Fitness and Exercise as Linked to Overall Stress and Physiological Stress Responses

Justine Monthony

Union College

Acknowledgements

I would like to thank my advisors Professor Lindsay Morton and Professor Brian Cohen for their assistance and guidance throughout the entirety of not only this study, but all the ideas and design process that went into it. I would also like to thank the entirety of Team Cohen and Professor Morton's research assistants all of whom were essential to the study as TSST panelists. Finally I would like to thank Union College for their Student Research Grant, without which I would have been unable to incite participants or assay the samples.

Table of Contents

Abstract

Exercise negatively correlates with physiological stress responses, but there is less research on short-term stress hormones for this response. For this thesis, I hypothesized that exercise and fitness levels would be negatively correlated with perceived stress and with physiological stress from a standard stressor. Undergraduate college participants reported their chronic stress, current mood, past week's physical activity (amount and intensity), perceived physical fitness, and demographic characteristics. Participants also engaged in a social stress task, on which they had limited time to prepare a speech with no notice or resources that was given in front of peers who were ostensibly analyzing and recording them. Then they performed a mental math task aloud for five minutes. To measure physiological reactions, participants' blood pressure, heart rate, and salivary amylase levels were assessed at baseline, immediately after the stressor, after ten minutes, and after thirty minutes. Perceived fitness and overall stress were negatively correlated, but there was no significant correlation between exercise engagement and overall stress. Participants were significantly emotionally stressed from the stress-inducing task, but participants who exercised more or had higher perceived fitness did not show significantly lower amylase, heart rate, or diastolic blood pressure responses to the stressful task. Participants who exercised more did show significantly higher systolic blood pressure than participants who did not exercise as much, which prompts a call for further research. These findings reinforce the theory that exercise reduces stress, but questions remain as to the effect of exercise and fitness on the physical stress response.

Keywords: stress reaction, norepinephrine, Trier Social Stress Test, perceived functional ability, Stanford 7-Day Recall

Fitness and Exercise as Linked to Overall Stress and Physiological Stress Responses

The human body is constantly reacting to external stimuli and the messages they convey. Some of these stimuli can convey messages that warn the body of potential harm. Stress is an example of one of these stimuli. From an evolutionary standpoint, it would make sense for more physically fit individuals to not need as much physiological activation to respond to a stressor, as those who are accustomed to running long distances quickly can do so with more ease than someone not accustomed to running at all. But can this be applied to our daily lives? After all, stress in humans is now more likely to be induced by social situations rather than a predator. In this study, I explored the relationship between exercise and physical responses to an induced social stress.

Stress is a force that causes the body to react in several ways. Anything that threatens the homeostasis of the body can be considered stress. When the body is exposed to stress, it first goes into the alarm phase (Martini, 1998). In the alarm phase, the "fight or flight" response is activated which activates the sympathetic nervous system and causes the adrenal medulla to release epinephrine and norepinephrine as a fast, initial response to the stress. The release of these hormones contributes to increased alertness, increased energy production and consumption, rerouting of the blood supply, and increased heart rate, blood pressure, and respiratory rate. A secondary response system is also activated at a more delayed rate, and this system causes the release of corticosteroids, which work to aid the body in restoring homeostasis and remaining healthy in the face of short-term stress (Straub, 2014). If the stressor remains for a long period of time, the body then begins what is called the resistance phase. During the resistance phase, glucocorticoids cause the release of fatty acids which are then broken down to release glucose.

This glucose is reserved for the brain to ensure proper functioning, which can lead to neglect of other organs if nutrition is not a constant. When the resistance phase ends, the exhaustion phase begins, and homeostasis deteriorates. Once the body enters the exhaustion phase, organ systems will fail, and this will prove fatal unless immediate action is taken (Martini, 1998). Although this physiological stress process is common to most humans, most often, when “stress” is discussed in everyday life, most people are referring to the alarm phase and the body’s immediate response to stress.

The body’s response to stress in the alarm phase can be measured in many different ways. However, the favored method used by researchers is the measurement of salivary cortisol (Holt & Hanley, 2012). Since it is in the saliva, the measurement is noninvasive, and it gives reliable data. Cortisol is a steroid stress hormone produced in the adrenal cortex that is slowly released during the alarm phase of stress. Cortisol stays in the body for a period of hours, possibly spanning to days, and it takes about 20 minutes for cortisol levels to change, making it known as a long-term stress hormone.

However, there are also short-term stress hormones. Specifically, epinephrine and norepinephrine are also released during the alarm phase. As part of the fast-acting sympatho-adreno-medullary (SAM) system, both are hormones secreted by the adrenal medulla (Straub, 2014). Researchers have recently found that salivary amylase, an enzyme that is secreted from the parotid gland, is both easily measured and correlates with norepinephrine. As shown in Figure 1, Norepinephrine is a peptide that functions as a short-term stress hormone and stays in the body for a period of minutes to hours. Since many studies on stress are short-term, this may allow for a more accurate representation of an individual's current stress level than cortisol (Holt & Hanley, 2012).

As example of this, Maruyama, Kawano, Okamoto, Ando, Ishitobi, Tanaka, Inoue, Imanaga, Kanehisa, Higuma, Ninomiya, Tsuru, Hanada, & Akiyoshi (2012) studied both salivary cortisol and salivary amylase in response to the Trier Social Stress Test (TSST). The TSST was created by Kirschbaum, Pirke, and Hellhammer in 1993. It is a fifteen minute task broken into three different portions of five minutes each. First, the participant is given five minutes to prepare a speech for a panel of their peers. Then, the participant gives the speech during the next five minutes, followed by a five minute mental math portion. Kirschbaum et al. (1993) found that the TSST showed a significant effect on the participants' prolactin, serum, salivary cortisol, adrenocorticotropin, and growth hormone. This means that the participants showed the expected physical stress response to the task, and the TSST is indeed a sufficient activity to induce stress on participants. Using this task, Maruyama and colleagues (2012) found that the reaction of salivary amylase to the TSST was not significantly different than the salivary amylase reaction to electrical stimulation. However, salivary amylase did show a rise directly after the stimulus and then a return to baseline, whereas the salivary cortisol continued to rise even 20 minutes after the stimulus. These results reiterate that measurements of cortisol and salivary amylase are differentially sensitive to the timing of particular stressors, such as the TSST.

A question then becomes how we can use a short-term measure of stress, such as salivary amylase, to explore how particular factors might moderate physiological stress reactions. Specifically, stress as seen in the alarm phase is experienced quite often, and there are a vast number of ways in which people seek to reduce it. One particular way is exercise, and thus research has sought to answer whether or not exercise actually reduces a person's response to stress both mentally and physically.

Much research has been done to investigate the connections between exercise and stress. For example, regular jogging has been found reduce hormonal responses to stress (Nabkasorn, Miyai, Sootmongkol, Junprasert, Yamamoto, Arita, & Miyashita, 2005). In this study, adolescent girls, who exhibited mild to moderate depressive symptoms, had their depression, cortisol, epinephrine, and resting heart rate measured at the beginning of the study. The girls then joined a fitness group that jogged for just under an hour five days per week for eight weeks. After the fitness intervention, the girls showed significantly lower cortisol, epinephrine, and depressive symptoms as compared to their control data during a "daily activity" intervention. They also observed a lower heart rate, but this was not significant (Nabkasorn et al., 2005). The experimental design of the research suggests that exercising regularly could actually cause a reduction in not only depression but also resting levels of cortisol and epinephrine.

In another study, it was found after a few days of aerobic, resistance, and/or power training, the participant's baseline salivary cortisol had decreased (Hayes, Grace, Baker, & Sculthorpe, 2015). A separate study found that not only does cortisol vary with physical exercise, but it also correlates with anxiety in athletes that is experienced before a performance or game. They also found that baseline salivary cortisol was significantly lower in athletes than in non-athletes (Gatti & De Palo, 2011).

These studies introduce the idea that the body compensates for the amount of strain it undergoes in order to prevent a repeated stressor . If the body only experiences mild and infrequent stress, then it will react more powerfully (e.g. release high levels of cortisol) to smaller stressful stimuli. However, if the body regularly experiences more serious stress such as exercise training on a daily basis, the body will not react as much to the same small stressor (e.g. release only small amounts of cortisol), leading the individual to feel less stressed about it and in fact have lower baseline cortisol.

Similar to the rest of the reviewed literature, Childs & de Wit (2014) designed a study that looked at exercise regularity and physiological response to stress. They determined exercise regularity via survey and measured physiological stress level by measuring cortisol levels, blood pressure, and heart rate in the participants. They had participants complete both a stressful task and a control task, one on each of two days, and measured physiological stress before, during, and after each task. The stressful task was the TSST, during which participants spoke on a topic that they were not familiar with for five minutes, and then did serial subtraction in their head for five minutes, all in front of an audience while being filmed (Childs & de Wit, 2014). Their goal was to find trends in overall stress as well as recovery from stress. The study also measured mood and affect of participants before and immediately after the task. They hypothesized that participants who were not regular exercisers would have a stronger physiological response to and slower recovery after the stressful task than participants who were regular exercisers. The results showed that regular exercisers were quicker to recover positive affect than non-exercisers were, and the participants who exercised regularly had a significantly lower baseline heart rate than those who did not.

Although Childs & de Wit (2014) had many significant findings, they used the long-term stress hormone cortisol as their main measure of physiological stress. While this is widely accepted in the scientific community, recording short-term stress hormones such as norepinephrine via salivary amylase concentrations as well as recording heart rate and blood pressure could give a more accurate view into what is currently happening in the human body during stressful experiences such as the TSST.

Current Research

The purpose of the current study was to examine self-reported exercise engagement and perceived fitness as related to short-term stress measures . Many studies have been done using the TSST, and quite a few of those have studied the relationship between exercise and stress using long-term stress measures such as salivary cortisol levels (e.g., Hayes et al., 2015; Gatti & De Palo, 2011; Perna et al., 1998). However, research has not looked at the relationship between exercise and stress using the short-term stress measures of heart rate, blood pressure, and salivary amylase together. This is precisely what this research was intended to evaluate.

The study examined self-reported exercise engagement and perceived fitness as related to short-term stress measures. I hypothesized that short-term stress, such as that induced by the TSST (Kirschbaum, Pirke, & Hellhammer, 1993), would be better measured by short-term stress hormones such as salivary amylase and short-term reactions to stress, such as that shown in changes in heart rate and blood pressure. This also allows researchers to obtain a more immediate indication of stress level, as salivary cortisol takes about 20 minutes to change. Heart rate and blood pressure have been shown to be different in exercisers and non-exercisers (Childs & de Wit, 2014), and salivary amylase has been shown to be responsive to the TSST (Maruyama et al. 2012), which was the cause of the stress in this study. I predicted that there would be a negative correlation between exercise engagement and the short-term stress measures of heart rate, blood pressure, and salivary amylase both overall and during a stressful situation. In addition, I predicted that there would be a negative correlation between perceived fitness and short-term stress measures both overall and during a stressful situation.

Method

Participants

A total of 86 undergraduate students participated in this study after providing informed consent. These participants were recruited through an online system and were provided with either 1.5 psychology course credits or $12 as compensation for completing the study. 28 participants were excluded from data analysis for various reasons. Five participants completed only the first day of the study (described below) and were compensated with 0.25 psychology course credits or $2, but were excluded from analysis. Four participants did not correctly answer survey questions that were designed to determine if the participant was reading the questions thoroughly. One participant responded to the survey question “Did you answer all questions honestly, accurately, and to the best of your ability?” with no. One participant finished the Trier Social Stress Test component of the study under the allotted amount of time, and one participant refused to do the Trier Social Stress Test and was therefore excluded from analysis. Four participants had incomplete data sets from either noncompliance or researcher error. Finally, 12 participants were excluded due to the fact that they were on prescription drugs that interfered with either the production or release of norepinephrine.

After exclusions, 58 undergraduate students were included in the analyses. These participants consisted of 18 men and 40 women between the ages of 18 and 22 (M=19.22, SD=1.439). Race and ethnicity were not taken into account in order to maintain anonymity, but 10 (17.24%) participants were not native English speakers.

Procedure

Participants came in for two consecutive days, first for fifteen minutes and then for an hour and fifteen minutes the next day. When the participants arrived on the first day, they were given an informed consent sheet and told that the study was looking at exercise, memory, and stress, and that there would be a task that induced moderate social stress. After the student agreed to participate, they were brought into an individual room to memorize a list of words. Participants were given a filler task of recalling a certain digit in a series of numbers and then were asked to recall as many of the words as they could. When they finished, participants were given a card with their assigned number and instructions for coming back the next day.

When the participants returned for the second day, they were asked for four samples of heart rate, blood pressure, and saliva at intervals throughout the study. They were also asked to complete various surveys that assessed their stress level, baseline mood, exercise habits, and a memory task for recall of words memorized on day one (either the memory recall or the exercise survey was be taken prior to the stressful task, depending on the condition assigned to the participant). Once these were completed, the participant would complete the Trier Social Stress Test, which consisted of a speech portion and a mental math portion. This was followed by a second mood survey as well as the exercise survey or memory recall, depending on the patient's condition. After these were completed, the participant was given a 20 minute relaxation period after which they completed a demographics survey. The participants were then debriefed and given compensation of their choice. The first day of the study as well as all memory recall tasks were irrelevant to the present study and will not be discussed in further detail.

Materials

Heart Rate and Blood Pressure. An Omron BP710 blood pressure and heart rate monitor was used to automatically collect and display heart rate and blood pressure data for each of the four samples taken from each participant. The cuff was placed on the participant's upper arm for each sample and then removed. If the monitor did not work after three attempts (n =2), a manual blood pressure cuff and stethoscope were used.

Saliva Sample. The participant was given a 1.5mL microcentrifuge tube and was asked to fill the bottom of the tube with saliva. Once collected, the salivary sample was analyzed for amylase concentration via assay. The enzymatic assay of specifically α-amylase was done by mixing starch and color reagent to the enzyme, boiling the solution, and then measuring enzyme concentration via spectrophotometer. Complete instructions can be found in Appendix A.

Measures and Tasks

Revised Undergraduate Student Hassles Scale (RUSH-S; Kanner, Coyne, Schaefer, & Lazarus, 1981). Participants were given 57 examples of everyday student hassles, which can be categorized into 11 subscales: Time pressures, financial constraints, race/ethnicity, gender, friendships, traffic, safety, religion, employment, physical appearance, and parental expectations. In validation work, the RUSH-S was predictive of mental health functioning, emotional health, and significant stress (Kanner et al., 1981). For this thesis, participants were told "For each hassle, please indicate how frequently it occurred in the past month on the 5 point scale (0 = did not occur to 4 = always occurred). If the hassle occurred at all in the past month, then, on the next 5 point scale please indicate how severe on average each hassle was (1= not at all severe to 5= extremely severe). If a particular hassle did not occur, leave the second question blank." From this, a total count of hassles encountered was tallied for each participant by counting the number of times the participant answered a one or higher for the frequency item. Using only the items on which participants reported a one or higher in frequency, an average monthly hassle score was also computed. Finally, only the severity items on which the participant reported a one or higher on the associated frequency item was averaged for a mean severity score. In the original sample of 965 undergraduate and graduate students, the average number of hassles experienced was 32.2, SD = 10.8. The original participants reported that the frequency of their experienced hassles was rare to occasional, M = 1.4, SD = 0.60, and that the average severity of their experienced hassles was moderate, M = 2.6, SD = 0.70 (Kanner et al., 1981). Women were also found to report more frequent and severe hassles than men. Kanner et al. (1981) provided evidence of good test-retest reliability for the frequency estimates and varied test-retest reliability for the severity estimates.

Attention Checks. Throughout the RUSH-S, there were four intermittent attention questions that told the participant which number to select to ensure they were paying attention.

Brief Mood Introspection Scale (Mayer, & Gaschke, 1988). Both immediately before and immediately after the TSST, participants were given sixteen adjectives (e.g., grumpy, tired, happy) and told to rate how much they were feeling that adjective at that moment. Participants rated each word with either “definitely do not feel,” “do not feel,” “slightly feel,” or “definitely feel.” For scoring, these responses were coded with the numbers 1-4 respectively. This allowed for a two dimensional view into how the participant’s arousal level and mood valence. Mayer and Gaschke (1988) reported an arousal range from aroused (maximum = 24) to calm (minimum = 0), M=17.5, SD=4.39, with adequate internal consistency for the subscale, a=0.58, and reported a valence range from positive (maximum = 18) to negative (minimum = -3), M=5.05, SD=7.4, with good internal consistency for the subscale, a=0.83.

Trier Social Stress Test (Kirschbaum, Pirke, & Hellhammer, 1993). The participants were asked to give a five minute speech on the politics behind the Syrian refugee crisis, given five minutes to prepare, and then brought in front of a panel of three peers who they were told would be recording the speech, as well as analyzing and rating it. After the five minute speech, the participant was asked to count backward from 1022 in intervals of thirteen. Every time a mathematical error was made, the participant was told to start again from 1022.

Manipulation Check. For a straightforward check of participant stress levels, we asked participants to rate their stress level on a scale of one to ten (1=very low, 10=very high), (Appendix B). We asked this before and immediately after the TSST in order to ensure the task had successfully induced stress in the participant.

Exercise Survey. The questionnaire we made and titled “Exercise Survey” (Appendix C) was composed of fourteen questions, two of which were the Stanford 7-day recall questions (Sallis, Haskell, Wood, Fortmann, Rogers, Blair & Paffenbarger 1985). These two questions asked the participant to recall the total number of hours (to the nearest 0.5 hours) in the past seven days during which they participated in moderate exercise and in vigorous exercise. Both moderate exercise and vigorous exercise were clearly defined, and the questions were short answer, not multiple choice. These questions were scored separately, not combined to form a score, as they are not shown to have a strong, positive correlation. Two more questions pertained to perceived functional ability (George, Stone, & Burkett, 1997). For these questions, participants were asked two questions that the participant responded to using one of thirteen options. The first question asked about which pace would be right for the participant (not too hard, not too easy) exercising continuously on an indoor track for one mile, and had answers ranging from 18 minutes per mile or more to 7 minutes per mile or less. The second question asked how fast the participant could cover a distance of three-miles and not become overly fatigued, and also included a 13-point response scale. The responses to these questions were summed to create an estimate of the physical fitness, based on previous findings that these items . correlate participant's maximal oxygen consumption s (George, Stone, & Burkett, 1997). The other questions were a variety of multiple choice and free response. These questions asked about how much participants had eaten, how much caffeine they had consumed, and if they had exercised the day of the study, as well as sleeping habits and whether they were a varsity or club athlete.

Demographic Questionnaire. This questionnaire consisted of eight questions which were both multiple choice and free response (Appendix D). These questions addressed major, class, age, sex, gender, native language, and any medications, vitamins, or supplements the participant was taking.

Results

RUSH-S and Self-Reported Exercise Scores

There was a significant correlation between the number of hassles encountered in the RUSH-S (M=36.96, SD=8.79)and perceived functional ability (M=7.82, SD=2.97), r=-0.416, $p \leq .000$. There was also a significant correlation between the frequency of hassles encountered in the RUSH-S (M=1.58, SD=0.67) and perceived functional ability, r=-0.437, $p \leq .000$, as well as a significant correlation between the severity of hassles encountered in the RUSH-S (M=1.88, SD=0.67) and perceived functional ability, r=-0.428, $p \leq .000$. All discussed means, standard deviations, and scale reliabilities can be found in Table 1. A correlation matrix for these variables is shown in Table 2.

There were no significant correlations between vigorous activity (M=3.62, SD=4.00) and RUSH-S hassles r=-0.085, p=0.484, RUSH-S frequency r=-0.136, p=0.266, or RUSH-S severity r=-0.096, p=0.428. Similarly, there were no significant correlations between moderate activity (M=4.04, SD=3.19) and RUSH-S hassles r=0.136, p=0.262, RUSH-S frequency r=0.089, p=0.465, or RUSH-S severity r=0.157, p=0.195.

Change in Stress after the TSST

Subjective affect changed significantly in participants after participation in the TSST. As seen in Figure 2, arousal as measured by the BMIS after the TSST (M=23.69, SD=7.08) was significantly higher than before the TSST (M=21.18, SD=8.26), $t(66)$=-3.473, p=0.001. As seen in Figure 3, valence as measured by the BMIS after the TSST (M=7.67, SD=13.85) was significantly higher than before the TSST (M=-1.58, SD=14.02), $t(65)$=6.255, p=0.000. As seen in Figure 4, objective stress as measured by the manipulation check after the TSST (M=6.61, SD=2.10) was also significantly higher than before the TSST (M=5.57, SD=1.91), $t(69)$=-3.990, p=0.000.

Only two of the physiological measurements of stress changed significantly after the TSST. Diastolic blood pressure after the TSST (M=77.03, SD=9.68) was significantly higher than before the TSST (M=73.74, SD=10.32), $t(57)$=-3.786, p=0.000, and heart rate after the TSST (M=77.77, SD=12.36) was significantly higher than before the TSST (M=81.63, SD=13.35), $t(57)$=2.050, p=0.045. On the contrary, salivary amylase after the TSST (M=26.84, SD=5.30) was not significantly higher than before the TSST (M=26.93, SD=7.00), $t(54)=0.743$, $p=0.461$, and systolic blood pressure after the TSST (M=125.76, SD=13.86) was not significantly higher than before the TSST (M=123.56, SD=14.72), $t(57)=-1.623$, $p=0.110$.

Exercise and Physical Stress Response

The correlation of all physiological data samples were analyzed with each of the three self-reported measures of exercise, the results of which are shown in Table 3. Interestingly, systolic blood pressure and perceived functional ability were found to correlate. While the correlation between the participant's first systolic blood pressure and perceived functional ability was not significant, $r=-0.220$, $p= .067$, the correlation between the participant's second systolic blood pressure and perceived functional ability was significant, $r=-0.267$, $p= .025$, as was the participant's third systolic blood pressure (M=120.04, SD=12.78) and perceived functional ability, $r=-0.254$, $p= .034$, and the participant's fourth systolic blood pressure (M=120.20, SD=12.87) and perceived functional ability, $r=-0.318$, $p= .007$.

However, when the data was split between men and women, this correlation was only reflected in women, but not significantly. In women, the first systolic blood pressure measurement (M=119.91, SD=13.63) and perceived functional ability (M=6.86, SD=2.87) was not significant, $r=0.229$, $p=0.126$, nor was the correlation between the women's second systolic blood pressure (M=122.33, SD=11.61) and perceived functional ability, $r=0.277$, $p=0.062$, nor the women's third systolic blood pressure (M=116.04, SD=11.32) and perceived functional ability, $r=0.147$, $p=0.330$, nor the women's fourth systolic blood pressure (M=116.09, SD=11.10) and perceived functional ability, $r=0.249$, $p=0.096$. None of these correlations proved significant at the 0.05 level, but there was a small effect size that may have been due to sample size.

Comparatively, in men, the first systolic blood pressure measurement (M=130.54, SD=14.48) and perceived functional ability (M=9.67, SD=2.22) was not significant, r=-0.182, p=0.405, nor was the correlation between the men's second systolic blood pressure (M=132.33, SD=15.61) and perceived functional ability, r=-0.043, p=0.845, nor the men's third systolic blood pressure (M=127.71, SD=12.08) and perceived functional ability, r=-0.072, p=0.743, nor the men's fourth systolic blood pressure (M=128.08, SD=12.54) and perceived functional ability, r=-0.002, p=0.992.

Discussion

As expected, participants who were more fit experienced less stress in daily life than participants who were less fit according to the perceived functional ability (PFA) questionnaire. The PFA is a strong, significant predictor of non-exercise VO_{2max} (George et al., 1996), and past research with objective measures of VO_{2max} have also demonstrated a significant negative relationship between stress and fitness (e.g., Thakur, 2016). Past research has also demonstrated a significant, negative correlation between the amount of various intensity levels of exercise and reports of daily hassles in college students (Nguyen-Michel, Unger, Hamilton, Spruijit-Metz, 2006). Thus, I also expected that participants who exercised more often would experience less stress overall than participants who did not exercise as often. However, no correlations were found between the RUSH-S, which was the measure of overall stress, and the Stanford 7-Day Recall, which was the measure of moderate- and vigorous-intensity exercise quantity over the past week. This might have been because the number of hours of exercise the participant did one week prior to the study is not as good of an indicator of fitness as the PFA.

The TSST was an effective method of stress induction in participants. Objective stress significantly increased after the task and 83.33% of participants answered "yes" when asked if they were more stressed immediately after the TSST than they were at the beginning of the study. Additionally, only two participants reported not feeling stressed either by the speech portion or the mental math portion of the TSST. However, the only physiological signs of stress that changed significantly after the TSST were heart rate and diastolic blood pressure. Diastolic blood pressure increased as was expected, but surprisingly heart rate decreased after the TSST. The decrease in heart rate is unexpected especially because the TSST has been shown to increase heart rate in past research (e.g., Childs & de Wit, 2014). This, coupled with the fact that systolic blood pressure and salivary amylase did not significantly increase suggests that the fight or flight response might not have been truly activated in our participants. In fact, there was no spike in amylase at all following the TSST, as was expected and shown in Childs & de Wit's (2014) study. It is unclear as to why this might be, but it is possible that the participants, the majority of whom participated in exchange for course credit (61.33%) were aware of the ethical limitations of studies using human participants. This means that the participants might have guessed that they were not actually being recorded or judged by the panelists and therefore not been as stressed by the TSST as someone who was unaware of the limitations. In addition, Childs & de Wit (2014) did not utilize college students as their participants. Because higher stress levels have been associated with being a college student (Kanner et al., 1981), it is possible that this may have skewed salivary amylase reactivity to additional stress. One possible explanation for this is that if hormone levels are elevated at baseline, it might be more difficult to elevate them further using an acute stressor.

The results regarding systolic blood pressure in this study were surprising. Specifically, there was a positive correlation between PFA and systolic blood pressure both in participants overall and specifically in women when the data was split by gender. This stands in contrast to the hypothesized negative correlation between PFA and blood pressure. This might have been dismissed as a type I error, as it contradicts the vast amount of literature that has shown that exercise lowers systolic blood pressure, had it not been for the clear pattern of the small effects across each measurement time period. In this study, participants with a higher PFA had a higher systolic blood pressure at every sample taken. Although not in line with the majority of work, there have been other researchers who have similar findings. Tsioufis, Kyvelou, Tsiachris, Tolis, Hararis, Koufakis, Psaltopoulou, Panagiotakos, Kokkinos, & Stefanadis (2011) found that in Greek adolescents, participants who had a higher physical activity level also had higher systolic blood pressure and lower heart rates than participants who had lower physical activity levels. This result was especially strong in males. Similarly, Bouchard, Blair, Church, Earnest, Hagberg, & Häkkinen (2012) found that there was a specific subset of people in whom increased exercise did indeed correlate with increased systolic blood pressure. The sample was diverse, and no correlation was seen between these adverse reactions and sex or ethnicity, so it is unclear as to what else this group might have in common (Bouchard et al., 2012). It is unclear why this group of people show quite the opposite physical reaction to regular exercise than most people for whom exercise would lower systolic blood pressure. However, this poses the question as to whether undergraduate students are part of the same subset as Bouchard et al.'s (2012) participants, or more specifically if Union College students are part of this group. This is a topic that should be explored in future studies, specifically examining what makes these individuals different from the general public and why regular exercise would adversely affect their systolic blood pressure.

There are a number of limitations that might have impacted this study. This study was done as a senior thesis at an elite, small, liberal arts college in the Northeast of the United States, and as such the sample was composed of Union College students. The population of Union College is 54.1% male, 72% white, and 99% age 24 and younger, all attending the same institution with a pricetag of over $64,000 per year (National Center for Education Statistics, 2017). This presents a homogenous sample that could have skewed the data in a way that sampling the general public would not. As college students, the participants in our study experience more stress than the general public (Kanner et al., 1981). Moreover, all have access to two gyms for free from 6am-12am, and the results suggested that the majority of participants exercised more often than the general public. Many of the women taking this study were on some form of birth control (44.9%), and while our survey only asked about medications, it is possible that there may have been variations in women that did not report birth control due to the increasing popularity of hormonal intrauterine forms of birth control. This is relevant because hormonal birth control has been shown to increase blood pressure, which was one of the dependent variables in this study (Fisch, Freedman, & Myatt, 1972).

The Trier Social Stress Test (TSST) had some limitations as a stress induction technique. It was originally designed to influence salivary cortisol levels in participants, but salivary amylase (and therefore norepinephrine) was analyzed in this study. While these hormones both respond to stress, norepinephrine is a “fight or flight” hormone whereas cortisol prepares the body for such a stress and provide a secondary, slower response to stressors. The TSST is said to provide a psychobiological stress (Kirschbaum et al., 1993), however it does so via social means. The stress comes from not wanting to look uninformed or perform badly in front of one’s peers. If a participant happened to be comfortable in this situation, or was not taking the study seriously, they may not have experienced a true fight or flight response, which would have limited the release of norepinephrine.

Our measure of participant exercise and fitness levels was measured by self-report via the Stanford 7 day recall questions as well as the perceived functional ability questions. Although all of these measures have been found to correlate with actual exercise levels and the participants were assured of their anonymity, there might have still been motivation for the participants to lie and exaggerate the amount of exercise that they have done in the past week in order to seem more healthy to the researcher. Such error variance may have impacted the results of this thesis.

Another limitation to our study is that our sampling of heart rate, blood pressure, and salivary amylase was taken in four intervals instead of continuously throughout the study, which would have allowed us to see exactly when the participant’s stress levels increased and decreased. The first of these samples was considered the participant’s baseline and was taken immediately upon arrival. This may have skewed our baseline levels, as the study was held on a second floor lab, meaning that participants were most likely climbing the stairs immediately before giving their baseline sample. Since physical exertion such as climbing a flight or two of stairs raises heart rate and blood pressure, the participant’s true baseline heart rate, blood pressure, and salivary amylase levels might actually be lower than what was recorded.

This study adds to the already remarkable base of literature suggesting that exercise has many health benefits, both physically and mentally. The mental benefit that correlated with exercise here is clear, those who reported higher levels of fitness also reported less stressed in everyday life. The physical benefits were less obvious, even though those who exercised more in this study did show increased physical benefits such as lower heart rate and diastolic blood pressure. However, the post-stress task increase in systolic blood pressure that was shown by those who had increased fitness levels in this study prompts a call for further research on the topic to assess if there might be a sub-group of individuals for whom high levels of exercise do not provide beneficial adaptations to stress-inducing situations. Further research should also be conducted to assess salivary amylase responses to the TSST in order to determine why this study did not show any significant change in amylase levels following induced stress. Overall, although this study found some support for the hypothesis that exercise and physical fitness might promote better responses to stress, it also leaves open questions regarding population-specific effects and possible negative stress-related outcomes associated with high levels of physical fitness.

References

Bouchard, C., Blair, S. N., Church, T.S., Earnest, C.P., Hagberg, J.M., Häkkinen, K., et al. (2012) Adverse metabolic response to regular exercise: Is It a Rare or Common Occurrence? *PLoS ONE, 7*. doi:10.1371/journal.pone.0037887

Childs, E., & de Wit, H. (2014) Regular exercise is associated with emotional resilience to acute stress in healthy adults. *Frontiers in Physiology. 5*:161. doi: 10.3389/fphys.2014.00161

Fisch I.R., Freedman, S.H., Myatt, A.V. (1972) Oral contraceptives, pregnancy, and blood pressure. *JAMA, 222,* 1507-1510. doi:10.1001/jama.1972.03210120013004

Gatti, R., & De Palo, E. F. (2011). An update: Salivary hormones and physical exercise. *Scandinavian Journal of Medicine & Science in Sports, 21*, 157-169.

George, J. D., Stone, W. J., & Burkett, L. N. (1997). Non-exercise VO2max estimation for physically active college students. *Medicine and Science in Sports and Exercise, 29*, 415-423.

Hayes, L. D., Grace, F. M., Baker, J. S., & Sculthorpe, N. (2015). Exercise-induced responses in salivary testosterone, cortisol, and their ratios in men: A meta-analysis. *Sports Medicine (Auckland, N.Z.), 45*, 713-726.

Holt, R. I. G. & Hanley, N. A. (2012). Essential Endocrinology and Diabetes. Sixth ed. *Blackwell Pub.*, Print.

Kanner, A. D., Coyne, J. C., Schaefer, C., & Lazarus, R. S. (1981). Comparison of two modes of stress measurement: Daily hassles and uplifts versus major life events. *Journal of Behavioral Medicine, 4*, 1-39.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test' – A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28*, 76-81.

Martini, Frederic. (1998). Fundamentals of anatomy & physiology. Fourth ed. *Prentice Hall*. Print.

Maruyama, Y., Kawano, A., Okamoto, S., Ando, T., Ishitobi, Y., Tanaka, Y., et al. (2012) Differences in salivary alpha-amylase and cortisol responsiveness following exposure to electrical stimulation versus the Trier Social Stress Tests. *PLoS ONE 7*. doi:10.1371/journal.pone.0039375

Mayer, J. D., & Gaschke, Y. N. (1988). The experience and meta-experience of mood. *Journal of Personality and Social Psychology, 55*, 102-111.

Nabkasorn, C., N. Miyai, A. Sootmongkol, S. Junprasert, H. Yamamoto, M. Arita, & K. Miyashita. (2005) Effects of physical exercise on depression, neuroendocrine stress hormones and physiological fitness in adolescent females with depressive symptoms. *The European Journal of Public Health, 16*, 179-84.

Nguyen-Michel, S.T., Unger, J.B., Hamilton, J., and Spruijt-Metz, D. (2006). Associations between physical activity and perceived stress/hassles in college students. *Stress and Health 22,* 179-88.

Pett, M. A., & Johnson, M. J. M. (2005). Development and psychometric evaluation of the revised university student hassles scale. *Educational and Psychological Measurement, 65*, 984–1010.

Sallis, J. F., Haskell, W. L., Wood, P. D., Fortmann, S. P., Rogers, T., Balir, S. N., & Paffenbarger, R. (1985). Physical activity assessment methodology in the Five-City Project. *American Journal of Epidemiology, 121*, 91-106.

Straub, R. O. (2014). *Health psychology: A biopsychosocial approach* (4th ed.). New York, NY: Worth Publishers.

Thakur, J.S. (2016) Relationship of perceived stress with the cardiovascular fitness of sportsmen. *International Journal of Physical Education, Sports and Health, 3,* 257-259.

Tsioufis, C., Kyvelou, S., Tsiachris, D., Tolis, P., Hararis, G., Koufakis, N., Psaltopoulou, T., Panagiotakos, D., Kokkinos, P., Stefanadis, C. (2011) Relation between physical activity and blood pressure levels in young Greek adolescents: The Leontio Lyceum Study. *Eur J Public Health, 21*, 63-68.

Union College. (2017, February 9). Retrieved from https://nces.ed.gov/collegenavigator/?q=Union%2BCollege&s=all&id=196866#general

Table 1

Compilation of mean (M), standard deviation (SD), and scale reliability (Cronbach's alpha) for measured variables

Measure	M	SD	Scale Reliability
Men's Perceived Functional Ability	9.67	2.22	0.88
Men's Sample 1 Systolic Blood Pressure	130.54	14.48	N/A
Men's Sample 2 Systolic Blood Pressure	132.33	15.61	N/A
Men's Sample 3 Systolic Blood Pressure	127.71	12.08	N/A
Men's Sample 4 Systolic Blood Pressure	128.08	12.54	N/A
Moderate Activity	4.04	3.19	N/A
Percieved Functional Ability	7.82	2.97	0.88
Post-TSST BMIS Arousal	23.69	7.08	0.57
Post-TSST BMIS Valence	7.67	13.85	0.65
Post-TSST Objective Stress	6.61	2.10	N/A
Pre-TSST BMIS Arousal	21.18	8.26	0.51
Pre-TSST BMIS Valence	-1.58	14.02	0.56
Pre-TSST Objective Stress	5.57	1.91	N/A
RUSH-S Hassle Count	36.96	8.79	N/A
RUSH-S Hassle Frequency	1.58	0.57	0.94
RUSH-S Hassle Severity	1.88	0.67	0.93
Sample 1 Diastolic Blood Pressure	73.74	10.32	N/A
Sample 1 Heart Rate	81.63	13.35	N/A
Sample 1 Salivary Amylase	26.93	7.00	N/A
Sample 1 Systolic Blood Pressure	123.56	14.72	N/A
Sample 2 Diastolic Blood Pressure	77.03	9.68	N/A
Sample 2 Heart Rate	77.77	12.36	N/A
Sample 2 Salivary Amylase	26.84	5.30	N/A
Sample 2 Systolic Blood Pressure	125.76	13.86	N/A
Sample 3 Systolic Blood Pressure	120.04	12.78	N/A
Sample 4 Systolic Blood Pressure	120.20	12.87	N/A
Vigorous Activity	3.62	4.00	N/A
Women's Perceived Functional Ability	6.86	2.87	0.85
Women's Sample 1 Systolic Blood Pressure	119.91	13.63	N/A
Women's Sample 2 Systolic Blood Pressure	122.33	11.61	N/A
Women's Sample 3 Systolic Blood Pressure	116.04	11.32	N/A
Women's Sample 4 Systolic Blood Pressure	116.09	11.10	N/A

Table 2

Correlation matrix of stress level as measured by the Revised Undergraduate Student Hassles Scale (RUSH-S) and exercise and fitness as measured by the Stanford 7-Day Recall and the Perceived Functional Ability Scale

		RUSH-S Hassle Count	RUSH-S Hassle Frequency	RUSH-S Hassle Severity	Vigorous Activity	Moderate Activity	Perceived Functional Ability
RUSH-S Hassle Count	Pearson Correlation	1	0.875**	0.857**	-0.09	0.14	-0.416**
	Sig (2-tailed)		0	0	0.48	0.26	0
RUSH-S Hassle Frequency	Pearson Correlation	0.875**	1	0.922**	-0.14	0.89	-0.437**
	Sig (2-tailed)	0		0	0.27	0.47	0
RUSH-S Hassle Severity	Pearson Correlation	0.857**	0.922**	1	-0.1	0.16	-0.428**
	Sig (2-tailed)	0	0		0.43	0.2	0
Vigorous Activity	Pearson Correlation	-0.09	-0.14	-0.1	1	-0.02	0.447**
	Sig (2-tailed)	0.48	0.27	0.43		0.84	0
Moderate Activity	Pearson Correlation	0.14	0.09	0.16	-0.02	1	-0.02
	Sig (2-tailed)	0.26	0.47	0.2	0.84		0.89
Perceived Functional Ability	Pearson Correlation	-0.416**	-0.437**	-0.428**	0.447**	-0.02	1
	Sig (2-tailed)	0	0	0	0	0.89	

*$p \leq 0.05$
**$p \leq 0.01$

Table 3

Correlation (Pearson's r) of physiological data at each sample time and exercise measures

Physiological Data	Sample Time	Perceived Functional Ability	Vigorous Exercise	Moderate Exercise
Salivary Amylase	1	0.072	0.024	0.080
	2	-0.024	-0.013	-0.161
	3	0.000	-0.087	0.132
	4	-0.016	0.035	0.153
Heart Rate	1	-0.066	-0.068	-0.004
	2	-0.175	-0.155	0.147
	3	-0.180	-0.191	0.143
	4	-0.167	-0.148	0.112
Systolic Blood Pressure	1	0.220	-0.202	-0.022
	2	0.267*	-0.135	0.082
	3	0.254*	-0.112	-0.066
	4	0.318**	-0.033	-0.081
Diastolic Blood Pressure	1	0.042	-0.176	-0.083
	2	0.073	-0.201	0.258*
	3	-0.098	-0.317**	0.116
	4	-0.117	-0.076	0.121

*$p \leq 0.05$
**$p \leq 0.01$

Figure 1

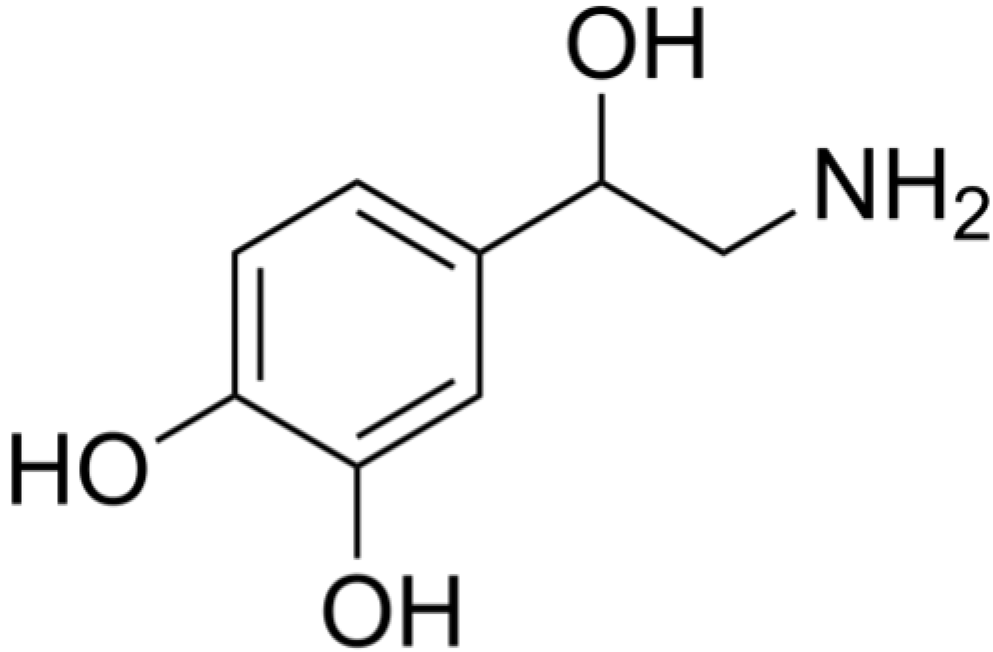

Figure 1: The chemical structure of norepinephrine

Figure 2

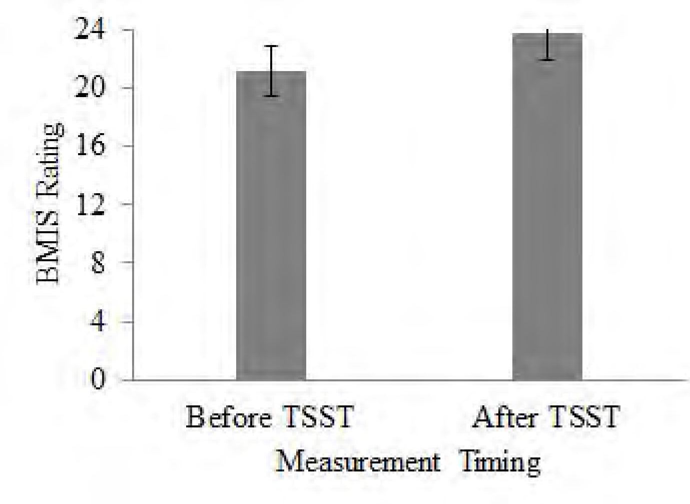

Figure 2. Mean arousal as measured by the Brief Mood Introspection Scale before and after the Trier Social Stress Test.

Figure 3

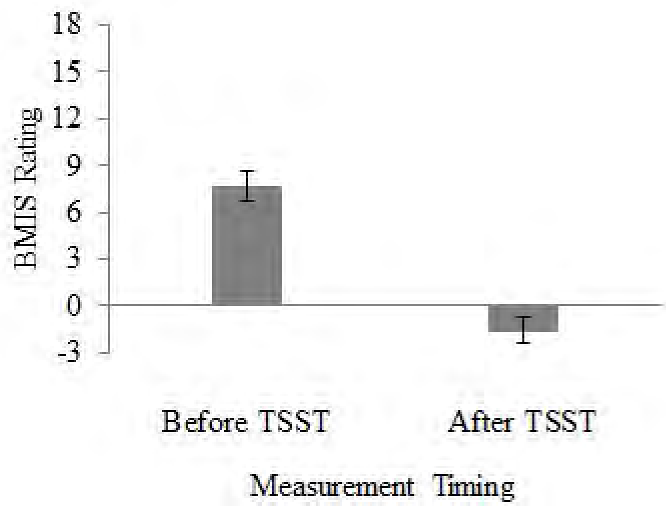

Figure 3. Mean valence as measured by the Brief Mood Introspection Scale before and after the Trier Social Stress Test.

Figure 4

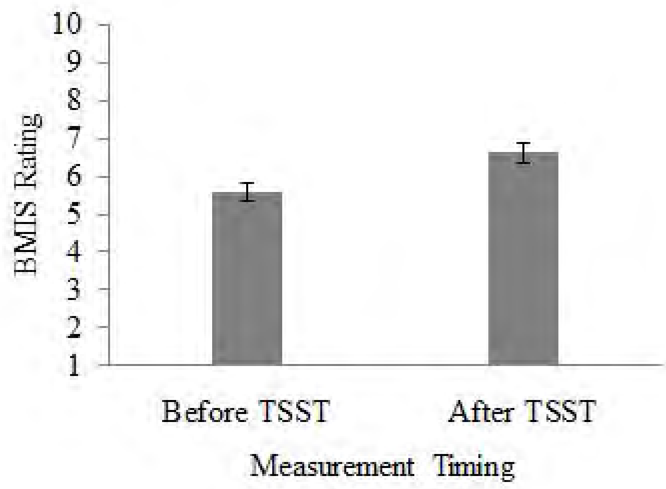

Figure 4. Mean objective stress as measured by the Brief Mood Introspection Scale before and after the Trier Social Stress Test.

Appendix A: Alpha-Amylase Assay

Soultions:

1. 20 mM Sodium Phosphate Buffer with 6.7 mM Sodium Chloride, pH 6.9 at 20°C (Buffer)
    a. Prepare 100 ml in purified water using Sodium Phosphate, Monobasic, Anhydrous, **Sigma-Aldrich Product Number S0751**and Sodium Chloride, **Sigma Product No S9888**. Adjust to pH 6.9 at 20°C with 1 M NaOH.
2. 1.0% (w/v) Soluble Starch Solution (Starch)
    a. Prepare 25 ml in Reagent 7.3.1 using Starch Potato Soluble, **Sigma-Aldrich Product Number S2630**. Facilitate solubilization by heating the starch solution in a glass beaker directly on a heating/stir plate using constant stirring. Bring to boil and maintain the solution at this temperature for 15 minutes. Allow the starch solution to cool to room temperature with stirring. Return the starch solution to its original volume (25 ml) by the addition of purified water and dispense aliquots for assay with stirring
3. Sodium Potassium Tartrate Solution
    a. Dissolve 12.0 g of Sodium Potassium Tartrate, Tetrahydrate, **Sigma-Aldrich Product Number S2377**, in previously heated 8.0 ml of 2 M NaOH, 50°C - 70°C. Heat directly on a heating/stir plate with constant stirring to dissolve. DO NOT BOIL.
4. 96 mM 3,5-Dinitrosalicylic Acid Solution
    a. Prepare 20 ml in purified water, 50°C - 70°C, using 3,5-Dinitrosalicylic Acid, **Sigma-Aldrich Product Number D0550**. Heat directly on a heating/stir plate with constant stirring to dissolve. DO NOT BOIL.
5. Color Reagent Solution (Clr Rgt Soln)
    a. To 12 ml of purified water, 50°C - 70°C, slowly add Solution 3 followed by Solution 4. If not completely dissolved, the reagents should dissolve when mixed. The solution should be stored in an amber bottle at room temperature. The Color Reagent Solution is stable for 6 months.
6. 0.2% (w/v) Maltose Standard (STD)
    a. Prepare 10 ml in purified water using Maltose, Monohydrate, **Sigma-Aldrich Product Number M5885** .

Assay:

1. Prepare four test tubes (Tube 1, 2, 3 & Blank) by adding 100uL of Solution 2 to each.
2. Mix by swirling and equilibrate to 20°C.
3. Add 50uL of saliva to Tube 1, 70uL to Tube 2, 100uL to Tube 3, and none to the Blank
4. Mix by swirling and incubate for exactly 3.0 minutes at 20°C
5. Add 100uL of Solution 5 to each tube
6. Cap with a vented cap and place in a boiling water bath
7. Add 50uL of saliva to Tube 1, 30uL to Tube 2, none to Tube 3, and 100uL to Blank
8. Boil for exactly 15 minutes, then cool on ice to room temperature, approximately 3 minutes
9. Add 900uL purified water to each tube
10. Mix by inversion and record the A_{540nm} for both the Test and Blank using a suitable spectrophotometer

Spectrophotometry:

11. Due to the short enzymatic incubation time of three minutes, each test lot must be run one at a time.
12. Standard Curve (uL):

	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std Blank
Solution 6	5	20	40	60	80	100	200	-----
Purified Water	195	180	160	140	120	100	-----	200
Solution 5	100	100	100	100	100	100	100	100

13. Place in a boiling water bath for exactly 15 minutes, then cool on ice to room temperature
14. Add 900uL of Purified Water to each Tube
15. Mix by inversion and record the A540nm for the Standards and Standard Blank using a suitable spectrophotometer.

Appendix B: Manipulation Check

How would you rate your current stress on a scale from 1-10? *

Mark only one oval.

	1	2	3	4	5	6	7	8	9	10	
Very low											Very high

Appendix C: Exercise Survey

Exercise Survey

Please answer each question honestly and to the best of your ability.

* Required

1. **Assigned Number** *

2. **How many meals have you eaten today?** *
   Mark only one oval.

0	1	2	3	4	5
◯	◯	◯	◯	◯	◯

3. **If possible, please list approximately how many calories each meal contained.** *

During the last seven days, how much total time did you spend doing VIGOROUS physical activity and MODERATE physical activity?

Record only time actually engaged in the activity (i.e., ignore breaks, rest periods, etc.). Please do not record any LIGHT physical activity (e.g., office work, light homework, very light sports such as bowling, or any activities involving sitting).

VIGOROUS ACTIVITY (jogging or running, swimming, strenuous sports such as singles tennis or racquetball, digging in the garden, chopping wood, etc.)

MODERATE ACTIVITY (bicycling on level ground, brisk walking, sports such as golf or doubles tennis, yard work, heavy housecleaning, etc.)

4. **Total hours of VIGOROUS ACTIVITY for the last 7 days to the nearest 0.5 hours:** *

5. **Total hours of MODERATE ACTIVITY for the last 7 days to the nearest 0.5 hours:** *

6. **Suppose you were going to exercise continuously on an indoor track for 1 mile. Which exercise pace is just right for you – not too easy and not too hard? Choose the appropriate number (any number: 1 to 13).** *

Mark only one oval.

- 1 Walking at a slow pace (18 minutes per mile or more)
- 2
- 3 Walking at a medium pace (16 minutes per mile)
- 4
- 5 Walking at a fast pace (14 minutes per mile)
- 6
- 7 Jogging at a slow pace (12 minutes per mile)
- 8
- 9 Jogging at a medium pace (10 minutes per mile)
- 10
- 11 Jogging at a fast pace (8 minutes per mile)
- 12
- 13 Running at a fast pace (7 minutes per mile or less)

7. **How fast could you cover a distance of 3-miles and NOT become breathless or overly fatigued?Please be realistic. Choose the appropriate number (any number: 1 to 13).** *

Mark only one oval.

- 1 I could walk the entire distance at a slow pace (18 minutes per mile or more)
- 2
- 3 I could walk the entire distance at a medium pace (16 minutes per mile)
- 4
- 5 I could walk the entire distance at a fast pace (14 minutes per mile)
- 6
- 7 I could jog the entire distance at a slow pace (12 minutes per mile)
- 8
- 9 I could jog the entire distance at a medium pace (10 minutes per mile)
- 10
- 11 I could jog the entire distance at a fast pace (8 minutes per mile)
- 12
- 13 I could run the entire distance at a fast pace (7 minutes per mile or less)

Have you exercised today? If yes, answer the next question. If no, please skip.

8. **For how many hours did you exercise?**
Mark only one oval.

- 0-1 hour
- 1-2 hours
- 2-3 hours
- 3-4 hours
- 4 hours or more

9. **When was the last time you exercised?**
Mark only one oval.

- 1-2 hours ago
- 2-4 hours ago
- 4-12 hours ago
- 12 or more hours ago

Are you a varsity athlete? If yes, answer the next two questions. If no, skip them.

10. **What sport do you play?**

11. **Are you currently in season? When is your next game?**

Are you a member of a club team? If so, please answer the next two questions. If not, please skip them.

12. **Which club team are you a member of?**

13. **Is your club team currently in season?**

14. **What time do you usually wake up?** *

Mark only one oval.

- Before 8am
- 8am-10am
- 10am-12pm
- 12pm-2pm
- Past 2pm

15. **What time do you usually go to sleep?** *

Mark only one oval.

- Before 9pm
- 9pm-11pm
- 11pm-12am
- 12am-2am
- Past 2am

16. **Have you consumed any caffeine today? How much? At what time?** *

Appendix D: Demographic Questionnaire

Demographic Questionnaire

Please answer honestly. All information provided will remain confidential. No information will be used to connect you to your data.

* Required

1. **Assigned Number** *

2. **What is your major?**

3. **What is your class year?**

4. **What is your age?**

5. **What is your biological sex?**
   Mark only one oval.
   - Female
   - Male
   - Intersex

6. **What gender do you identify as?**
   Mark only one oval.
   - Female
   - Male
   - Other
   - Prefer not to answer

7. **Is English your native language?**
   Mark only one oval.
   - Yes
   - No

8. **Please list any medication(s) you are taking, including birth control.**

9. **Please list any vitamins or supplements you are taking.**