood Pressure Covaried with PSS

Diastolic BP Finals

stresscat	score	obs	sum of ranks
1	1	3	42.5
2	2	16	200
3	3	5	57.5

z = -0.51
Prob > |z| = 0.612

Diastolic BP Mid-semester

stresscat	score	obs	sum of ranks
1	1	4	38.5
2	2	11	101.5
3	3	5	70

z = 1.19
Prob > |z| = 0.233

Correlations

Correlation statistics revealed few statistically significant correlations between variables. For data collected during finals, systolic blood pressure correlates with diastolic blood pressure (Table 11). Correlation analysis of data collected during mid-semester suggests that Perceived Stress Scale score correlates with systolic blood pressure which correlates with diastolic blood pressure (Table 12).

Table 11: Correlation Matrix- Finals

	PSS1	Systol1	Diastol1	Cortisol1
PSS1	1.000			
Systol1	0.2003	1.000	0.3480	
Diastol1	0.1679	0.4569*	1.000	
Cortisol1	0.2856	0.2154	-0.0644	1.000

Table 12: Correlation Matrix- Mid-semester

	PSS2	Systol2	Diastol2	Cortisol2
PSS2	1.000			
Systol2	0.5178*	1.000	0.0194	
Diastol2	0.3536	0.6852*	1.000	
Cortisol2	-0.1695	-0.3383	-0.0992	1.000

DISCUSSION

We hypothesized that self-identified stress categories would significantly predict stress biomarker levels. Some of our results and analyses did support this hypothesis. One measure of stress, the Cohen Perceived Stress Scale did significantly vary by stress category for the measurement during finals. However, no trends were observed in the mid-semester measurements. It is possible that a higher PSS score observed in a few individuals at mid-semester slightly skewed

this measure. A Paired T-Test also revealed that the mean for all PSS scores varied significantly between finals and mid-semester collections.

Individuals that self-identified with high stress levels did have higher PSS scores than those who identified with low stress levels. The PSS scores of those with medium stress levels were higher than both low and high stress levels for the mid-semester measurement (Graph 1). This pattern is difficult to explain and may be a result of an accumulation of stressful events around the mid-semester collection time for several individuals who identified with a medium stress level. A relatively small increase in PSS score for a few individuals in this category would be enough to skew the mean upward.

Cortisol concentration did present interesting comparative trends (Graph 2). However, statistical tests revealed mixed results for the significance of these trends and differences. A Paired T-Test between the means of cortisol concentration during finals and cortisol concentration at mid-semester did not produce any significant results, suggesting that cortisol concentrations hold relatively steady in this health group of college students. In addition, analysis of variance tests did not show any significant differences by stress category. However, nonparametric trend analysis did reveal statistically significant differences between cortisol concentrations during finals between stress categories in precisely the pattern we would expect. Individuals who self-identified as low stress responders showed the most consistent cortisol levels from measure one to measure two with the lowest overall salivary cortisol concentration. The results of these statistics are difficult to interpret. The sample

size is small enough for non-parametric statistics, and thus the use of the Kruskal-Wallis test, but because the sample sizes for high and low stress categories are so low, a trend analysis is a useful supplement. A larger sample size would likely reveal clearer trends in correlation and association between cortisol and self-identified stress level. However, statistical significance achieved at a low sample size is a robust measure. The cortisol data does follow trends in the literature illustrating increased cortisol concentration in regards to academic and psychosocial stressors (Campisi et al. 2012).

Systolic blood pressure did not vary significantly between stress categories. No significant trends were found for systolic blood pressure between finals and mid-semester measurements. Mean diastolic blood pressure did vary significantly between finals and mid-semester. This is potentially due to numerous academic and other psychosocial stressors present during finals week that are not present at mid-semester. However, this could potentially be due to "white-coat effect," and participants may have been more comfortable during the second meeting and biomarker collection. This seems unlikely because the collections were months apart. Diastolic blood pressure did not significantly vary by stress category. This blood pressure data does not follow previous studies in which blood pressure of college student increases in response to stress (Campisi et al. 2012; Hughes, 2005; Zhang et al. 2011). It should be noted that existing literature examined changes in blood pressure in response to acute psychosocial stressors while this study sought to look over a longer time frame. It is likely that differences in blood pressure between finals and at mid-semester are not

observed in college students that are young enough so that DBP is not responsive over that length of time.

It was also hypothesized that constantly stressed individuals in the high stress category would have similar cortisol concentrations to those who identify as rarely stressed in the medium stress category during finals, a time of high stress. This is difficult to determine from the collected data. A slightly significant trend was observed between stress category and cortisol concentration with a negative trend from higher mean cortisol concentration in the higher stress category to lower mean concentration in the low stress category. However, as discussed above, there were no significant differences for blood pressure, systolic or diastolic, by stress category. A potential interpretation is that individuals in the high stress category do not have a blunted stress response so they produce more cortisol than those in the other categories.

Finally, we did not find the Cohen Perceived Stress to correlate with most of the stress biomarker data. The only exception to this was that PSS score correlated with systolic blood pressure at mid-semester. While this is expected, it is unusual that a similar pattern was not observed during finals. This is difficult to explain because the Cohen PSS has been found to be an accurate predictor of perception of stress, which should correlate with biomarkers such as blood pressure (Cohen et al. 1983). A possible explanation is that blood pressure is not a sensitive biomarker for this age group. Systolic blood pressure was significantly correlated with diastolic blood pressure at both finals and mid-semester.

Limitations

It is important to recognize some limitations of this study. First, the sample size was relatively small. The majority of participants identified with the periodically, or medium, stress category while few identified with the constantly or rarely stressed categories. This reduced the power of statistical comparisons between groups. While the statistical significance achieved was robust, the sample size likely masked trends in correlation/association. There was also a sex bias in the sample with a much larger number of female participants and very few males. This made any comparison by sex meaningless, if not impossible.

A lack of control data regarding diet and preexisting health conditions that might elevate any of the collected biomarkers also presents a problem. Due to time constrains, relative homogeneity was assumed between participants--that is, diets and activities levels were assumed to be similar enough between participants to forgo collection of this data. However, given more time and resources in addition to a larger sample of willing participants, this information would prove useful as potential control data.

There were important limitations with the biomarkers collected as well as the number of collections. Blood pressure and salivary cortisol were selected for cost-effectiveness and ease of collection. Due to financial constraints and time limitations with the participants, only two meetings and collections were conducted. Ideally, many more samples would have been collected. Recent studies suggest that to accurately compare cortisol between groups or individuals

between 10 and 15, cortisol samples need to be collected per person (Nepomnaschy et al. 2012). This presents serious implications for the cortisol data used in this study. Blood pressure has shown to be sensitive in this age group (Campisi et al. 2012; Hughes, 2005; Zhang et al. 2011). While many studies have examined longer-term changes in blood pressure in response to psychosocial stressors in other populations, such as health professionals, few have examined college students. However it is possible that blood pressure is not a sensitive enough biomarker to semi long-term psychosocial stressors in this age group.

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