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Do Athletes Respond Differently to Academic and Social Stress? An Examination of Cortisol and Perceived Stress Throughout a Semester in College Athletes and Typical College Students

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An Examination of Cortisol and Perceived Stress Throughout a Semester in College Athletes and Typical College Students

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Abstract

In order to be a successful athlete, you must be able to perform well under stressful situations. Are athletes also better at responding to stress under other circumstances such as social and academic stress? The present study investigated the impact of exercise on salivary cortisol and perceived stress in college students. Cortisol was sampled throughout a semester as well as before and after a laboratory-based stress test during the final exam period. It was found that athletes had the largest increase in cortisol between baseline and the final exam period and the sedentary students had the smallest increase. Also, cortisol levels and perceived stress were correlated in the athlete group and in a second group of students who work out regularly. These findings suggest that perhaps since athletes are often in competitive situations their HPA axis is physiologically conditioned to raise their cortisol to an optimal level in order to achieve their personal best possible results in stress provoking situations.

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Do Athletes Respond Differently to Academic and Social Stress?

An Examination of Cortisol and Perceived Stress Throughout a Semester College Students Athletes and Typical College Students

Cortisol

Cortisol is commonly accepted as a biomarker of stress, anxiety, and depression in human psychobiological studies. The hormone cortisol is a glucocorticoid that affects every system in the body. Glucocorticoids play an important role in the body's response to physiological and psychological stressors. They also suppress the immune system and play an important role in many brain activities, such as cognitive function. Glucocorticoids also affect human behaviors such as sleep patterns, mood, and the reception of sensory input (Kirschbaum & Hellhammer, 1989,1994; Levine *et al,* 2006).

Cortisol is the end product of the hypothalamic-pituitary-adrenal (HPA) axis. When the hypothalamus is stimulated, it secretes corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to secrete adrenocorticotrophic hormone (ACTH). Then ACTH stimulates the secretion of cortisol from the cortex of the adrenal gland. Overall, the HPA axis is self-regulating because of a negative feedback loop in which elevated levels of cortisol lead to a suppression of CRH and ACTH, which in turn reduces cortisol production. Cortisol levels follow a pattern of peaking prior to waking, and decreasing throughout the day until they reach low levels in the afternoon and evening. Both stress and the circadian cycle are associated with the HPA axis, however the central pathways by which they are linked to the hypothalamus are not completely understood (Kirschbaum & Hellhammer, 1989, 1994; Levine *et al*, 2006).

The "Free Hormone Hypothesis" predicts that the biological activity of a given steroid correlates with the free protein-unbound concentration rather than the total concentration of the steroid. The physiological background is that non-polar steroid hormones have a low solubility in aqueous extracellular fluid, circulate in the blood stream bound to specific high affinity, low capacity carrier proteins, as well as binding to lower affinity, high capacity non-specific proteins. Therefore, only free cortisol is available for movement out of capillaries and into cells. The "Free Hormone Hypothesis" is the commonly accepted view of how steroid hormones function (Levine et al, 2006).

This hypothesis is the reason it is commonly accepted to use cortisol from saliva instead of blood as a biomarker. Measuring cortisol from saliva allows for frequent and rapid sampling. It is also a non-invasive and stress-free procedure. Salivary cortisol is a reliable reflection of total plasma values and circulating free cortisol. Salivary cortisol has been used in endocrinology, psychobiology, and behavioral medicine research studies since the early 1980s (Levine et al, 2006). Hellhammer, Wust, and Kudielka (2009) confirm obtaining cortisol levels through saliva is the preferred method of obtaining cortisol and an accurate biomarker of stress. Therefore, it can be assumed that obtaining cortisol levels through salivary samples will give an acute measure of stress.

Simpson and colleagues (2008) investigated the relationship between cortisol, perceived stress, and mood. Forty-one healthy adults from Northern

Ireland were recruited for this study. Participants were excluded if they had been diagnosed with depression or if they smoked more than 10 cigarettes a day because depression and smoking are known to affect the normal rhythm of cortisol. Salivary samples were collected at 2:30pm and 10:30pm (avoiding meal times) for 7 consecutive days. However, it is interesting that the experimenters didn’t choose a time that was closer to waking. The Positive Affect and Negative Affect Schedule (PANAS) and the Perceived Stress Scale (PSS) were completed four times a day for the same 7 consecutive days. Packets containing all of the containers for cortisol and scales were mailed to the participants 10 days before their appointment at the research center. The experimenter called the participants on the second day of collecting samples to see if there were any problems. After the completion of the 7 days, the participants brought the cortisol samples and the completed questionnaires to the research center. Overall, cortisol levels in the participants decreased between the afternoon and evening sample, which illustrates cortisol’s circadian rhythm. This study illustrated that there were no sex differences between the cortisol levels of males and females. Also, there was no correlation between cortisol and negative mood. Additionally, there was no correlation between cortisol and perceived stress scores. This study confirm the circadian pattern of cortisol and also suggests that it may be difficult for people to predict their own stress since there was no a correlation between cortisol and perceived stress.

Laboratory Stressors

In order to examine the relationship between psychological stress and salivary cortisol in young adults, Takai and colleagues performed a study in 2004.

There were 83 healthy volunteers with a mean age of 24. The psychological stressor was a video recording of a corneal transplant surgery, which involved scenes of injections into the cornea eyeball and incisions of the cornea with scissors for 15 minutes. There was also a three-minute scenic beauty video as a soother. Forty-eight subjects viewed only the stressful video, 19 subjects viewed only the soothing video, and 16 subjects viewed both videos. The videos were followed by 15 minutes of silence. Saliva was collected every three minutes throughout the session. The stressful video was shown to increase cortisol and the soothing video did not affect cortisol levels. These results support the hypothesis that cortisol rises during situations that people would identify as stressful and remains constant in more comfortable situations.

Another example that investigates the effect of laboratory stress is when Roy (2004) had 82 male fire fighters complete several questionnaires and a mental arithmetic task as well as a speech task. Saliva samples were collected before and after each task. After the speech task samples were taken 10, 20, and 30 minutes after the start of the task. The overall pattern of cortisol response in the study was not as extreme as had been seen in previous research of parachute jumping or public speaking but may correspond more closely with daily stress. Some participants showed an increase in cortisol in response to the stressor while others demonstrated a decrease in cortisol, which may reflect differences in feedback mechanisms. The mean cortisol response was not correlated with mood. However, high recent stress exposure was associated with lower cortisol levels, this has also been seen in military personal and individuals with occupational stress.

Additionally, larger cortisol responses were associated with more control of anger. Therefore, it can be suggested that higher cortisol levels in response to an acute stressor can be seen as a more adaptive and flexible method of coping instead of a negative reaction to an acute stressor.

In addition to examining cortisol in firefighters, a 2009 study by Smeets, Dziobek, and Wolf investigated sex differences in social cognition during a period of stress. In this study, 32 men and 32 women filled out the Autism Spectrum Quotient (AQ) and the Positive and Negative Affect Schedule (PANAS). Then the participants were exposed to the Trier Social Stress Test (TSST), which involves public speaking and mental math, or a non-stressful control test. After the TSST or control test, the participants completed the PANAS again and then were exposed to the Reading the Mind in the Eyes test (RMET-R) and the Movie for the Assessment of Social Cognition (MASC-MC), which measure the response to social cues and levels of social cognition. Salivary cortisol samples were taken 5 minutes before the TSST or control test as well as 20, 30, and 60 minutes after. There were sex specific differences in cortisol levels in reference to the MASC video. Males with high cortisol scored higher on the MASC than the males who had low cortisol. In contrast, women who had higher MASC scores had lower cortisol. Therefore, women performed better when their cortisol was low but men performed better when their cortisol was high. This study illustrates that high levels of cortisol may yield opposite effects in men and women. This supports a theory by Taylor et al. (2000) that women exhibit “the tend and befriend” response to behavioral stress whereas men exhibit a “fight or flight” response. This theory gives the interesting suggestion that men perform better in social situations when they are stressed but women perform better when they are calm.

Kudielka, Hellhammer, and Wust (2009) reviewed why there are differences in salivary cortisol in response to challenges. They found that age usually did not affect the level of cortisol in response to speech task and psychosocial stress. It was also found that cortisol levels either remained the same or decreased after physical exercise. However, sex differences have been found consistently. In response to the TSST, men's cortisol levels increase twice as much as women's. Men's levels have also been found to rise in anticipation of a stressful activity, which is not seen in women, which could be due to testosterone levels. However, it has been seen that there is an effect of the menstrual cycle and oral contraceptives on women's responses. Women in the luteal phase show a similar response to men, whereas women in the follicular phase and taking oral contraceptives show a lower cortisol response. In addition to age and sex differences, it has also been seen that chronic alcohol consumption can cause a blunted cortisol response.

Coffee and energy supplements have also been seen to increase cortisol responses to stress. Men demonstrated slightly lower cortisol responses when their partner supported them. However, women showed slightly higher cortisol levels when supported by their life partner. In response to an acute stressor, salivary cortisol has been found to peak 15-20 after the initial exposure to the stressor. Repeated stress studies illustrate the possibility of a correlation between personality and salivary cortisol response. However, through twin studies it has been found that there is not a genetic component. Overall, there are many factors other than the actual stressor that affect the cortisol response.

Trier Social Stress Test (TSST)

In 1993, Kirschbaum, Pirke, and Hellhammer invented a standardized method of inducing stress, which has become the predominant approach in this area of research and is known as the Trier Social Stress Test (TSST). In the original series of 5 studies, there were 155 subjects of both sexes. The participants ranged from 15-33 years old and were medication-free, refrained from smoking, physical exercise, meals, alcoholic beverages, and low pH soft drinks for at least one hour prior to testing. When the subjects arrived, they rested for either 30 or 10 minutes in room A, then they were taken to room B and introduced to the task they would be participating in. The participants were blind to the task. In room B, 3 people were already sitting at a table and a video camera was installed. The subject was asked to stand at a microphone in front of the three people. Next, the investigator asked the participant to take over the role of a job applicant who was invited for a job interview with the company's staff managers. They were told that after a preparation period that they should introduce themselves to the managers and convince the managers that they are they perfect person for the position. The managers were introduced as being trained in reading nonverbal behavior. In addition, participants were told that a video analysis would be performed.

After receiving the instructions, the subjects returned to room A to prepare their speeches. They were given paper and a pencil but were not allowed to bring the paper into the room. After 10 minutes, the participant was brought back to room

B to deliver their speech. If they finished before time, the one of the mangers would say "you still have more time please continue" and if they finished a second time the managers would precede with a series of prepared questions. After the 5 minutes was over, the participant was then asked to subtract 13 from 1,022 as quickly and as accurately as possible. If the participant made a mistake, one member of the committee would say "stop, 1022" and then the participant had to start over; this continued for 5 minutes. After the mental math was completed, hormone samples were taken and the participant was debriefed. Blood or saliva samples were obtained in 10-30 minute intervals depending on the analysis of cortisol of serum in saliva. Cortisol peaked in saliva 10 minutes after cessation of stress. High reproducibility was seen across all five different studies. After 90 minutes, cortisol levels returned to baseline levels. The TSST reliably induces a 2-4 fold increase in salivary cortisol with a similar peak concentration in different populations studied. One element of the TSST that seems to be very important is the perceived scrutiny of one's abilities, which is referred to as "social-evaluative threat". In addition, lack of control is also seen to raise cortisol levels (Dickerson and Kemeny, 2004).

Optimal Stress

In addition to the hormone cortisol, the noradrenergic and dopaminergic neurons change their firing rates according to arousal state and according to the relevance of events in the environment as reviewed by Arnsten (2009). In the locus coeruleus, noradrenaline neurons do not fire during REM sleep and have low firing during slow wave sleep. When waking, they fire in response to relevant stimuli. However, they can also respond to irrelevant stimuli during fatigue or stress.

Additionally, dopamine neurons are usually fired as a reward mechanism. But dopaminergic neurons in the midbrain also increase their firing when presented with aversive stimuli. Also, noradrenaline is released in the prefrontal cortex (PFC) and dopamine is released from the ventral tegmental area (VTA) during exposure to acute stress.

Dopamine and noradrenaline each have a shape that looks like an inverted U related to their effect on working memory with the optimal stress level at the top of the inverted U. Too much or too little of either impairs the function of the PFC. Each of these neurotransmitters provide excitatory influences that put the PFC into a state that allows the neurons to process information. Additionally, dopamine and noradrenaline have modulatory influences that affect the strength of the PFC connections as these networks engage in working memory. This information suggests that there is a certain amount of each of these neurotransmitters and perhaps other neurotransmitter and hormones necessary to achieve an optimal stress level, which in turn provides an optimal performance (Arnsten, 2009).

Exercise and Stress

A 2009 study by Milani and Lavie looked at the impact of reducing psychosocial stress though exercise in cardiac rehabilitation patients. There were 522 participants in this study and all of them had completed cardiac rehabilitation and exercise training. Also, all of the patients started the program between 2 and 6 weeks after a coronary event such as acute myocardial infarction, coronary bypass, and percutaneous coronary intervention. Twenty-seven participants with high social stress scores made up a control group because they were not participating in the cardiac rehabilitation. The Kellner Symptom Questionnaire was used to assess behavioral characteristics such as symptoms of depression, anxiety, somatization, and hostility. The exercise session consisted of approximately 10 minutes of warm-up exercises, then 30-40 minutes of aerobic and dynamic exercises such as walking, jogging, rowing, or biking, which was followed by 10 minutes of cool down.

At baseline, all participants were educated about the American Heart Association Step II diet with a Mediterranean modification. All of the health providers frequently encouraged the participants to comply with the diet. Daily lectures were also given in the hospital on coronary health. Participants with high psychosocial stress demonstrated improvements in exercise capacity, high-density lipoprotein cholesterol, and all behavioral parameters, including psychosocial stress. The participants with low psychosocial stress revealed improvements in BMI, exercise capacity, high lipoprotein cholesterol, triglycerides, high sensitivity C-reactive protein and all behavioral parameters including psychosocial stress. After cardiac rehabilitation, psychosocial stress was decreased from 10% to 4%. Participants with high psychosocial stress had 22% mortality as opposed to the participants with low psychosocial stress who had a mortality of 5%. The control group of participants who had high psychosocial stress had the mortality for 19%, illustrating that psychosocial stress is a very strong risk factor for mortality. The participants were divided into two groups based on their degree of exercise change during the cardiac rehabilitation and exercise training. At a follow-up, the participants with high exercise change had 4% mortality as oppose to the lower exercise change, which had 10% mortality. Additionally, participants who had low psychosocial stress and high exercise didn't differ significantly from the group with low psychosocial stress and low exercise change. However, participants with high psychosocial stress and high exercise change had a mortality of 0% whereas the participants with high psychosocial stress and low exercise change had a mortality of 19%. This finding suggests that exercise had a large effect on those with high psychosocial stress.

Additionally, a 2007 study by Anshel and Sutarso also investigated sex differences in response to stress. However, the present study only used participants who has participated in high school athletics. There were 176 men and 156 women in this study ranging from 18-23 in age all of whom had competed on his or her high school sports team. Participation on a high school sports team suggests moderate skill level and similar sources of acute stress (SAS). First, the experimenters tried to identify the sources of stress perceived as highly intense and then tried to determine the degree to which they used similar coping strategies following two different acute stressors. Each item on the criterion was something commonly experienced in a sport setting. Participants were asked to indicate their level of stress after a particular event. Next, the participants were assessed. The respondents were asked to indicate the usual way that they responded to a particular situation that they indicated was stressful. The SAS were generated and categorized in to "performance related" and "coach related." The relationship between both of these types of stress significantly showed that males and females had different coping strategies in response to stress. Females tended to discuss their problems with others more whereas males tried to deal with their stressors on their own. Therefore, gender had more of an effect on coping than the commonality of participating in high school sports.

Cortisol and Exercise

In addition to the response to laboratory stress and exercise being studied separately as in the previous studies, the relationship between cortisol and exercise has also been investigated. In a 2009 study by Vale and colleagues, the relationship between blood cortisol and exercise in elderly women was explored. The women were divided into three groups: strength training, aerobic exercise, and control. Each of the experimental groups completed a 12-week intervention designed by the experimenters, which consisted of an exercise routine the participants were not familiar with, and the control group agreed not to engage in physical activity for the 12 week period. There were no differences in the cortisol levels after the 12-week intervention. However, there was a decrease in cortisol. Psychological states of the participants were not taken into account in this study. Also, since this study involves the elderly the physical activity was not as intense as what young people would perform. This suggests that exercise must be performed at a certain intensity to change cortisol levels.

In contrast with the elderly, Karkoulias and colleagues examined hormonal responses of marathon runners in 2008. There were 11 non-elite marathon runners in this study. Blood cortisol samples were taken 1 week before the race, 1 hour after completion of the race, and a week after the race. Cortisol levels increased significantly immediately after the race and almost returned to baseline a week after the marathon suggesting that athletic competition may cause an increase in cortisol.

Salvador (2005) performed a review of stress in competitive situations. In this review, Salvador found that men had an increased cortisol level after a sporting event regardless of if they won or lost. It was also seen that those who had a high self-efficacy and lost had a larger rise in cortisol than those who had a low self-efficacy and lost suggesting that the shock of losing increased their cortisol. In studies with women and sports, there are also not significant differences in cortisol levels based on winning or losing. However, there is not enough research presently on female athletes, most of the research is currently done on males. Additionally, it was found that cortisol increases in athletes in anticipation of competitive events, which would indicate an adaptive response. This suggests that cortisol might help an athlete succeed in a competitive event.

In addition to investigating the elderly and marathon runners, a different study explored the effect of different lengths of rest time between lifting sets and cortisol. There were 12 healthy females in this study with a mean age of 26, whom were randomly assigned different rest times, 30 seconds, 60 seconds, and 120 seconds, on three separate occasions. Blood samples were drawn before, immediately after each training session, and after 5 minutes, 15 minutes, and 30 minutes of each training session. Cortisol levels 5, 15, and 30 minutes after each training session were significantly higher than baseline. Cortisol tended to be lower as rest intervals increased. (Bottaro et al, 2007). This suggests that the rise in cortisol is not limited to aerobic exercise but also could rise in response to anaerobic exercise.

The effect of the combination of laboratory stress and exercise has also been studied in conjunction with cortisol. A 2006 study by Lovallo and colleagues looks at the relationship of exercise, mental stress, caffeine, and cortisol. The reason caffeine is significant is because it is known to increase cortisol and epinephrine during stress and at rest. This study had 96 participants and was conducted for 4 weeks. The study began with 5 days of self-administration of either a placebo lactose pill or a lactose pill with 300 mg/day of caffeine. This was followed by a laboratory test day, when the participant received a placebo pill on week one and the caffeine pill on the other three weeks. Mental stress testing was performed on 49 of the participants (24 women) and exercise testing was performed on 47 of the participants (24 women). The mental stress consisted of 15 minutes of work on a demanding reaction time task followed by 15 minutes of mental arithmetic. This task combination has been known to be “mildly aversive”. Exercise consisted of 30 minutes of stationary biking. Overall, men had higher cortisol than women. It was found that caffeine did not increase cortisol on its own but in conjunction with stress caffeine did increase cortisol significantly. This effect was similar in men and women. In contrast, neither men nor women had an acute cortisol response to exercise. Also, caffeine did not alter the cortisol response during exercise but it did produce a more delayed response of the cortisol release over the course of time. Therefore, the over-consumption of caffeine might delay the hormonal cascade that ends with the release of cortisol. However, the combination of exercise and caffeine may be beneficial.

Since there are so many positive effects of exercise, Foley and colleagues (2009) investigated exercise as a treatment of depression. There were 23 men and women between the ages of 18 and 55 years old who were currently experiencing a major depressive episode, un-medicated or antidepressant medication for over four weeks, and sedentary (exercise less than 30 minutes, three times per week). The Beck Depression Inventory (BDI-II) and Montgomery-Asberg Depression Rating Scale were used to assess depression. The Depression Coping Self-Efficacy Scale (DCSES) was also used. Episodic memory was also examined. A list of 32 nouns was read aloud and participants had 3 minutes to recall without cues. Then they were given 40 seconds to recall words with cues. Salivary cortisol samples were also taken at waking, 30 minutes after waking, and before bed. The cortisol waking response (CAR) was determined by subtracting the waking cortisol from the 30 minutes after waking cortisol. The participants were randomized into either aerobic exercise (n=10) or stretching (n=13). Then the participants completed the 12-week program at the Exercise and Health Psychology Laboratory (EHPL) of either mild-intensity stretching or moderate intensity aerobic exercise. Participants in the exercise group completed significantly more weeks of the intervention than those in the stretching group. The CAR significantly decreased in the aerobic exercise group at 6 and 12 weeks. In the stretching group, the CAR decreased at 6 weeks and increased at 12 weeks. The BDI and MADRAS were significantly correlated and the BDI and the DCSES change scores were inversely correlated. There were significant decreases in depression in both groups over the 12 weeks. Both groups had significant increases in coping efficacy and in episodic memory performance over 12 weeks. This may mean that the self-efficacy produced by completing an exercise program also had effects in the rest of the participants' life. Both groups also had significant increases in episodic memory over 12 weeks. The memory improvement may relate to the hippocampus, improved depression symptoms, or improved motivation. Overall, stretching and exercise were positively associated with improvements in depressive symptoms suggesting that this might also be correlated with the return of cortisol to the circadian rhythm.

Cortisol, Exercise, and TSST

In addition to studying laboratory stress and exercise, Rimmele (2007) and colleagues investigated the combination of different levels of athletic participation and stress in relation to cortisol. The participants were 22 elite sports men and 22 untrained men. The elite sportsmen were mostly recruited from endurance trained sports and had participated in the Olympics and/or were members of the Swiss national team. The untrained men were participants who exercised for less than 2 hours a week. Three of the original subjects were excluded. Participants were asked to refrain from eating, drinking, and physical activity for 2 hours before the experiment. In order to refrain from over training, the elite sportsmen followed a 10-day recovery phase training schedule prior to the experiment. The psychological stress was induced by TSST and comprised for a 5-minute public speaking task followed by a 5-minute mental arithmetic task in front of an unknown panel of one man and one woman. After entering the TSST room, subjects remained standing throughout the 10 minutes. Both groups were confronted with subjectively important situations, the elite sportsmen were instructed to apply for a contract with a sponsor and the untrained men were asked to convince the audience that they were the right person for the job of their choice. Under both conditions, the panel was presented as experts in nonverbal behavior.

Following the completion, subjects were instructed to rest for 90 minutes until saliva sampling was complete. Saliva sampling occurred immediately before and after stress exposure, there was one sample taken one minute before and samples were taken 10, 20, 30, 45, 60, and 90 minutes after. The samples were stored at -20 degrees Celsius. Participants also completed questionnaires to measure personality characteristics, psychopathological symptoms, self-efficacy, perceived stress, and overtraining. The questionnaires that were included were the Symptom Checklist, State-Trait Anxiety Inventory, Inventory on Competence and Control Belief, Perceived Stress Scale (PSS), Recovery-Stress Questionnaire for Athletes, and the Multidimensional Mood Questionnaire.

The TSST significantly increased the salivary free cortisol in both groups. The cortisol levels did not differ between groups at baseline. The trained men showed lower cortisol responses to the stressor compared with the group of untrained men. The TSST worsened the mood in both groups and significantly worsened the mood of the untrained subjects more. Also, state anxiety significantly increased in both groups but the trained men showed a trend toward lower levels of state anxiety. In terms of calmness, the trained men demonstrated higher levels than the untrained men throughout the entire session. In addition, differences in calmness and state anxiety correlated significantly with an increase in cortisol in the total group of subjects.

Building off of the previous study, in 2008 Rimmele and colleagues performed another similar study. The difference in this experiment is that in addition to elite sportsmen and untrained sportsmen, they included a group of amateur sportsmen. Therefore, this study consisted of 8 elite sportsmen, 50 amateur sportsmen, and 24 untrained men. The participants were recruited by the Swiss Federal Office of Sports, local sports clubs, and through advertisements in newspapers and at local universities. Participants were evaluated on physical fitness tests and self-report questionnaires. This study also used the TSST, which involved public speaking and mental arithmetic in front of two evaluators. Salivary samples were taken to evaluate cortisol level 1 minute before the TSST and 10, 20, 30, 45, 60, and 90 minutes after the TSST as in the previous study. The psychological measures used in this study were the Competitive Index (CI), the Sports Orientation Questionnaire (SOQ), the State-Trait Anxiety Inventory (STAI), and the Multidimensional Mood Questionnaire.

The cortisol and psychological data was analyzed on SPSS using a two-way ANOVA with repeated measurement. The groups did not differ in their perceived stress levels and there was no difference in perceived exertion of the elite athletes and the amateur athletes. The cortisol levels did not differ at baseline but the groups differed significantly in their response to the TSST. The group of the elite sportsmen exhibited the lowest cortisol response. The untrained men and amateur sportsmen had more similar levels with the amateur sportsmen being slightly higher. All groups exhibited the same pattern of reactivity, which involved a peak at approximately 18 minutes after stress exposure followed by a steady decline. The recovery did not differ significantly between groups. State anxiety increased in all groups. However, the highest anxiety was in the untrained men and the lowest in the elite sportsmen. The stress protocol significantly worsened mood in all groups. The highest negative mood and lowest calmness scores were when the subjects were anticipating the stressor. The highest level of competitiveness was found in the elite athletes, however it did not mediate the response to cortisol.

Research Design

Although research has been done with elite athletes, the connection between cortisol and exercise has not been examined in college athletes. Since college athletes need to succeed athletically and academically, their response to academic and social stress would be of interest. The present study obtained salivary cortisol samples from student athletes, students who work out regularly, and sedentary students throughout the semester and during finals period. In addition, salivary samples were collected before and after a social stress test during finals period from each of the participants.

Hypotheses

First, I hypothesize that cortisol levels will be higher during finals for all participants, to reflect the greater perceived stress expressed by students in a preliminary questionnaire, compared with a more relaxed time earlier in the semester. Additionally, I hypothesize that there will be a rise in cortisol after the TSST in comparison to before in the TSST in all participants. In addition, I hypothesize that sedentary students will have the greatest rise in cortisol after the stress test, athletes will have the lowest rise, and students who regularly exercise but are not on a sports team will have a cortisol level in between the other two groups in response to the stress test. Additionally, I hypothesize that these responses to stress will be consistent will the stress levels throughout the semester. I also hypothesize that student athletes will most accurately perceived their stress levels, sedentary students will least regularly perceive their stress levels, and students who regularly exercise but are not on a sports team will have a cortisol level in between the other two groups in accuracy of stress perception.

Methods

Participants

The preliminary questionnaire consisted of 34 males and females whom were freshmen and sophomore college students at a small New England liberal arts school. The follow-up study consisted of 15 college students, which consisted of 12 females and 3 males. In this sample, 5 participants were freshmen and 10 participants were sophomores. None of the students selected for the follow-up study were known to have any major health problems. The Connecticut College IRB approved the procedures.

Materials

A preliminary questionnaire was prepared by the experimenter in order to recruit the proper number of participants for each group (see Appendix A). In order to perform an enzyme immunoassay a kit purchased from Salimetrics, State College, Pennsylvania for measurement of salivary cortisol was used.

Procedure

A preliminary questionnaire was given to 34 students. During the preliminary questionnaire, the participants were informed that if selected, they would be contacted via email by the experimenter if they were selected for the follow-up study. The participants were divided into categories based on their exercise habits as determined by the questionnaire. Participants were then randomly selected in order to have 5 participants in each group.

The selected participants were taught how to properly obtain a salivary sample by the experimenter. These participants took a salivary sample as close to waking as possible and an afternoon sample on two days that they had anticipated being least stressed. Participants were instructed to wait at least two hours after eating to collect a sample. Samples were collected and refrigerated by the experimenter. During finals period, the participants participated in the Trier Social Stress Test (TSST). The schedule is shown in Appendix B. After the participant arrived for the TSST, an initial cortisol sample was taken. After the first sample was taken, the following was read to the participant by the experimenter.

"You will be participating in a 10 minute stress provoking activity. In the first 5 minutes, you will be asked to speak for 5 minutes about why you should be chosen as a Student Advisor next year at Connecticut College. You will be given 5 minutes to prepare this speech and may use scrap paper to prepare but may not use any notes while you are delivering this speech. You will be presenting this speech to two people who have been trained in reading body language and nonverbal cues. Then during the second 5 minutes, you will be doing a different activity, which will be explained after you are done with the speaking portion. During the entire 10 minutes you must remain standing the whole time. Do you have any questions?"

After receiving these instructions and any remaining questions were answered by the experimenter, the participants were given 5 minutes to prepare their speech. They were provided pencil and paper to outline. After 5 minutes, the participant was taken into a different room by the experimenter. First, one of the confederates read, "as you have been told you have 5 minutes to explain to us why you should be chosen as student advisor. You may begin now and we will let you know when it has been 5 minutes." If the subjects finished before the 5 minutes were over, one of the confederates looked down at their watch and told them how much time they had left. If the subject finished a second time before the 5 minutes were over, the confederates waited 20 seconds and then asked prepared questions (see Appendix C). Once the allotted 5 minutes for the speech was up, the participant was asked to serially subtract 13 from 1,022 as fast and as accurately as possible. If the participant failed, the participant had to restart after one of the confederates said "Stop. 1,002." This task continued for 5 minutes total. After, the participant was then brought to a different room by the experimenter. Then one cortisol sample was immediately taken and then another sample was taken 10 minutes later. After the last cortisol sample, the experimenter debriefed the participant and given a debriefing form, which is shown in Appendix E.

After all the samples were collected, an enzyme immunoassay was performed on all salivary samples during finals period and one baseline day from each participant. First, the plate layout was determined (see Appendix D). Next, the non-specific binding wells (NBS), replaced the original wells, in H1 and H2. The original wells were coated with monoclonal antibodies to cortisol, which allows the cortisol in the standards and the unknowns to compete with the cortisol linked with horseradish peroxiadase for the antibody binding sites. Then, 25 uL of the standards, controls, and unknowns was pipetted into the appropriate wells. Additionally, 25 uL of assay diluent was pipetted into the wells G1 and G2 to serve was zero values and well as 25 uL of assay diluent into each NSB well. Next, 15 uL of the conjugate was added to 24 mL of assay diluent and 200 uL of this solution was pipetted into each well using a multichannel pipette. Then, the plate was mixed on a rotator for 5 minutes at 500 rpm and incubated at room temperature for an additional 55 minutes. After this period of incubation, the plate was washed 4 times by pipetting 300 uL of wash buffer into each well and using a plate washer each time. This washing process ensures that the unbound components are washed away. Next, 200 uL of tetramethylbenzidine (TMB) solution was added to each well with a multichannel pipette, which produced a blue color based on the reaction of the peroxidase enzyme with the TMB. The plate was then mixed on a plate rotator for 5 minutes at 500 rpm and incubated in the dark at room temperature for 25 minutes. Next, 50 uL of stop solution was added to each well by a multichannel pipette, which causes a yellow color to form. Then, the plate was mixed for 3 minutes at 500 rpm on the plate rotator. Then, the plate was placed in the plate reader at 450 nm. The intensity of the yellow color is directly proportional to the amount of cortisol present.

The average optical density of the NSB wells was averaged and subtracted from all the other wells. The amount of cortisol present was then determined by the average of the duplicates based on a 4-parameter sigmoid minus curve fit. The data was analyzed using SPSS.

Results

In order to demonstrate the circadian rhythm of cortisol, a paired-samples t-test was performed between AM and PM cortisol values for all participants. As shown in Figure 1, the test revealed that there was a statistically significant difference between AM (M=0.42, SD=0.03) and PM (M=0.22, SD=0.13) cortisol values, $t(14)=6.78$, $p<.05$. Therefore time of day had a significant effect on cortisol level.

The hypothesis that the cortisol levels during the semester would be lowest in the athlete group and highest in sedentary group was not supported. In order to illustrate the differences of AM and PM cortisol between groups, a repeated measures ANOVA was preformed. There was not a significant effect of time of day between AM means for the athlete (M=0.47, SD=0.19), work out (M=0.39, SD=0.4), and sedentary (M=0.40, SD=0.07) groups and the PM mean for the athlete (M=0.23, SD=0.14), work out (M=0.21, SD=0.16), and sedentary (M=0.21, SD=0.12) groups, Wilks' Lambda = 0.93, $F(2,12) = 0.467$, $p=0.638$. These results are illustrated in Figure 2.

In seek of more results, the athlete and work out group were combined into a group labeled "active" and sedentary group was labeled "non-active". A repeated measures ANOVA showed there was no a significant effect of time of day between the AM means for the active (M=0.43, SD=0.14) and the non-active (M=0.40, SD=0.07) group and the PM means for the active (M=0.22, SD=0.14) and the non-active (M=0.22, SD=0.12) group, Wilks' Lambda =0.988, F (1,13) = 0.161, p=0.695. These results are illustrated in Figure 3.

Additionally, the work out group and the sedentary group were combined into a group labeled "non-athlete" and the athlete group retained its original label. A repeated measures ANOVA revealed there was not a significant effect of time of day between AM means for the athlete (M=0.47, SD=0.19) and the non-athlete (M=0.40, SD =0.06) groups and the PM means for the athlete (M=0.23, SD=0.14) and non-athlete (M=0.21, SD=0.14) groups, Wilks' Lamba = 0.928, F (1,13) = 1.00, p=0.335. These results are illustrated in Figure 4. Overall, the early-semester baselines did not significantly differ between the original groups. The early-semester baselines also did not differ between either combination of the newly formed groups.

The hypothesis that cortisol levels would be higher during finals for all participants was supported. In order to show that cortisol levels would be higher during finals period than an early-semester baseline, a paired sampled t-test was performed. The early-semester baseline cortisol value was adjusted for the time of day the participant was scheduled for their TSST testing during finals period because all participants were scheduled for different times of day, therefore either their AM or PM value was chosen based on the time of day they took the TSST. The finals value is an average of all the cortisol values on the TSST day for each participant. As shown in Figure 5, the test revealed that there was a significant difference between the early-semester baseline (M=0.30, SD=0.20) and finals period

(M=0.63, SD=0.35) cortisol values, $t(14)=-4.72$, $p<.05$. Therefore, finals period had an effect on cortisol levels on all participants.

Additionally, to examine the differences between groups a one-way ANOVA was performed on the difference between groups. In order counteract for the differences in times of day, a difference score was calculated between the finals period and early-semester baseline for each group. As shown in Figure 6, a one-way ANOVA illustrated that there was a significant difference between groups, $F(2,12) = 5.54$, $p <.05$. The post hoc comparisons using Tukey HSD test revealed that the mean cortisol value for the athlete group (M=0.51, SD=0.22) significantly differed from the sedentary group (M=0.08, SD=0.09). However, the athlete group did not differ significantly from the work out group (M=0.39, SD=0.28). Also, the work out and sedentary group did not differ significantly from each other.

As shown in Figure 7, the difference scores between the early-semester baseline and finals period cortisol values were compared for the active (M=0.45, SD=0.24) and non-active (M=0.08, SD=0.10) groups. A one-way ANOVA demonstrated that there was a significant difference between groups, $F(1,13) = 10.44$, $p <.05$. As shown in Figure 8, the difference scores between the early-semester baseline and finals period cortisol levels were compared for the athlete and non-athlete groups. A one-way ANOVA illustrated that there was not a significant difference between the athlete (M=0.51, SD=0.22) and non-athlete (M=0.24, SD=0.25) groups, $F(1, 13) = 4.22$, $p=0.61$. Therefore, significant differences were seen when comparing the athlete and sedentary group and the active and non-active group.

The hypothesis that cortisol levels would be higher after the TSST compared to before the TSST for all participants was not supported. As shown in Figure 9, the paired-samples t-test comparing Pre TSST with Post 1 TSST illustrated that there was a significant difference but in the opposite direction than expected, t (14) = 4.01, p<.05. The mean for Pre TSST (M=0.67, SD=0.35) was higher than the mean for Post 1 TSST (M=0.58, SD=0.34). As also shown in Figure 9, the paired-samples t-test comparing Pre TSST with Post 2 TSST (M=0.63, SD=0.38) demonstrated that there was not a significant difference, t (14)= 0.77, p=0.46. Therefore, cortisol either increased or remained the same after the TSST instead of the expected increase.

The hypothesis that the comparison of cortisol before and after the TSST would differ among groups with athlete group having the least difference and sedentary group having the largest difference was not supported. Difference scores comparing the Pre TSST to Post TSST were examined to determine if the TSST manipulation had a greater effect on the sedentary students compared to the exercising students. A one-way ANOVA demonstrated that there was not an effect of exercise in the comparison of Pre TSST and Post 1 TSST difference scores between the athlete (M=-0.12, SD=0.11), work out (M=-0.12, SD=0.05) and sedentary (M=-0.05, SD=0.11) groups, F(2,12)=.99, p=.40. An ANOVA was also performed on the difference between Pre TSST and Post 2 TSST. These difference scores between the athlete (M=0.0260, SD=0.22), work out (M=-0.09, SD=0.29), and sedentary (M=-0.06, SD=0.09) groups were also not significant, F (2,12)=.38, p=.69. These results are illustrated in Figure 10.

With the data re-grouped into active and non-active, the one-way ANOVA was repeated. There were not significant differences between Pre TSST and Post 1 TSST between the active (M=-0.12, SD=0.08) and non-active (M=-0.05, SD= 0.10) groups, F(1,13)=2.14, p=.17. In addition, there were not significant differences between active (M=-0.03, SD=0.25) and non-active (M=0.06, SD=0.09) groups compared between Pre TSST and Post 2 TSST, F(1,13)=.076, p=.79. These results are illustrated in Figure 11.

The same one-way ANOVA was performed with the athlete and non-athlete groups. The comparison between Pre TSST and Post 1 TSST between the athlete (M=-0.12, SD=0.11) and non-athlete (M=-0.08, SD=0.086) group was not significant, F(1,13)=.510, p=.49. The contrast of Pre TSST and Post 2 TSST between the athlete (M=0.03, SD= 0.22) and non-athlete (M=-0.07, SD=0.20) groups was also not significant, F (1,13)=.779, p=.39. These results are shown in Figure 12. Therefore, there was no difference in any combination of groups in their overall cortisol response to the TSST.

In order to determine if there was a difference between the early-semester baseline levels and levels before and after the stress test, several one-way ANOVAs were performed. First, a one-way ANOVA was performed to compare the differences between Pre TSST and the early-semester baseline between groups. There was a significant difference between groups, F (2,12)=4.38, p<.05. Post hoc comparisons using Tukey HSD test indicated that the mean difference score of the athlete group (M=0.54, SD=0.17) was significantly higher than the mean difference score of the sedentary group (M=0.12, SD=0.13). There was not a significant difference between athlete group and the work out group (M=0.46, SD=0.35) or between the work out group and the sedentary group. Another one-way ANOVA was performed to assess the difference between Post 1 TSST and the early-semester baseline between the athlete (M=0.42, SD=0.21), work out (M=0.34, SD=0.37) and sedentary (M=0.07, SD=0.12) groups. There was not a significant difference between these groups, F(2,12)=2.61, p=.11. An additional one-way ANOVA was performed comparing the difference between the Post 2 TSST and early semester baseline. There was a significant difference between groups, F(2,12)=8.02, p<.05. Post hoc comparisons using Tukey HSD test indicated that the mean difference of the athlete group (M=0.57, SD=0.30) was significantly higher than the sedentary group (M=0.06, SD=0.7). However, there was not a significant difference between the work out (M=0.37, SD=0.17) and athlete group or the work out and sedentary group. These results are summarized in Figure 13.

The data was regrouped into the active and non-active groups and the same ANOVAs were performed. The first one-way ANOVA compared the Pre TSST baseline with early semester baseline and a significant difference was found between the active (M=0.50, SD=0.27) and non-active (M=0.12, SD=0.13) groups, F(1,13)=8.94, p<.05. The next ANOVA compared the Post 1 TSST with early semester baseline and there was a significant difference between active (M=0.38, SD= 0.29) and non-active (M=0.07, SD=0.12) groups, F(1,13)=5.28, p<.05. The following ANOVA compared Post 2 TSST with early semester baseline and there was a significant difference between active (M=0.47, SD=0.25) and non-active (M=0.06, SD=0.07) groups, F(1,13)=12.53, p<.05. These results are summarized Figure 14.

In addition, the data was also regrouped into the athlete and non-athlete groups and the same ANOVAs were performed, which are shown in Figure 15. The first one-way ANOVA compared the Pre TSST with early-semester baseline, there was not a significant difference between the athlete (M=0.54, SD=0.17) and non-athlete (M=0.29, SD=0.31) groups, F(1,13)=2.82, p=.12. The next ANOVA compared the Post 1 with early-semester baseline and there was not a significant difference between the athlete (M=0.42, SD=0.21) and non-athlete (M=0.20, SD=0.30) groups, F (1, 13)=2.10, p= .17. The next ANOVA compared Post 2 with early-semester baseline and there was a significant difference between the athlete (M=0.57, SD=0.30) and non-athlete (M=0.22, SD=0.20) groups, F (1,13)=7.20, p<.05. Therefore, there were the most significant differences when the active and non-active groups early-semester baseline and TSST cortisol values were compared.

The hypothesis that athletes would most accurately perceive and the no workout group would least accurately perceive their stress levels was partially supported. All of the perceived stress levels are summarized on Figure 16. As shown in Table 1, there are no significant Pearson correlations between cortisol levels and perceived stress when all participants are considered together. Next, these correlations between cortisol and perceived stress were preformed by groups, which is shown in Table 2. There was significance for the athletes in Pre TSST, r (3)=-.81, p<.05. There was also significance in the workout group at Post 2 TSST, r (3)=.82, p<.05. As shown in Table 3, the data was regrouped into the active and non-active groups. No significance was shown in these correlations. The data was again

Table 1

Correlation Between the Cortisol Level and Perceived Stress Level

Cortisol	AM	PM	Pre TSST	Post 1 TSST	Post 2 TSST
			(n = 15)		
Perceived Stress					
AM	-.055	-	-	-	-
PM		.122	-	-	-
Pre TSST			-.121	-	-
Post 1 TSST				-.097	-
Post 2 TSST					-.222

* Correlation is significant at the 0.05 level (1-tailed)

Table 2

Correlation Between the Cortisol Level and Perceived Stress Level by Group

Cortisol	AM	PM	Pre TSST	Post 1 TSST	Post 2 TSST
		Athletes (n = 5)			
Perceived Stress					
AM	-.037	-	-	-	-
PM		-.445	-	-	-
Pre TSST			-.810*	-	-
Post 1 TSST				-.651	-
Post 2 TSST					-.353
		Work Out (n = 5)			
Perceived Stress					
AM	.398	-	-	-	-
PM		.224	-	-	-
Pre TSST			-.443	-	-
Post 1 TSST				.126	-
Post 2 TSST					.822*
		No Work Out (n = 5)			
Perceived Stress					
AM	.093	-	-	-	-
PM		-.745	-	-	-
Pre TSST			.437	-	-
Post 1 TSST				.734	-
Post 2 TSST					.681

* Correlation is significant at the 0.05 level (1-tailed)

Table 3

Correlation Between the Cortisol Level and Perceived Stress Level by Group (Active vs. Non-Active)

Cortisol	AM	PM	Pre TSST	Post 1 TSST	Post 2 TSST
		Active (n = 10)			
Perceived Stress					
AM	-.009	-	-	-	-
PM		-.236	-	-	-
Pre TSST			-.184	-	-
Post 1 TSST				-.347	-
Post 2 TSST					-.156
		Non-Active (n = 5)			
Perceived Stress					
AM	-.702	-	-	-	-
PM		-.745	-	-	-
Pre TSST			.437	-	-
Post 1 TSST				.734	-
Post 2 TSST					.681

* Correlation is significant at the 0.05 level (1-tailed)

regrouped into the athlete and non-athlete groups and the correlations were performed. No new significant correlations were found, which is shown in Table 4.

Table 4

Correlation Between the Cortisol Level and Perceived Stress Level of by Group (Athlete vs. Non-Athlete)

Cortisol	AM	PM	Pre TSST	Post 1 TSST	Post 2 TSST
		Athlete (n = 5)			
Perceived Stress					
AM	-.037	-	-	-	-
PM		-.445	-	-	-
Pre TSST			-.810*	-	-
Post 1 TSST				-.651	-
Post 2 TSST					-.353
		Non-Athlete (n = 10)			
Perceived Stress					
AM	-.120	-	-	-	-
PM		-.286	-	-	-
Pre TSST			-.232	-	-
Post 1 TSST				.407	-
Post 2 TSST					.520

* Correlation is significant at the 0.05 level (1-tailed)

Discussion

This study presented some results that were very different than expected, but proved to be of interest nonetheless. The present study demonstrated the circadian rhythm of cortisol for all participants. Since there were significant differences overall and within each group, the difference in the AM and PM levels is a global response not exclusively correlated with any particular group. As also shown by Levine and colleagues (2006), salivary cortisol was highest in the AM sample and decreased significantly by time of the PM sample, which is consistent with the overall pattern of cortisol activity. The difference between the AM and PM levels is evidence of a healthy pattern of cortisol activity in the participants of this study. Therefore, the participants in this study have a HPA axis that accurately releases cortisol appropriately based on time of day.

The hypothesis that the cortisol levels during the semester would be lowest in the athletes and highest in the sedentary group was not supported in the present study. There was no difference shown between the groups or when the groups were regrouped into active and non-active. This suggests that there are not differences in cortisol levels during periods of relation between groups based on level of physical activity.

The hypothesis that cortisol levels would be higher for all participants during finals period compared to an early-semester baseline day was supported. Since there were significant differences overall and within each group, the rise in cortisol is therefore a global response not exclusively correlated with any particular group. This result supports previous research that salivary cortisol is released more readily during periods of stress compared to less stressful periods. Therefore, salivary cortisol is an appropriate biomarker to measure stress in humans. This allows researchers and health care providers to use salivary cortisol levels to determine if a person is appropriately responding to stress.

However, the athlete group had a significantly larger cortisol difference between the early-semester baseline and finals period compared to sedentary groups. Although this is opposite to what was expected, it might illustrate the phenomenon of optimal stress levels. As illustrated by Arnsten (2009), the optimal stress level is the peak level of stress in the human body in which dopamine and norepinephrine are released in the prefrontal cortex so that optimal performance can be achieved. If stress levels or cortisol levels are higher or lower than this ideal level, optimal performance is inhibited. Therefore, this difference in cortisol levels could be a positive response, which promotes the athletes to perform during finals period to their optimal ability by releasing neurotransmitter that help with cognition and working memory. This could be a learned physiological response to stressors, which athletes more readily experience since they are more often involved in competitive activities. This theory is also validated by the fact that during periods of non-stress the cortisol levels did not differ between groups, suggesting that athlete’s cortisol levels changed in response to stressor of final exams.

The hypothesis that cortisol would rise in all participants after the TSST compared to before the TSST was not supported. Also, the hypothesis that the comparison of cortisol before and after the TSST would differ among groups with athletes having the lowest cortisol difference between before and after the TSST and the sedentary group having the highest cortisol difference was not supported. The analysis of the difference scores between Post 1 and Pre TSST and Post 2 and Pre TSST did not only show any differences between groups but also did not cause an increase in cortisol in any groups. After regrouping, there were still no significant results. These results are contrary to Rimmele and colleagues (2008) but similar to Vale and colleagues (2009). Since there was a rise in cortisol overall during finals period but not after the stressor, it could be suggested that finals period was a larger source of stress for the participants than the TSST. It is also possible that the anticipation of the stressor was a larger source of stress than the stressor itself. Since, cortisol level dropped after the stressor it can be suggest that the participants anticipation having to do something stressful increased cortisol and once they found out what the test consisted of, there cortisol decreased.

There were significant differences between the groups between the early-semester baseline and Pre TSST and the early-semester baseline and Post 2 TSST. However, the differences in cortisol were also contrary to what was expected. Instead of the athletes being the least stressed in comparison of the early-semester baseline and the stressor they had the largest difference in cortisol levels. The athletes were followed by the workout group, which had the next largest difference scores, who were then followed by the no workout group which had the smallest difference scores. These results are consistent with the optimal stress theory and follow the same pattern of the cortisol level during finals period overall.

The hypothesis that athletes would most accurately perceive and the sedentary group would least accurately perceive their stress levels was partially supported. Since there were no significant correlations overall, there was not a global accuracy of perception of cortisol levels at any point in time. In addition, there were no significant correlations with the sedentary group at any point in time. Thus, illustrating the sedentary group did not have the ability to accurately determine their actual stress. It is possible that this could relate to the optimal stress level pervious discussed. Perhaps that since the sedentary group could not accurately predict their stress level, they also do not have the ability to physiologically achieve their optimum stress level, which could be due to a lack of competitive situations compared to athletes. In contrast, the athlete group and workout group each accurately predicted their stress levels on one occasion. The athlete group accurately predicted their stress levels before the TSST. This could also be related to their optimal stress level. Since athletes often take part in competitive activities, perhaps they are more aware of how to physiologically achieve the level of optimum stress and therefore more accurate at predicting their stress level before a stressful event because they try to achieve this optimal level more often. In addition, the workout group accurately predicted their stress level 10 minutes after the conclusion of the social stress test. Since their differences from the baseline were only slightly lower than the athlete group perhaps the workout group is also good at achieving their optimal stress level. Even though they are not on a sports team, they might have competitive workouts with friends or compete against their own times when working out. Also, these participants could have been athletes before college, therefore have experience a large amount of athlete competition in their lives. Perhaps these students were good at predicting their stress levels after the stress test because they are used to achieving their optimal stress level after exercising and anticipating studying, which is similar to the situation after the stress test during finals. This group of participants must be able to manage their schoolwork and personal workouts. Therefore, even if not on a sports team, working out might be beneficial to have students more accurately predict their stress levels after a period of stress and in anticipation of more stress.

This study had several limitations. The most relevant limitation is the small sample size. Since there were only 5 participants in each group, it is very difficult to make any generalizations. It also makes it very difficult to exclude any subjects. More participants would have given a more complete picture of the difference in cortisol between groups. There also were only 3 males in the entire sample. More males and an equal amount of males and females could have increased the reliability of this study. This is especially relevant because as demonstrated by Kudielka, Hellhammer, and Wust (2008) and Salvador (2005) cortisol is higher in males than in females. Therefore, having two males in the athlete group and no males in the workout group could have affected the results. However, these levels were not significantly higher than the females in the athlete group, so maybe there was not an effect of gender. Also, the confederates in the study were female college students. These students were possibly not intimidating enough to the participants since they were also college students.

There are many changes that could be made for future research. In addition to having more participants overall, there could be a more drastic difference between groups. For example, the sedentary group could work out less than two days a week perhaps even zero. The present study did not differentiate between in-season and out-of-season athletes. Future research could make different groups for in-season and out-of-season athletes or limit the study to only in-season athletes. Also, the current workout group contained participants who worked out 5-7 days a week. Future research could only include students who workout over 6 days a week, so this group is more similar to the athlete group. Also the present study did not ask the workout group the length of time of their exercise. Future research could also only include students in the work out group who workout over 2 hours a day to also more closely simulate the athlete group. Increasing the specifications of each group has the possibility of increasing accuracy of the results.

In addition to changing the specificity of the groups, several other changes could be made. Changing the order of the participants on test day could allow for comparisons between groups before computing the difference score, instead of not being able to use the original test day data. Either the schedule could be altered by alternating between each group for the order of participants or each group could be run at the same time on consecutive days. Additionally, more cortisol samples could be taken after the stress test. If more samples were taken, then it would be possible to see if the athlete group returns to their baseline level more quickly than the other groups even though there was a bigger increase. This idea would support the optimal stress level theory because if the athlete group was able to return to their baseline quicker then it would support the idea that they have more control over their stress level than the other groups and more of an ability to achieve their optimum stress level in comparison to the other groups. In addition, future research could collect more cortisol sample over a longer period of time in order to achieve a more accurate representation of the actual cortisol levels of each participant. It would also have been nice to have more information about the participants, to be able to address how factors such as competitive personality and coping styles relate to the cortisol levels.

The most prominent implication of this research is that the cortisol response pattern to stress is much more complicated than only comparing the amount of increase in cortisol in response to a particular stressor, which is the common theme in the current literature. Cortisol is not that simple. There are many more factors to consider such as how well someone can predict their own stress, how much a person's cortisol changes throughout the day, and how long it takes a person to return to their baseline after responding to a stressor. Factors such as how long a person is in a stressful state should also be considered. Therefore, an increase in cortisol is not a response anyone should try to avoid but rather an adaptive mechanism that should be embraced to help achieve optimal performance.

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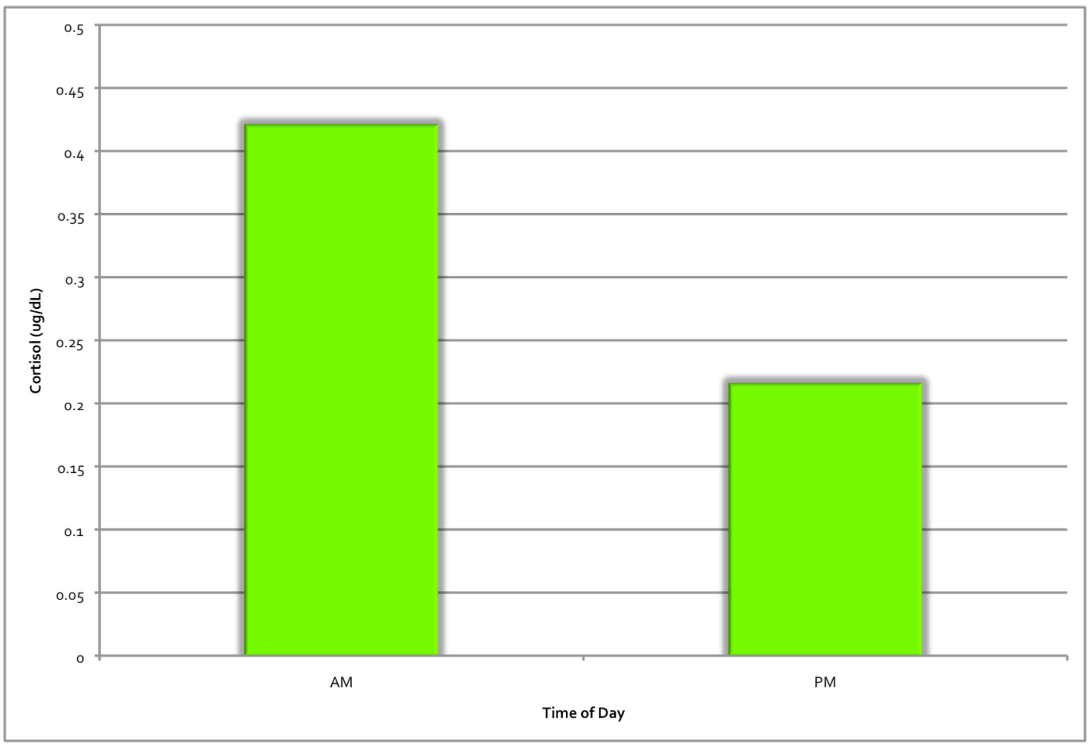

Figure 1. Cortisol (*ug*/dL) means as a function of time of day for all participants.

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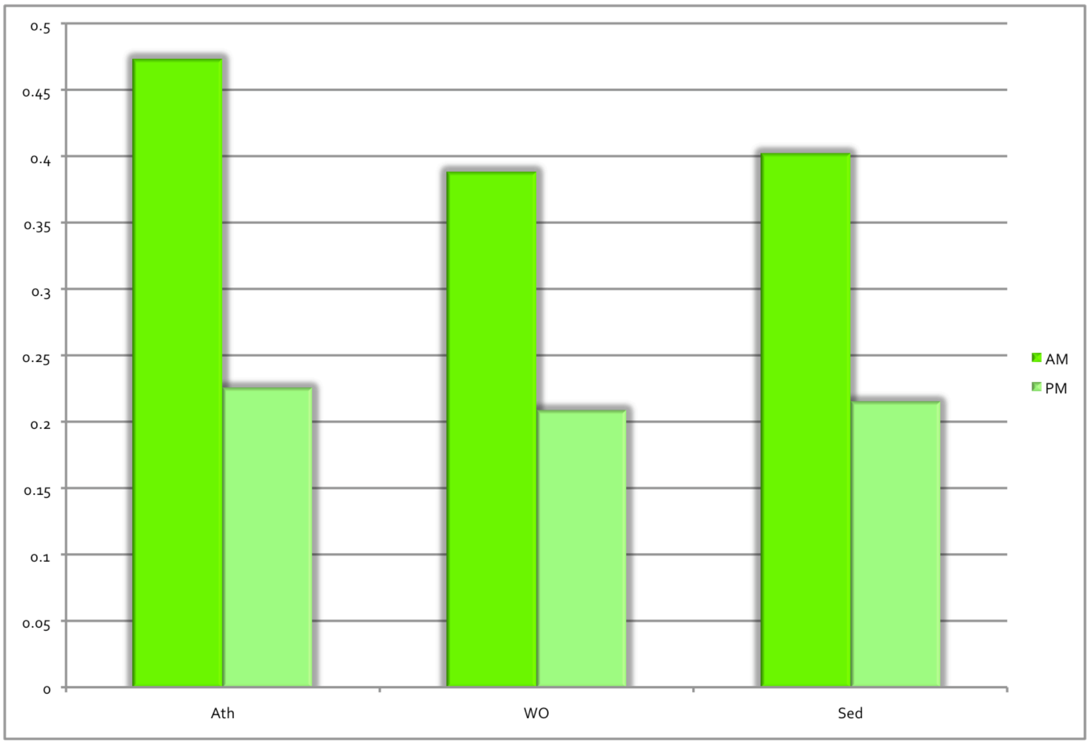

Figure 2. Cortisol (*ug*/dL) means as a function of time of day for the athlete, workout, and no work groups.

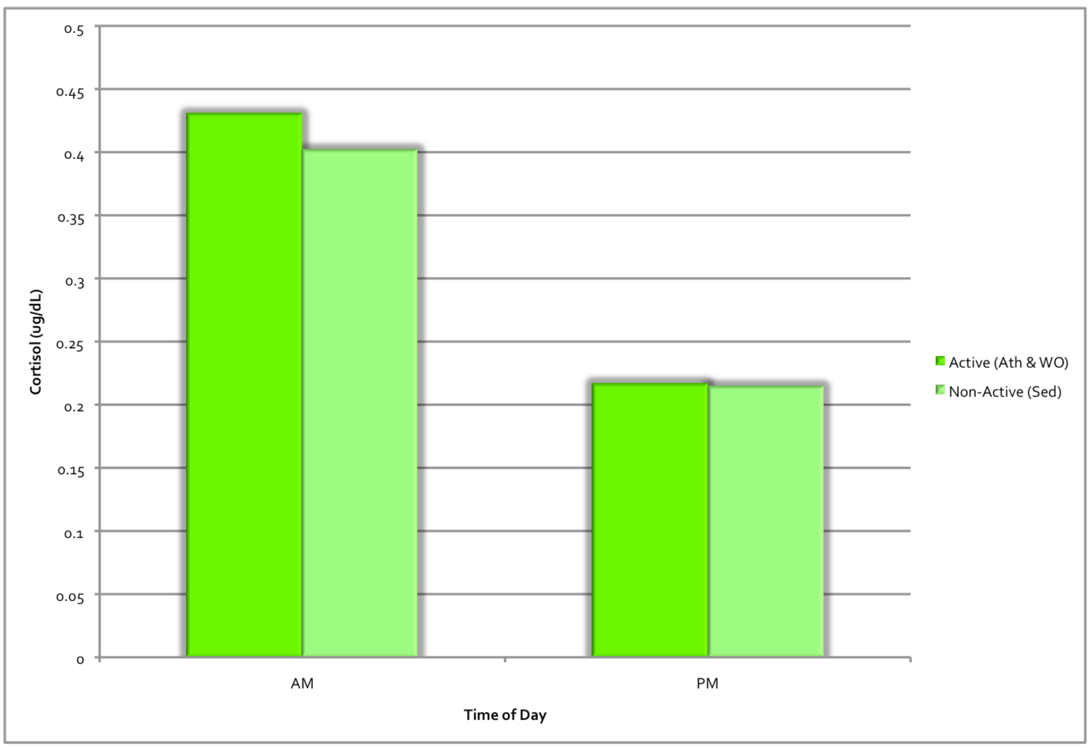

Figure 3. Cortisol (*ug*/dL) means as a function of time of day for the active and non-active groups.

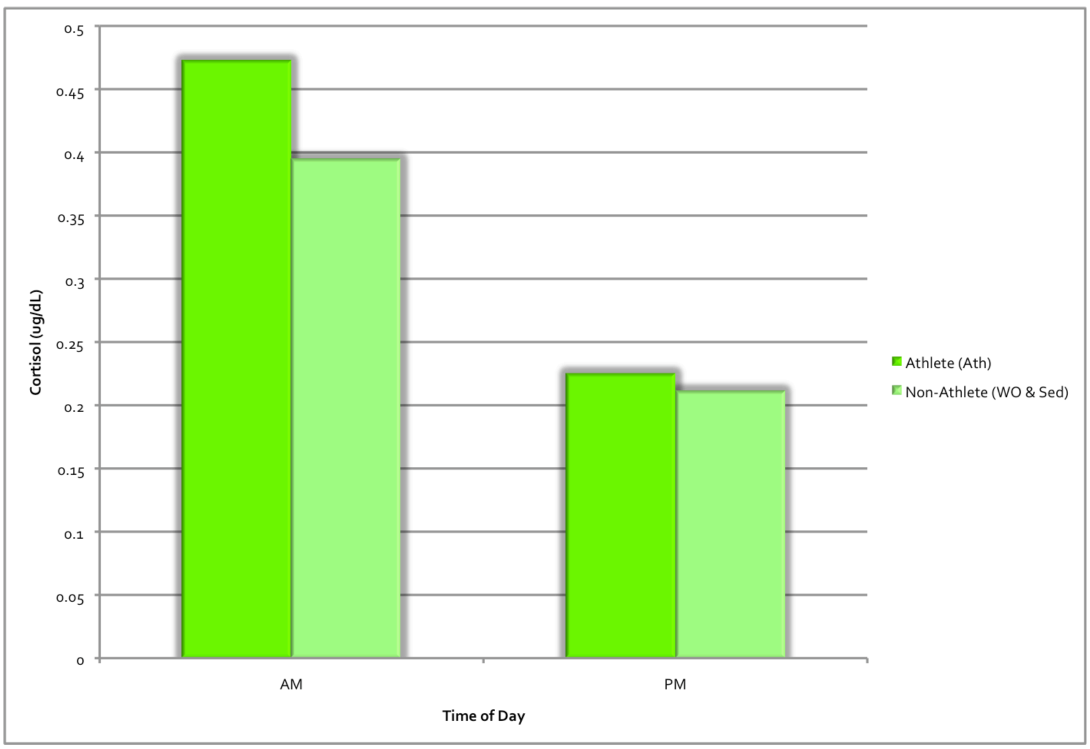

Figure 4. Cortisol (*ug*/dL) means as a function of time of day for the athlete and non-athlete groups.

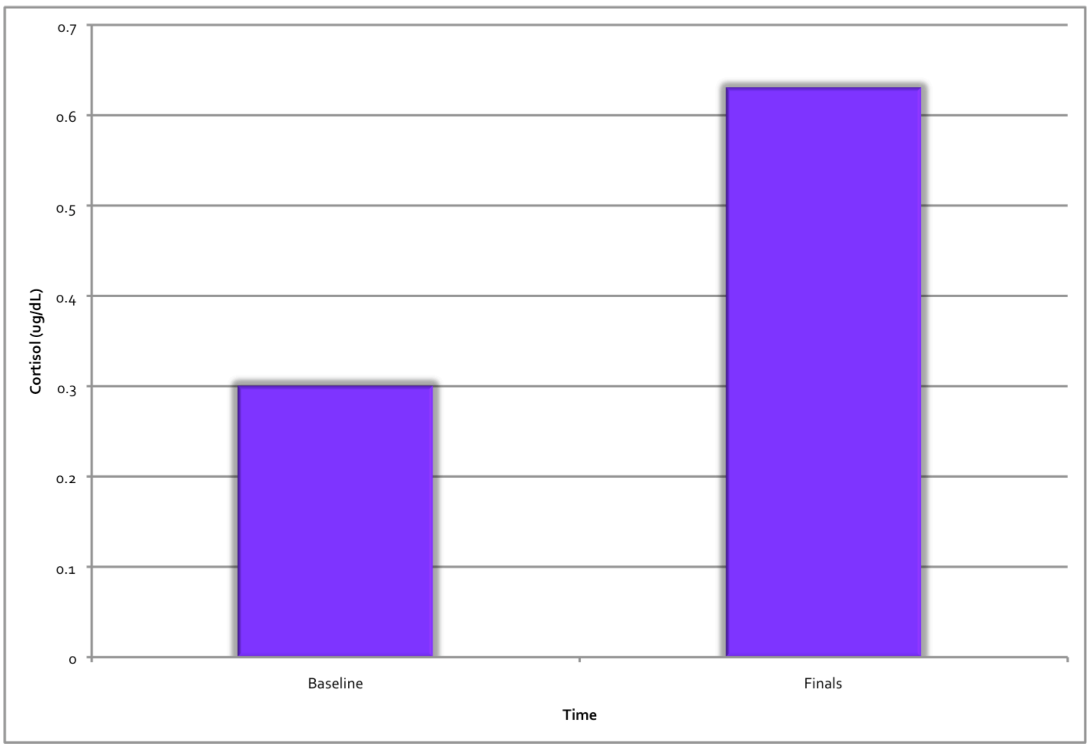

Figure 5. Cortisol (*ug*/dL) means as a function of time for all participants.

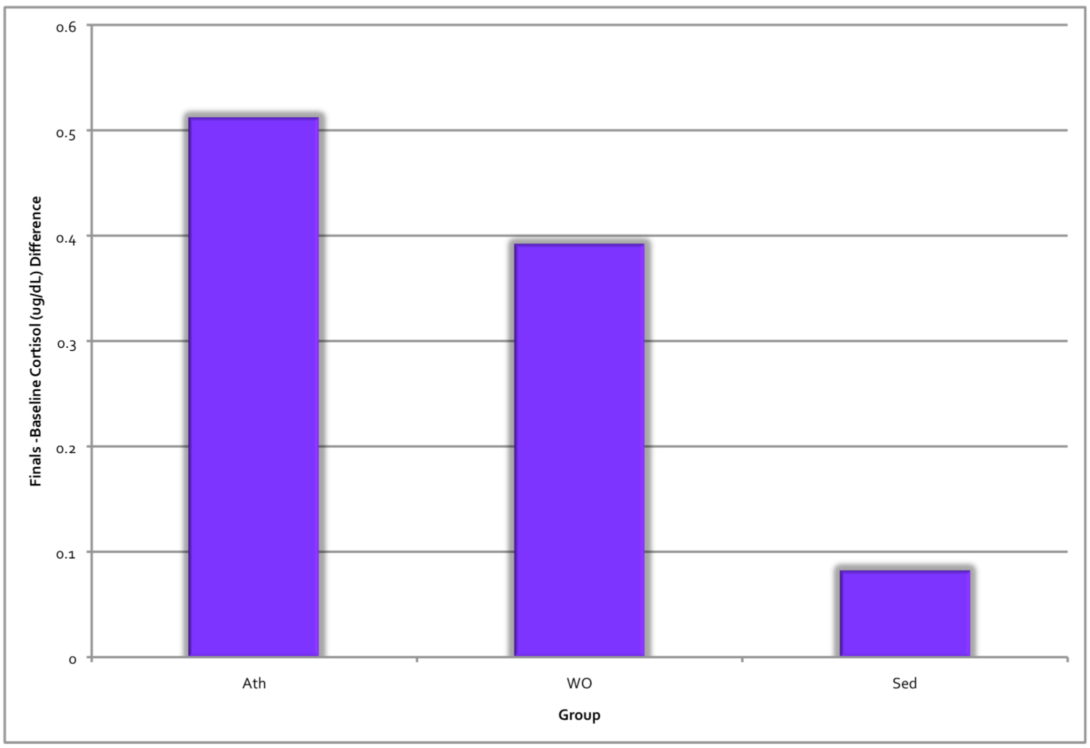

Figure 6. Finals – Baseline Cortisol (uh/dL) Difference between the athlete, work out and sedentary groups.

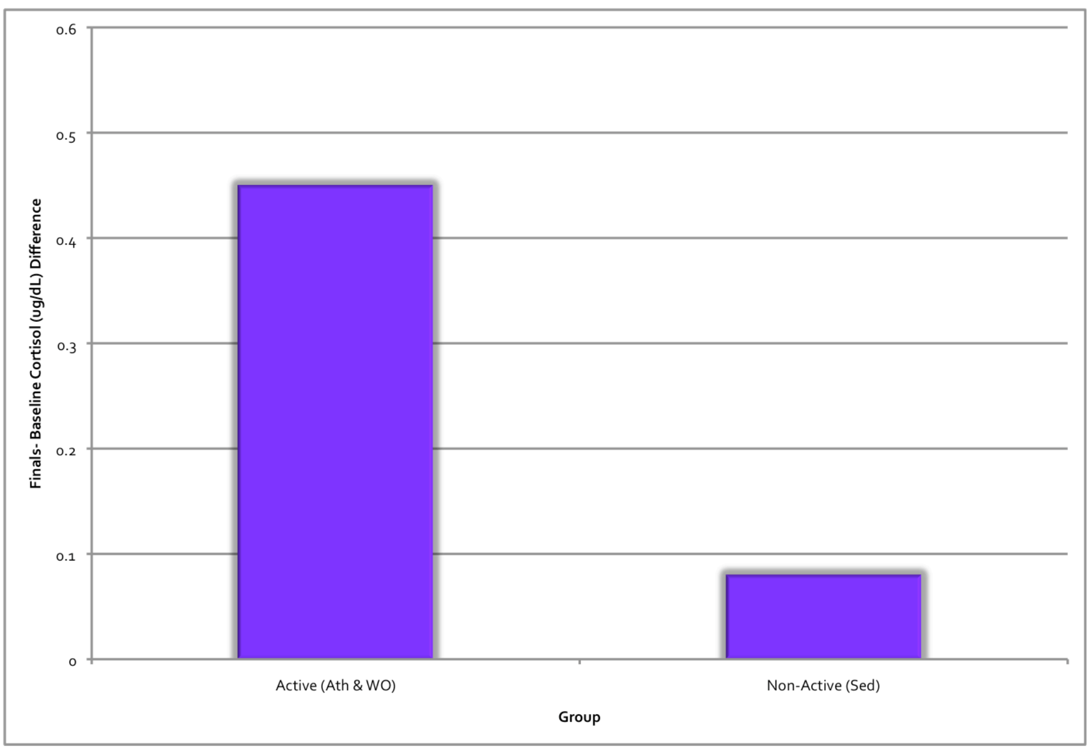

Figure 7. Finals – Baseline Cortisol (uh/dL) Difference between the active and non-active groups

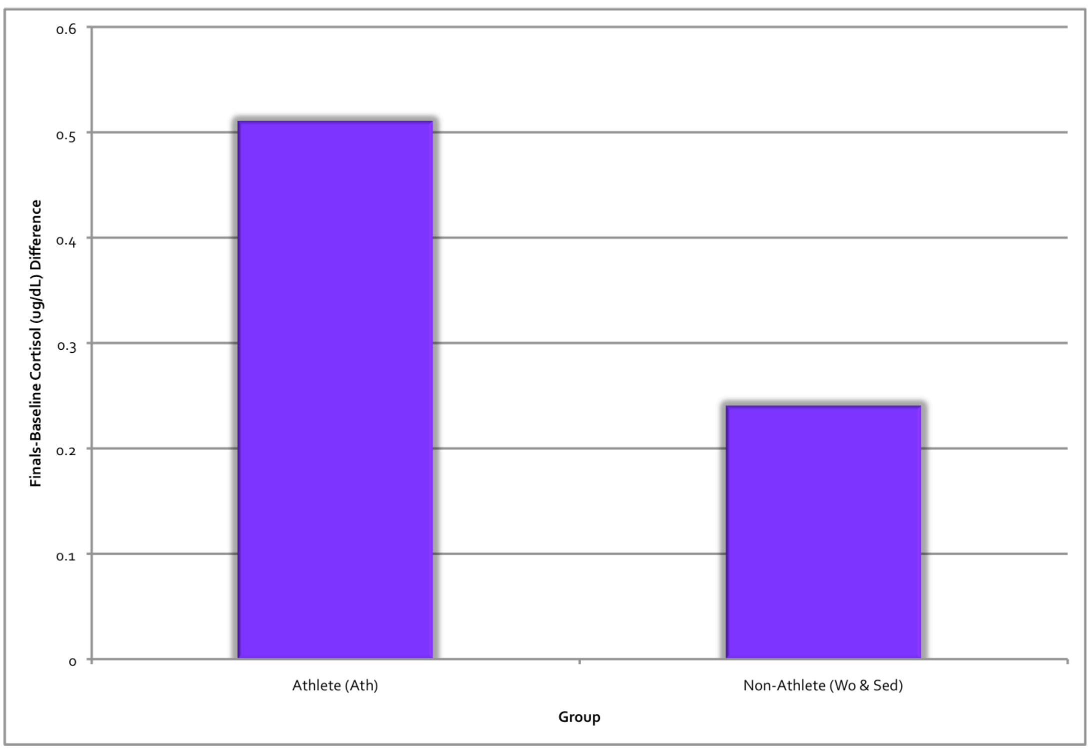

Figure 8. Finals – Baseline Cortisol (uh/dL) Difference between the athlete and non-athlete groups

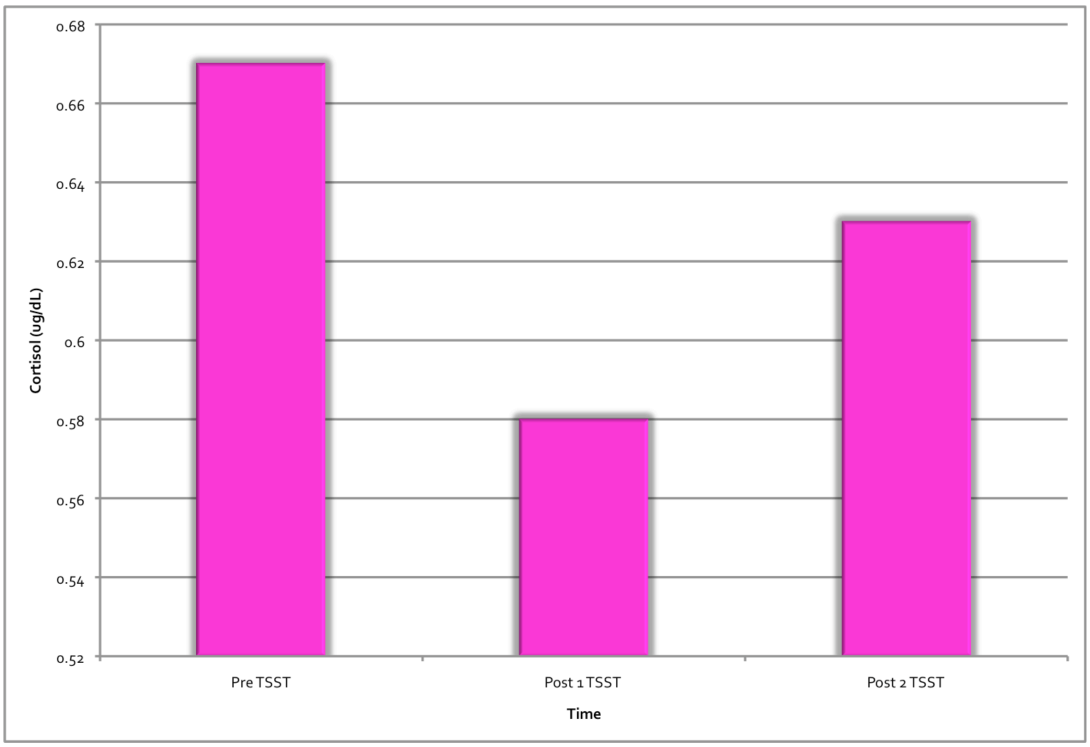

Figure 9. Cortisol (ug/dL) as a function of time in relation to TSST.

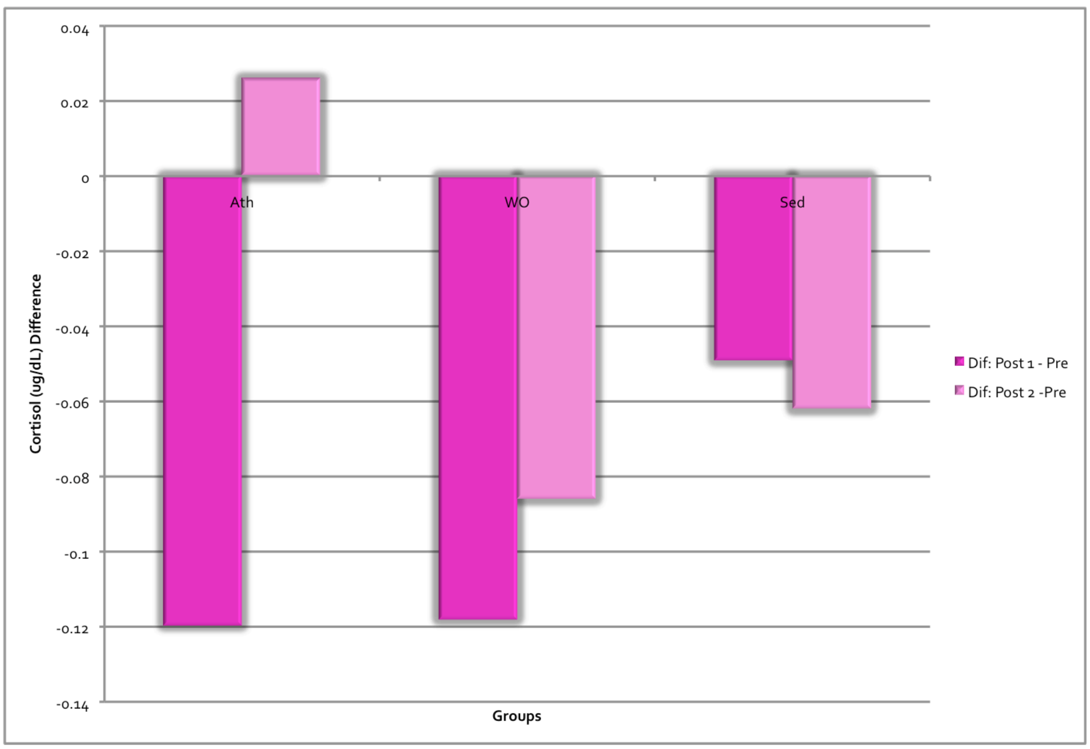

Figure 10. Cortisol (*ug*/dL) difference scores between Post 1 and Pre TSST and Post 2 and Pre TSST as a function of the athlete, workout, and no workout group.

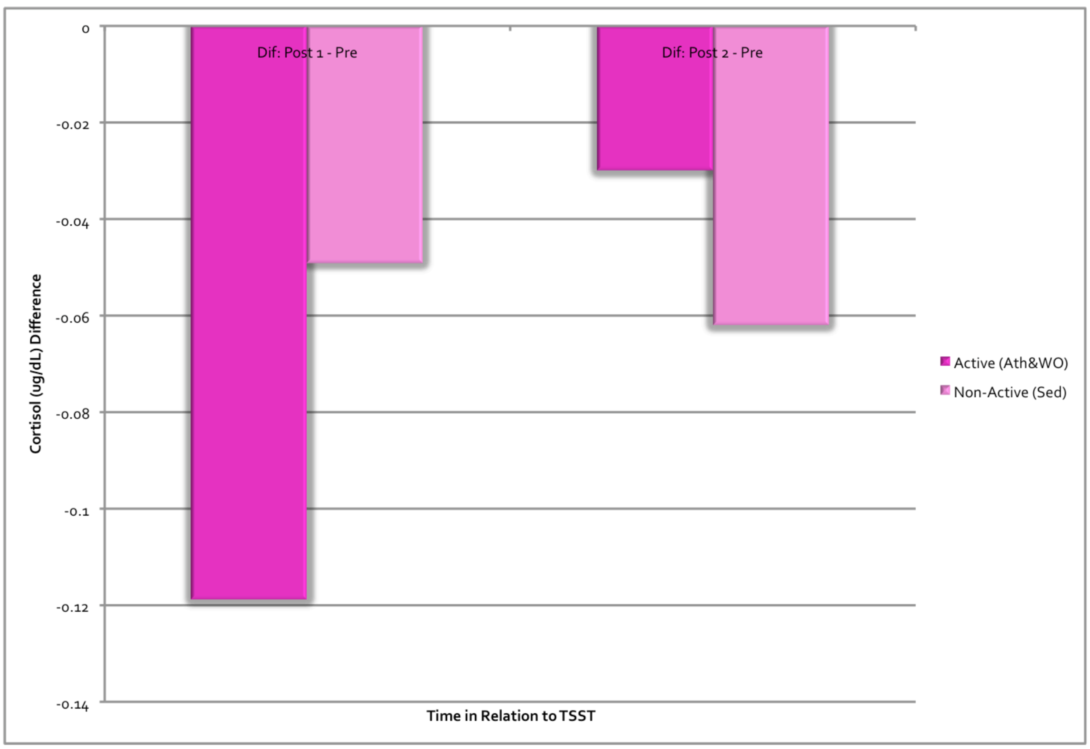

Figure 11. Cortisol (*ug*/dL) difference scores between Post 1 and Pre TSST and Post 2 and Pre TSST as a function of the active and non-active groups.

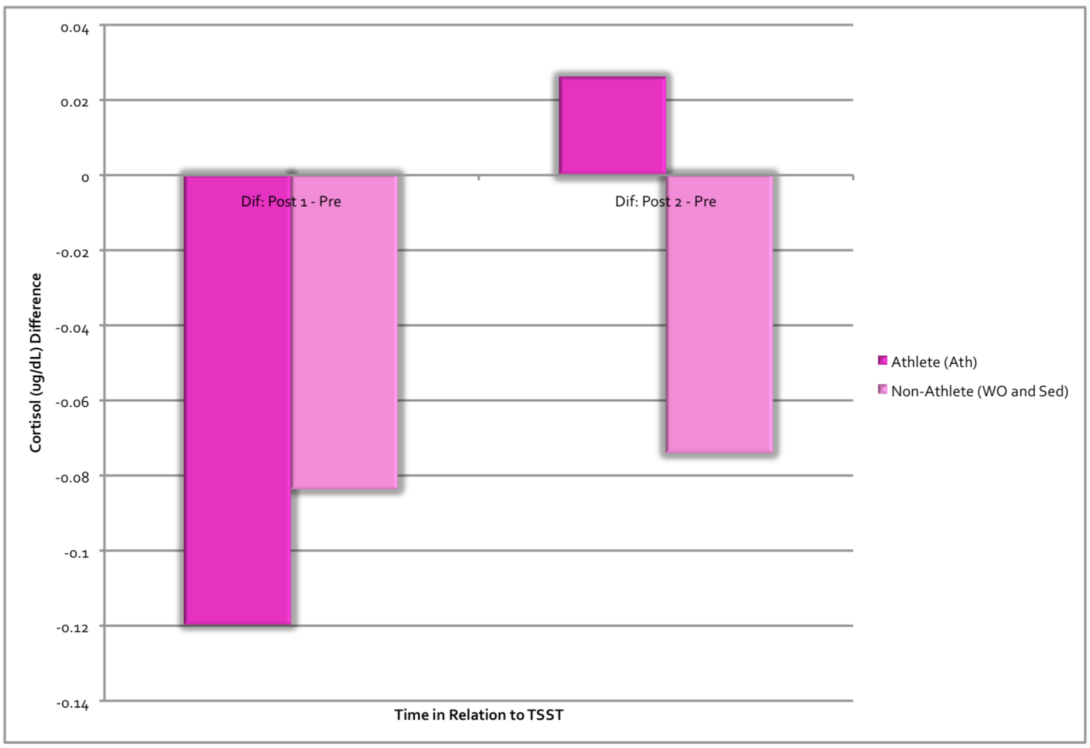

Figure 12. Cortisol (*ug*/dL) difference scores between Post 1 and Pre TSST and Post 2 and Pre TSST as a function of the athlete and non-athlete groups.

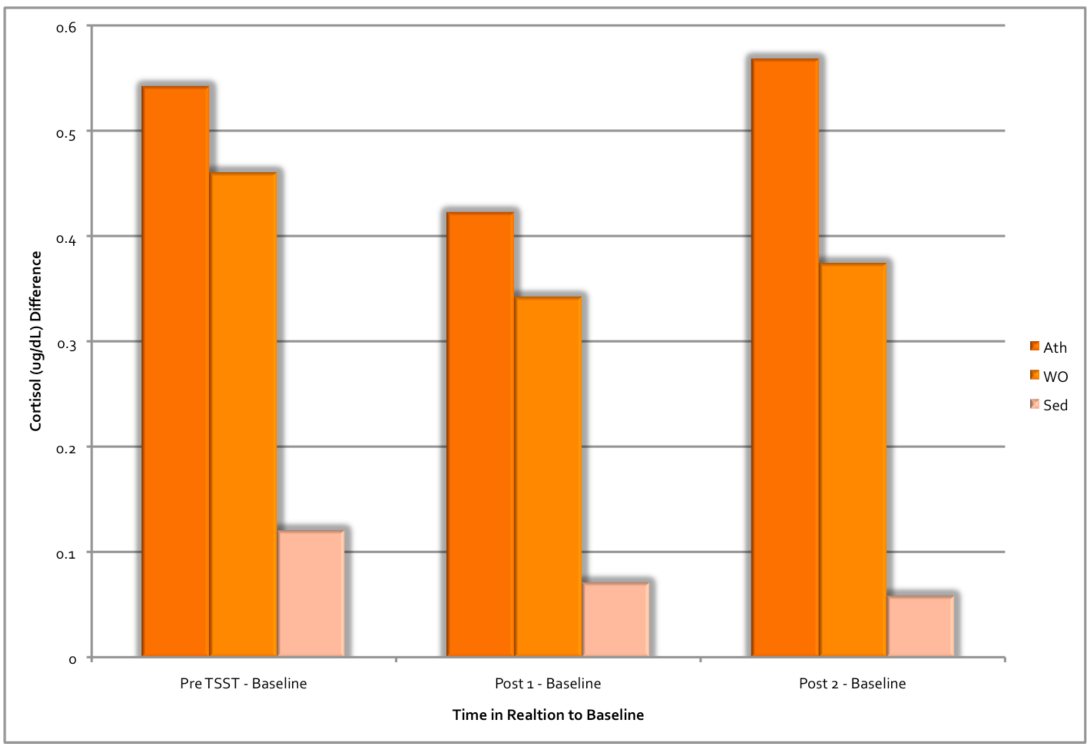

Figure 13. Cortisol (*ug*/dL) difference scores between Pre TSST and Baseline, and Post 1 and Baseline, and Post 2 and Baseline as a function of the athlete, workout, and no workout groups.

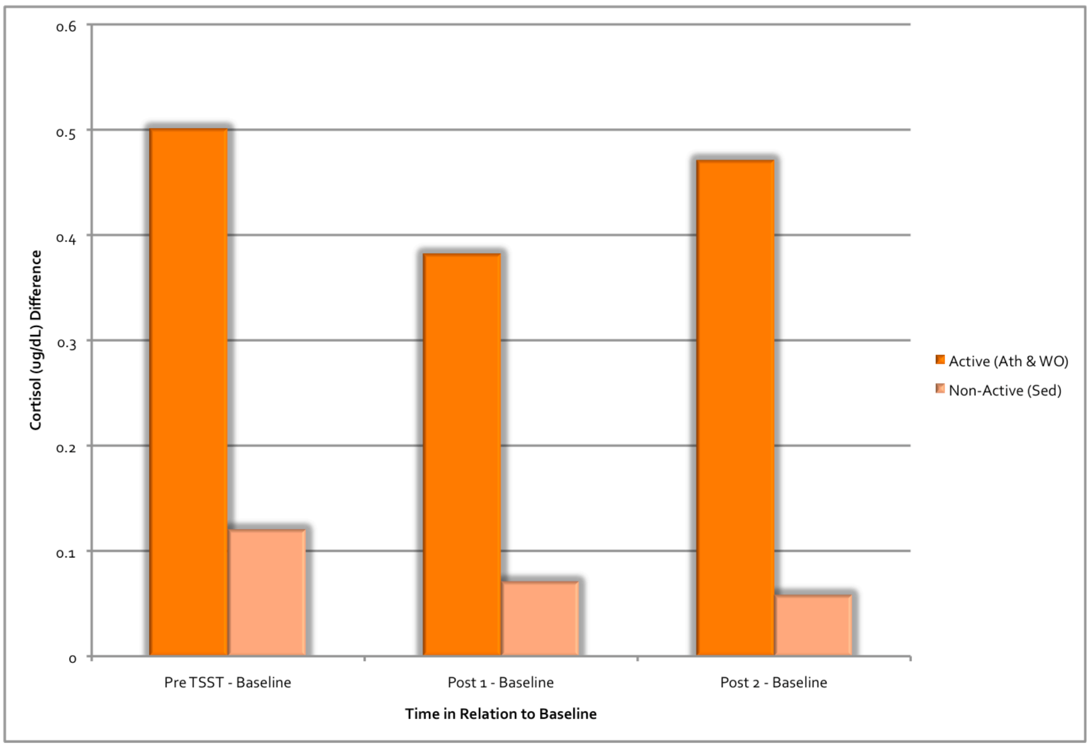

Figure 14. Cortisol (*ug*/dL) difference scores between Pre TSST and Baseline, and Post 1 and Baseline, and Post 2 and Baseline as a function of the active and non-active groups.

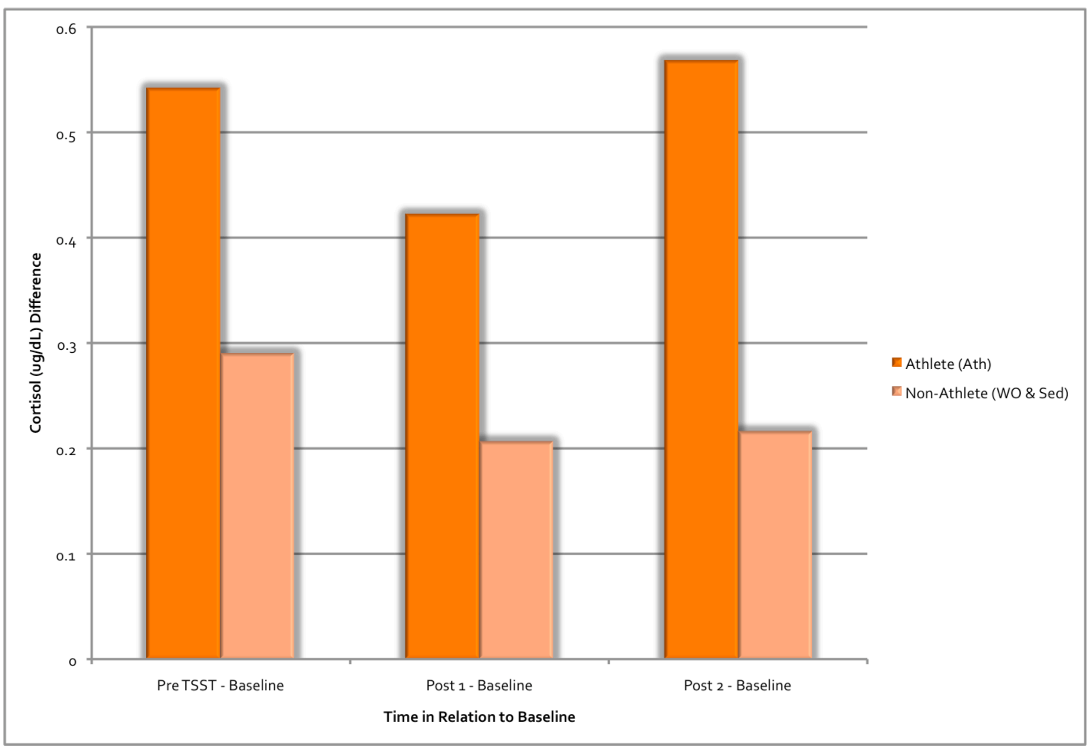

Figure 15. Cortisol (*ug*/dL) difference scores between Pre TSST and Baseline, and Post 1 and Baseline, and Post 2 and Baseline as a function of the athlete and non-athlete groups.

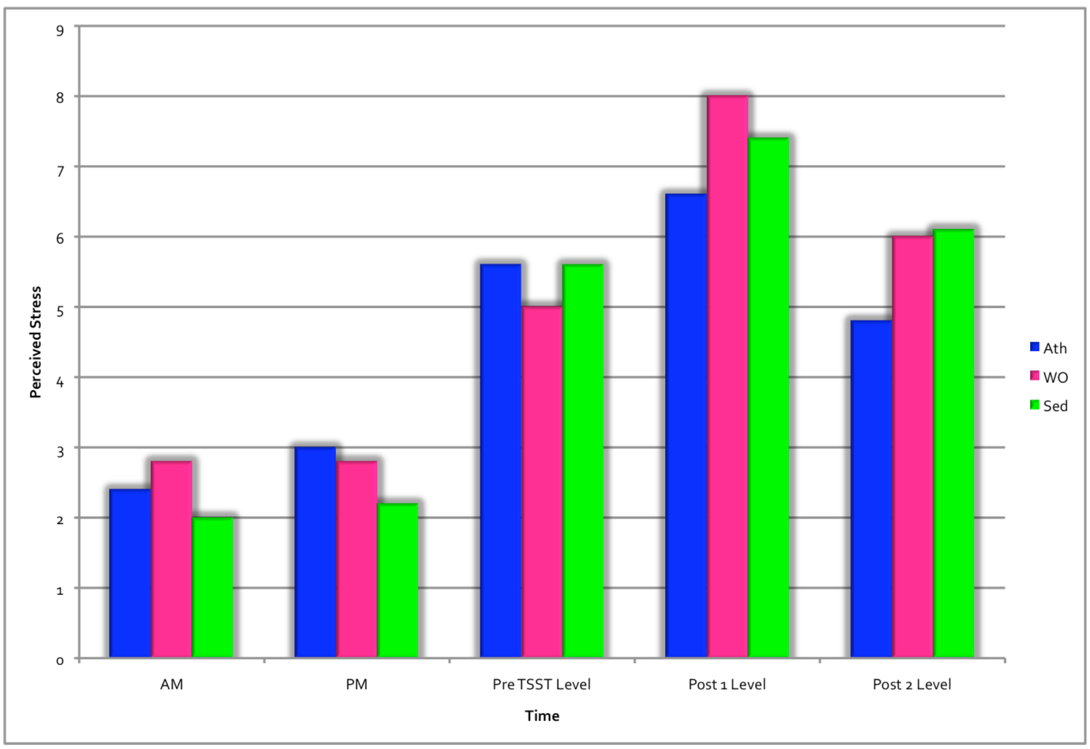

Figure 16. Perceived stress levels as a function of time for the athlete, workout, and no workout groups.

Appendix A

I hereby consent to participate in Rita Holak's research about stress levels in college students.
I understand that this research will involve completing a brief questionnaire.
While I understand that the direct benefits of this research to society are not known, I have been told that I may learn more about stress levels.
I understand this research will take about 30 minutes.
I have been told that are no known risks or discomforts related to participating in this research.
I have been told Rita Holak can be contacted at rholak@conncoll.edu.
I understand that contact information will be retained in order to enroll people who qualify in a follow up study.
I understand that in the follow up study, I will be tracked by a number not my name and that all contact information will be kept confidential.
I understand that I may decline to answer any questions that I see fit, and I may withdraw from the study without penalty at any time.
I understand that all information will be identified with a code number and not my name.
I have been advised that I may contact the researcher who will answer any questions that I may have about the purposes and procedures of this study.
I understand that this study is not meant to gather information about specific individuals and my responses will be combined with other participants' data for the purpose of statistical analyses.
I consent to publication of the study results as long as the identity of all the participants is protected.
I understand that this research has been approved by the Connecticut College Human Subjects Institutional Review Board (IRB).
Concerns about any aspect of this study may be addressed to Associate Professor Audrey Zakriski Chairperson of the Connecticut College IRB (439-5734).

I am at least 18 years of age, and I have read these explanations and assurances and voluntary consent to participate in this research about stress levels.
Name (printed)____.
Signature____.
Date

Appendix B

Name:______________________________________.

Age:____.

Sex: Male Female

Grade: First Year Sophomore Junior Senior

Do you play a Varsity Sport at Connecticut College?

Yes No

If yes, what type?

Individual Team

If yes, which season?

Fall Winter Spring

If yes, which sport?

Basketball
Cross Country
Field Hockey
Ice Hockey
Lacrosse
Rowing
Sailing
Soccer
Squash
Swimming and Diving
Tennis
Track and Field
Volleyball
Water Polo

If No, how many days do you exercise a week?

0 1 2 3 4 5 6 7

If No, how many people do you exercise with?

0 2-4 5+

When do you expect to be least stressed between now and the end of the semester?
(circle two)

Early November
Mid November
Late November
Early December
Finals Period

When do you expect to be most stressed between now and the end of the semester?
(circle one)
Early November
Mid November
Late November
Early December
Finals Period

Appendix C

First if all, thank you for participating in this research on stress levels in college students. The purpose of this research is to gather preliminary data in order to recruit participants of different backgrounds for a follow up study on stress levels of college students. You will be notified via email if you have been randomly selected to participate in a follow up study examining stress levels of college students.

If you are interested in this topic and want to read the literature in this area, please contact me (Rita Holak) at rholak@conncoll.edu.

Listed below are two sources you may want to consult to learn more about this topic:

Andews, J et. al. (2007). Effects of manipulating the amount of social-elaluative threat on the cortisol stress respinse in young healthy men. *Behavioral Neuroscience*, 121, 871-876.

Kirshlbaum et al. (1995). Sex-specific effects of social support on cortisol and subjective responses to acture psychological stress. *Psychosomatic Medicine*, 57, 23-31.

Appendix D

I hereby consent to participate in Rita Holak's research about stress levels in college students.
I understand that this research will involve taking my levels salivary cortisol at 2 different times throughout the semester, before and after a stress test, and completing a series of questionnaires.
I understand that samples will be destroyed after the study is complete.
While I understand that the direct benefits of this research to society are not known, I have been told that I may learn more about stress levels.
I understand this research will take about 5 hours.
I have been told that are no known risks or discomforts related to participating in this research.
I have been told Rita Holak can be contacted at rholak@conncoll.edu.
I understand that I may decline to answer any questions that I see fit, and I may withdraw from the study without penalty at any time.
I understand that all information will be identified with a code number and not my name.
I understand that my contact information has be retained in order for the researcher to communicate with me but all information will be kept completely confidential.
I have been advised that I may contact the researcher who will answer any questions that I may have about the purposes and procedures of this study.
I understand that this study is not meant to gather information about specific individuals and my responses will be combined with other participants' data for the purpose of statistical analyses.
I understand that this study involves participating in a stress inducing experience and I have no known medical issues that will prevent me from participating in this stress inducing experience.
I consent to publication of the study results as long as the identity of all the participants is protected.
I understand that this research has been approved by the Connecticut College Human Subjects Institutional Review Board (IRB).
Concerns about any aspect of this study may be addressed to Associate Professor Audrey Zakriski, Chairperson of the Connecticut College IRB (439-5134)

I am at least 18 years of age, and I have read these explanations and assurances and voluntary consent to participate in this research about stress levels.
Name (printed)______________________________.
Signature___________________________________.
Date_______________________________________.

Appendix E

First if all, thank you for participating in this research dealing with stress levels in college students. In this research, I am comparing the stress levels in people who vary in their amount and type of exercise. Members of the Psychology 101 and 102 classes at Connecticut College are participating in this research. One of the issues in the literature is the amount of salivary cortisol that athletes produce in response to a stress test compared to non-athletes. Typically researchers have looked at the cortisol levels of elite athletes. They have found that elite athletes have a reduced response to a psychosocial stressor compared to non-elite athletes. To my knowledge, no research has actually focused on college athletes or has compared athletes on teams to college students who regularly exercise.

I hypothesize that students who do not regularly exercise will have the biggest rise in cortisol after the stress test. I also hypothesize that student athletes will have the lowest rise in cortisol after the stress test. Therefore, I additionally hypothesize that students who regularly exercise but are not on a team will have a cortisol level in between the other two groups in response to the stress test. Additionally, I hypothesize that these responses to stress will be consistent will the stress levels throughout the semester. These are standard testing procedures.

I chose to do this study throughout this semester because I thought it would be easiest to keep track of people during one semester rather than two. I was also very interested in the cortisol levels of students during finals period and if I had waited until next semester, I would not have time to analyze my data. Gathering my data during first semester will allow me to have second semester to analyze it.

If you are interested in this topic and want to read the literature in this area, please contact me (Rita Holak) at rholak@conncoll.edu.

Listed below are two sources you may want to consult to learn more about this topic:

- Rimmele, U. et al. (2007). Trained men show lower cortisol, heart rate, and psychological responces to psychosocial stress compared with untrained men. *Psychoneuroendocrinology*,32, 627-635.
- Lippi, G. (2009). Measurement or morning saliva cortisol in athletes. *Clinical Biochemistry.* 904-906.

  Student Counseling Services:
  Phone: 860-439-4587
  Fax: 860-439-2317
  Warnshuis Building
  270 Mohegan Avenue
  New London, CT 06320-4196

National Suicide Crisis Line: 1-800-273-TALK (8255)

Appendix F

Letter:

Tube #	Date/Time	Stress Level (1-10)	Gum: Y or N
1			
2			
3			
4			

Tube 1 and 2 should be on the same day, 1 in the morning and 2 in the afternoon. Follow the same procedure with tubes 3 and 4 aka 3 in am and 4 in the pm.

Take your morning sample as close to waking as possible.

Do not eat for 2 hours before taking a sample.

Try to get to the 0.5 line on the tube.

Chew gum if you can't produce enough spit but try to get enough without it.

Refrigerate or freeze your samples immediately and keep them in the refrigerator/freezer until you give them to me.

My email is rholak@conncoll.edu if you have any questions.

Appendix G

Letter:

12/17/09

Tube #	Time	Stress Level (1-10)	Gum: Y or N
6			
7			
8			

Appendix H

Participant	Cort. 1	Prep	TSST	Cort. 2	Cort. 3
A **Ath**	10:00	10:05	10:15	10:25	10:35
B **Ath**	10:15	10:20	10:30	10:40	10:50
C **Ath**	10:30	10:35	10:45	10:55	11:05
D **Ath**	10:45	10:50	11:00	11:10	11:20
E **Ath**	11:00	11:05	11:15	11:25	11:35
F **WO**	11:15	11:20	11:30	11:40	11:50
G **WO**	11:30	11:35	11:45	11:55	12:05
H **WO**	11:45	11:50	12:00	12:10	12:20
I **WO**	12:00	12:05	12:15	12:25	12:35
J **WO**	12:15	12:20	12:30	12:40	12:50
K **No WO**	12:30	12:35	12:45	12:55	1:05
L **No WO**	12:45	12:50	1:00	1:10	1:20
M **No WO**	1:00	1:05	1:15	1:25	1:35
N **No WO**	1:15	1:20	1:30	1:40	1:50
O **No WO**	1:30	1:35	1:45	1:55	2:05

Appendix I

- What do you like about Connecticut College? Why or why not?
- Are you happy you decided to go here? Why or why not?
- Why would you be a good role model?
- How do you feel about underage drinking?
- How many finals do you have left? What is your plan for studying for them?
- What book are you reading right now?
- What do you think you are going to major in? Why?
- What type of job do you want to have?
- What are you going to do this summer?
- What is the most stressful experience you have gone through?
- Please explain an experience in which you have successfully overcome a stressful situation.
- What is your least favorite food? Why?
- Do you have a good relationship with your parents?
- What are you involved in at Connecticut College?
- What is your favorite TV show? Why?
- What has been your favorite class at Conn?
- Describe a project you are working on or just completed?

Appendix J

	1	2	3	4	5	6	7	8	9	10
A	**3.00 std**	**3.00 std**	**Ctrl-H**	**Ctrl-L**	D5a	F5a	H5a	J5a	L5a	N5a
B	**1.00 std**	**1.00 std**	**Ctrl-L**	**Ctrl-H**	D5b	F5b	H5b	J5b	L5b	N5b
C	**0.333 std**	**0.333 std**	A5a	B7a	D7a	F7a	H7a	J7a	L7a	N7a
D	**0.111 std**	**0.111 std**	A5b	B7b	D7b	F7b	H7b	J7b	L7b	N7b
E	**0.037 std**	**0.037 std**	A7a	C5a	E5a	G5a	I5a	K5a	M5a	O5a
F	**0.012 std**	**0.012 std**	A7b	C5b	E5b	G5b	I5b	K5b	M5b	O5b
G	**Zero**	**Zero**	B5a	C7a	E7a	G7a	I7a	K7a	M7a	O7a
H	**NSB**	**NSB**	B5b	C7b	E7b	G7b	I7b	K7a	M7b	O7b

	1	2	3	4	5	6	7	8	9	10	11	12	1	2
A	**3.00 std**	**3.00 std**	**Ctrl-H**	**Ctrl-L**	C3	C3	E6	E6	H2	H2	K1	K1	M6	M6
B	**1.00 std**	**1.00 std**	**Ctrl-L**	**Ctrl-H**	C4	C4	F1	F1	H6	H6	K2	K2	N1	N1
C	**0.333 std**	**0.333 std**	A1	A1	C6	C6	F2	F2	I1	I1	K6	K6	N2	N2
D	**0.111 std**	**0.111 std**	A2	A2	D3	D3	F6	F6	I2	I2	L1	L1	N6	N6
E	**0.037 std**	**0.037 std**	A6	A6	D4	D4	G1	G1	I6	I6	L2	L2	O3	O3
F	**0.012 std**	**0.012 std**	B1	B1	D6	D6	G2	G2	J1	J1	L6	L6	O4	O4
G	**Zero**	**Zero**	B2	B2	E1	E1	G6	G6	J2	J2	M3	M3	O6	O6
H	**NSB**	**NSB**	B6	B6	E2	E2	H1	H1	J6	J6	M4	M4		