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Interactions between Aerobic Exercise Volume, Academic Stress, and Immune Function

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INTERACTIONS BETWEEN AEROBIC EXERCISE VOLUME, ACADEMIC
STRESS, AND IMMUNE FUNCTION

A Thesis
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The Faculty of the School of Kinesiology, Recreation, and Sport
Western Kentucky University
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In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

By
Teresa Wiczynski

May 2018

INTERACTIONS BETWEEN AEROBIC EXERCISE VOLUME, ACADEMIC STRESS, AND IMMUNE FUNCTION

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTERACTIONS BETWEEN AEROBIC EXERCISE VOLUME, ACADEMIC
STRESS, AND IMMUNE FUNCTION

Teresa Wiczynski   May 2018   60 Pages

Directed by: Lee Winchester, Cody Morris, Scott Arnett, and Mark Schafer

Department of Kinesiology   Western Kentucky University

Many college students exercise individually or participate in collegiate
and intramural sports in addition to fulfilling their stressful academic
requirements. The combination of accumulated stress and vigorous exercise
could result in an impaired immune system, prompting the onset of disease and
absences in class and sports practice.

Twenty-six male and female participants aged 18 to 23 were recruited
for this study. Over the course of an academic semester, participants completed
weekly electronic surveys documenting stress levels, aerobic exercise, and
symptoms related to upper respiratory tract infections. Participants were
evaluated at four different time points (Baseline, Post-Midterm Exam, Baseline
Reassessment, and Post-Final Exam) for body fat percentage, cardiovascular
fitness, heart rate, blood pressure, and a 10mL blood draw. Blood samples were
used to measure blood glucose, cortisol, IL-6, and CD11b levels. Analysis of
cortisol and IL-6 concentrations required ELISA kits for protein quantification
in plasma samples. CD11b levels in peripheral blood mononuclear cell samples
were measured by Western Blot analysis.

There was a significant increase in blood pressure during the final exam compared to rest for systolic (p=0.005) and diastolic (p=0.004) blood pressures. There was a significant decrease in anxiety during the final exam compared to anxiety during the mid-term exam (p=0.022). The acute stress of an exam was strong enough to illicit physiologic blood pressure change, but the chronic stress throughout the semester was not intense enough did not illicit physiologic or immune responses. The volume of aerobic exercise in the vigorous workout group was not great enough to influence immune responses nor disease incidence.

Chapter 1: Introduction

Natural selection for the sympathetic "flight or fight" response and the immune response began in prehistoric times as the first organisms were subjected to environmental stressors such as fleeing a natural disaster or fighting a predator (Segerstrom, 2004). Immunological responses triggered by stress that accelerate pathological defense and wound repair would therefore be selected along with other physiologic changes to ensure survival of the best fit (Segerstrom, 2004). Activation of the stress response provides many beneficial and vital physiologic changes in the face of acute stress; however; today`s stressors tend to be psychological, such as taking an academic exam, rather than physiological, such as escaping a predator. Although the "flight or fight" response is no longer needed for the majority of today`s stressors, the immune system continues to respond with the same equivalence. Such evidence of this continued reaction is found in a study on dental students, where cortisol levels and perceived stress were evaluated during a major exam period and four weeks post exam. Cortisol and reported stress levels were significantly elevated during the exam period compared to the later time point (Johannsen, 2009). Additionally, prolonged sympathetic activation and prolonged cortisol production is associated with suppression of the immune system, creating alterations in lymphocyte and cytokine release (cell-signaling proteins) thereby leaving the individual more susceptible to disease (Dickerson, 2004).

While chronic stress resulting from prolonged activation of the sympathetic response may have suppressive effects on the immune system, habitual exercise has been reported to be beneficial to counteracting disease. In a study by Nieman et al. (1990), sedentary women aged 25 to 45 years were randomly assigned to an exercise or non-exercise group. After six weeks, natural killer cell activity had increased by 56.9 ±

10.5% in the exercise group compared to a $3.4 \pm 8.1\%$ increase in the sedentary group. Additionally, exercisers experienced significantly fewer days with symptoms of upper respiratory infections throughout the 15 week study.

The previous study may suggest that the greater the volume of exercise, the greater the protection against disease; however, when comparing immunological benefits between exercise groups, extreme exercise volume and intensity proves to be a disadvantage. Multiple studies focusing on endurance runners tabulate lower incidence of disease among runners who record low to moderate mileage compared to high and elite mileage athletes (Gleeson, 2013, Heath, 1991). Additionally, risk of infection was found to be five times higher for marathon runners one week after completing a marathon race compared to pre-race conditions, and ultramarathon runners were at twice the risk of contracting an upper respiratory infection two weeks after an ultramarathon competition compared to prerace conditions (Nieman, 1990a; Peters, 1983). Based off these findings, the benefits of exercise on immune function follow a J-shaped pattern (Nieman, 1990a). Sedentary and highly trained individuals experience immune impairment due to nonexistent or extreme exercise volumes while moderate exercisers benefit from the immunological changes caused by mild exertion (Nieman 1990a; Nieman 1990b).

As previously mentioned, studies exist that link immune response and academics or immune response and exercise intensity, but to the best of the authors' knowledge, no study has investigated the combined effects of academic stress and exercise on immune function. Many college students exercise individually or participate in collegiate and intramural sports in addition to fulfilling their stressful academic requirements. The combination of accumulated stress and vigorous exercise could result in an impaired

immune system, prompting the onset of disease and absences in class and sports practice. Additionally, studies focusing on stress responses singularly examine acute stress or chronic stress scenarios. This study followed a unique procedure by tracking students through a chronic stressor, an academic semester, followed by an acute stressor, a final exam. Therefore, the purpose of this study was to measure the effects of academic stress and habitual aerobic exercise intensity on immune function. This study measured reported levels of stress, disease, and aerobic exercise as well as levels of glucose, cortisol, IL-6, and CD11b in the blood over the course of an academic semester in sedentary and aerobically active participants. It was hypothesized that individuals who engaged in moderate aerobic exercise would demonstrate a more effective immune response to chronic and acute stressors than their sedentary counterparts, while individuals who engaged in regular vigorous exercise would have impaired immune responses.

Chapter 2: Literature Review

Origins of immune response

Natural selection for the immune response began in prehistoric times as the first organisms were subjected to environmental stressors (Segerstrom, 2004). The ability to respond to predation and natural disaster increased the likelihood of reproduction and the passing down of the "fight or flight" response. Such a response includes increased oxygen and glucose to the heart, lungs and skeletal muscles, thereby prepping the body to exert an exceptional amount of energy fighting or fleeing from danger (Segerstrom, 2004). It is likely that increased immune activity was also a component of this response (Segerstrom, 2004). Fighting a predator or fleeing a natural disaster increases the likelihood of injury, providing pathogens with an entry to the blood stream via open wounds. Immunological responses triggered by stress that accelerate pathological defenses and wound repair would therefore be selected along with other physiologic changes to ensure survival of the best fit (Segerstrom, 2004).

Basic immune response to infection

The immune system is commonly divided into natural and adaptive immunity. Natural immunity is a response in mammals as well as in lower order organisms that is a non-specific attack against all foreign particles. This response is activated within minutes to hours in stressful situations. Adaptive immunity targets specific invaders by creating cells that solely identify and attack the current pathogen. This process, known as cell proliferation, requires several days, so the body must continue to rely on the natural response during this time (Segerstrom, 2004).

Natural Immunity

The first immune cell to arrive at the site of disease or damage is the neutrophil. Second to arrive is the monocyte which is drawn towards the proinflammatory signaling proteins released by the neutrophil (McDonald, 2010). Neutrophils and macrophages phagocytize, or eat, invaders and damaged tissue after the release of toxic free radicals (Segerstrom, 2004). Additionally, macrophages release communication molecules known as cytokines that promote fever, inflammation, and wound healing (Segerstrom, 2004).

Another cell involved in natural immunity is the natural killer cell. Natural killer cells inhibit viral infection before adaptive immunity proliferates an effective response. Natural killer cells recognize "the lack of a self-tissue molecule" on the surface foreign cells. These foreign cells are then lysed by the release of toxic substances. Natural killer cells also play a role in attacking self-cells that have become malignant, such as cancerous cells (Segerstrom, 2004).

Adaptive Immunity

Adaptive immunity is characterized by a specialized attack on a specific invader. Coordinating such attacks are lymphocytes, which possess receptor cites on their surfaces that correspond to a single molecular shape. Such shapes, or antigens, are present on particular invaders. When an antigen binds to the corresponding cite on a matching lymphocyte, the antigen-specific lymphocyte begins clonal proliferation, or cell division, to create an army of lymphocytes with the same antigen specificity (Segerstrom, 2004).

Three types of lymphocytes are involved in adaptive immunity: T-helper cells, T cytotoxic cells, and B cells. T-helper cells produce cytokines to direct and amplify immune response. T-cytotoxic cells lyse virally-infected cells and cancer cells by recognizing their expressed antigens. B cells produce antibodies, which are soluble proteins that can neutralize bacterial toxins, bind to free viruses to prevent entry into cells, and bind to foreign particles in a process known as opsonization to increases the attraction of phagocytic cells belonging to natural immunity (Segerstrom, 2004).

Stress Response

Physiological Response to Acute Psychological Stress

The hypothalamic-pituitary-adrenocortical (HPA) axis is a system comprised of the hypothalamus, pituitary gland, and the adrenal glands is the mechanism responsible for eliciting the stress reaction when an organism is subjected to acute stressors (Dickerson, 2004). The thalamus and prefrontal cortex evaluate environmental stimuli, leading to emotional responses evoked by connections to the amygdala and hippocampus. The amygdala and hippocampus connect to the hypothalamus, which is the activator of the HPA axis (Feldman, 1995; Lovallo, 1997). The hypothalamus in turn releases argenine vassopressin and corticotropin-releasing hormone (CRH). CRH is transported to the pituitary gland, resulting in the release of adrenocorticotropin hormone. This triggers the release of cortisol, the primary corticosteroid responsible for the stress response, into the bloodstream by the adrenal cortex (Lovallo, 2000; Sapolsky, 2000).

Cortisol is responsible for regulating levels of glucose in the blood stream. Cortisol stimulates gluconeogenesis, the formation of glucose, by accelerating the

6

expression of enzymes responsible for converting glucose from proceeding substances. Glucose is the body`s most immediate form of energy, which is imperative in flight or fight situations. In times of metabolic stress, such as food shortages or fasting, cortisol ensures a steady supply of glucose in the blood stream, allowing the body to continue vital functions on a low caloric intake (Dickerson, 2004).

Another vital hormone is epinephrine, commonly known as adrenaline, which is secreted by the adrenal glands as part of the sympathetic-adrenal-medullary axis (Padgett, 2003). Epinephrine is responsible for increasing heart rate, blood pressure, and cardiac output and for the bronchioles and arteries leading to muscles (Curtis, 2002). Such alterations prepare the body for fighting an enemy or fleeing from danger (Curtis, 2002).

Physiological Response to Chronic Psychological Stress

While activation of the HPA system provides many beneficial and vital physiologic changes in the face of acute stress, prolonged activation of the HPA system and cortisol production is associated with suppression of the immune system including decreased lymphocyte and cytokine production. (Dickerson, 2004). For example, cortisol has been shown to prevent the proliferation and mobilization of T-cell (Choi, 2008; Dimitrov, 2018; Patterson, 2013). Such effects may render individuals more vulnerable to disease in times of chronic stress. In mice studies by Dhabhar et al. (1997) and McEwen et al. (2001), T-cells redistributed to the skin during acute stress, thought to be advantageous during the flight-or-fight response when wound acquirement is likely and protectors are most vital at the skins surface to fight incoming invaders. Conversely, when subjected to chronic stress, T-cells were shunted away from the skin and the

immune response was diminished. Acute stress enhances while chronic stress suppresses the immune response.

Additionally, prolonged cortisol secretion elicits downregulation of cortisol receptors located on white blood cells. The downregulation reduces the cells` response to anti-inflammatory signals, allowing inflammatory processes mediated by cytokine signaling to go uncontrolled (Miller, 2002). It is additionally thought that cortisol participants in a negative feedback loop. In a normally functioning individual, high cortisol levels inhibit the release of CRH, the hormone responsible for activating the pituitary gland`s release of cortisol. Chronic stress has been speculated to disrupt this negative feedback loop, resulting in an uncontrolled production of cortisol, further fueling the decreased sensitivity of receptors to cortisol (Yehuda, 2006).

Modern Stressors

While today`s stressors tend to be psychological, such as taking an academic exam, rather than physiological, such as escaping a predator, the immune system responds with the same equivalence. A review study by Segerstrom et al. has evaluated the outcome of 300 studies, concluding "that psychological challenges are capable of modifying various features of the immune response" (Williams, 1998).

It is regularly seen that heart rate and blood pressure increase before and during academic exams (Elwess, 2005, Florence, 2014; Simic, 2011; Steptoe, 2001). In a study on dental students, cortisol levels and perceived stress were evaluated during a major exam period and four weeks post exam. Cortisol and reported stress levels were significantly elevated during the exam period compared to the later time point

(Johannsen, 2009). Additionally, Dickerson et al. (2004) found that academic exams with oral and mental components (such as answering question from the audience after giving a presentation) provoke the highest degrees of physiological stress, measured by cortisol and ACTH, compared to other forms of academic exams that lack elements of social threat and uncontrollability.

Today`s stressors can be chronic as well. An example of chronic stress eliciting immune suppression occurred in elderly women whose partners suffered from Alzheimer`s. Caretaking for such partners was documented to create high, chronic stress. When comparing a caregiving group to a group with healthy spouses, the caregivers reported significantly elevated rates of emotional distress and disease compared to non-caregivers. On the molecular level, the cytokine interleukin 6 (IL-6) was found in significantly elevated levels in the caregiver group compared to the control group, possibly indicating that elevated IL-6 levels is an indicator for chronic stress (Lutgendorf, 1999; Kiecolt-Glaser, 1999).

Interleukin-6

The cytokine Interleukin-6 (IL-6) is a cell-signaling molecule released by immune and non-immune cells (Akira 1990; Judd, 1990; Spangelo 1989; Spangelo 1990). IL-6 is released as a result of elevated cortisol levels during the inflammatory response to tissue injury, a common cause of HPA axis activation via the sympathetic response, as found in trauma and surgery patients (Di Padova, 1991). Increased levels of cortisol are also found in such individuals, providing a marker of psychological stress (Di Padova, 1991). Zhou et al. (1993) confirmed that physiologic and mental stress without tissue damage can

cause the release of IL-6 by subjecting a group of rats to a series of foot shocks (physiologic stressor) and another group to prolonged bodily restraint (mental stressor). Both groups produced a significant increase in IL-6 compared to a control group, with the foot shock group surpassing the restraint group. Additionally, the concentration of IL-6 was found to peak after the release of corticosterone, a hormone released due to mental stress. In a study by Cohen et al. (1999) subjects were experimentally infected with an influenza virus and monitored for symptom severity, levels of psychological stress, and IL-6 production. Higher psychological stress scored reported before the experimental infection resulted in greater severity of upper respiratory infection symptoms and IL-6 production.

The findings of Zhou et al. (1993) and Cohen at al. (1999) indicate that stress hormones in the absence of tissue injury can influence the release of IL-6. IL-6 can be produced by the hypothalamus (Spangelo, 1990), anterior pituitary (Spangelo, 1989), and adrenal cortex (Judd, 1990), key neuroendocrine and endocrine glands to the stress response and activation the sympathetic nervous system. Release of IL-6 by the endocrine system promotes B cells to form antibodies and promotes T lymphocyte proliferation and differentiation (Akira, 1990), equipping the immune system for potential pathological invasion. It has been shown that psychological stress alone can trigger such a response rather than the more threatening physiological damage that would result in such a foreign invasion.

Effect of Aerobic Exercise on Immunity

Moderate levels of Aerobic Exercise

While stress may have suppressive effects on the immune system, habitual exercise has been reported to be beneficial to counteracting disease. In a geriatric study by Kahut et al. (2002), participants that completed at least 20 minutes of vigorous or moderate exercise three times per week in the previous year responded more favorably to an influenza vaccine than sedentary participants. Two weeks after the influenza vaccination, blood samples were incubated with influenza virus. Participants completing vigorous and moderate exercise were found to have a greater cell-mediated response to clearing infection compared to sedentary individuals, indicating that the cell-mediated response for clearing infection is more proficient in exercisers than sedentary individuals.

The same pattern holds true for younger individuals. In study by Nieman et al. (1990b), 36 mildly obese, previously sedentary women aged 25 to 45 years were randomly assigned to an exercise or non-exercise group. Exercisers were subjected to a 15-week walking plan comprised of 45 minutes of brisk walking five days per week at 60% of heart rate reserve. After six weeks, natural killer cell activity had increased by $56.9 \pm 10.5\%$ in the exercise group compared to a $3.4 \pm 8.1\%$ increase in the sedentary group. Additionally, walkers experienced significantly less days with symptoms of upper respiratory infections throughout the 15 week study.

Vigorous Levels of Aerobic Exercise

The previous two studies may suggest that the greater the volume of exercise, the greater the protection against disease; however, when comparing immunological benefits between exercise groups, extreme exercise volume and intensity proves to be a disadvantage. Multiple studies focusing on endurance runners tabulate lower incidence of disease among runners who record low to moderate mileage compared to high and elite mileage athletes (Gleeson, 2013; Heath, 1991; Spence, 2007). Spence et al. (2007) monitored symptoms, throat swabs, and nasopharyngeal swabs for upper respiratory tract infections in elite and recreational triathletes over a five-month competition season. The elite and sedentary control group had significantly higher incidents of infection compared to the recreational group. Furthermore, the elite group possessed the highest number of infectious agents, such as human rhinovirus or influenza A, as determined by the throat swabs and pharyngeal swabs. Additionally, risk of infection was found to be five times higher for marathon runners one week after completing a marathon race, and ultramarathon runners were at twice the risk of contracting an upper respiratory infection two weeks after an ultramarathon competition (Nieman, 1990a; Peters, 1983). Based off these findings, the benefits of exercise on immune function follow a J-shaped pattern. Sedentary and highly trained individuals experience immune impairment due to nonexistent or extreme exercise volumes while moderate exercisers benefit from the immunological changes caused by mild exertion.

Causes of Decreased Immunity

A suggested cause for decreased immunity with high levels of training is the fluctuation in cytokine levels due to exercise-induced tissue trauma (Smith, 1999). Physical activity causes microtrauma in the muscle, bone, and connective tissue (Smith, 1999). When combined with the natural inflammatory response to trauma and proper rest, adaptive healing occurs, leaving stronger tissue that is more suitable for the activity resulting from the original microtrauma (Clarkson, 1998). Similar to major tissue damage as seen in surgery patients, microtrauma instigates an inflammatory response, beginning with the release of pro-inflammatory cytokines from the damaged tissue to recruit neutrophils and macrophages to the injury site (Biffl, 1996). Neutrophils are initially attracted to damaged tissue by the release of proteins, nucleic acids, extracellular matrix components and lipid mediators from dying cells (Kono, 2008; McDonald, 2010; Shi, 2003). Upon arrival at the injury site, neutrophils release proteases that breakdown damaged or dead tissue (Tidball, 2005; Tiidus, 1998). Known as the *respiratory burst*, the neutrophils also release reactive oxygen species such as superoxide ($-O_2$), hydrogen peroxide (H_2O_2), hydroxyl (OH), and hypochlorous acid (HCl) (Blake, 1987; Bleakley, 2010) Overall, the major importance of neutrophils in the inflammatory response is the breakdown of necrotic cells for the later phagocytosis via incoming macrophages. The release of pro-inflammatory signaling proteins produced by neutrophils and other white blood cells attract the presence of monocytes to the injury site (McDonald, 2010). At the site of the injury, monocytes move out of the blood stream and into the damaged tissue. Through a process of differentiation, the monocyte then becomes a macrophage, which are responsible for the phagocytosis of foreign particles and dying tissue (Mills, 2012;

Sica, 2012; Winchester, 2015). An activated macrophage is a "complex, powerful, and mobile cell that is capable of secreting over 100 difference chemicals and is central to the local and systemic inflammatory process" (Simpson 1997). While this is a natural process for all exercise, continuous levels of hyper-inflammation due to chronically intense exercise sessions can cause an overabundance of proinflammatory cytokines (Biffl, 1996). Such cases are paired with the release of anti-inflammatory cytokines as the body attempts to contain the response (Biffl, 1996). For example, IL-6 is regarded as a proinflammatory cytokine, but it has also been found to play an anti-inflammatory role by inhibiting certain proteases and the inflammatory cytokines IL-1 b and TNFa (Biffl, 1996). The overall effect of prolonged, counter-regulation of the inflammatory response is suppression of the immune system, suppressing its reactivity to invasion (Biffl, 1996).

Another suggestion for impaired immune function is a depletion of the amino acid glutamine. Glutamine is one of the most abundant amino acids in blood plasma and the muscle-free amino acid pool, and it is used in metabolism and as a precursor to liver gluconeogenesis (Wagermakers, 1998). Glutamine is also a key component of lymphocyte proliferation and function (Smith, 1999). Depletion of glutamine due to excess exercise leads to lower fuel levels for lymphocytes, therefore decreasing immune cell activity levels and leaving the body susceptible to foreign pathogens (Smith, 1999).

For individuals at higher risk of infection due to less than ideal exercise volumes, an indicator of impending disease may be elevated levels of CD11b (cluster of differentiation molecule 11b), which is a cell surface marker of activated monocytes, neutrophils, and natural killer cells often seen during disease onset that indicates an inflammatory response (Kawai, 2005). Studies by Nupponen et al. (2001) and Weirich et

al. (1998) found significantly elevated levels of CD11b in newborns who were at the early stages of infection. Similarly, elevated CD11b levels were found in adults with sepsis or septic shock (Lin, 1993).

Summary

The acute stress response elicits a variety of responses that prepared the body for flight or fight situations. Chronic stress has been shown to suppress these responses, leading to immune deficiency and illness. Furthermore, stress in the form of moderate exercise boosts immune function, but absent or extreme exercise volumes impair the immune system. The current study provides a unique perspective of looking at the acute stress response after exposure to chronic stress. Additionally, the interaction of immune function with exercise or academic stress has been examined, but the interrelated effects of all three elements has yet to be investigated. Therefore, information gathered from this study will provide knowledge on physical and mental stressors that students regularly encounter during an academic semester. Such findings may result in better maintenance of stress and disease states resulting from an academic setting.

Hypothesis and Specific Aims

Key Objective

The objective of this study was to determine the effects of varying aerobic exercise intensity on susceptibility to disease and investigate if academic stress levels can influence these effects.

Hypothesis

Vigorous aerobic exercise volumes will increase incidence of disease and academic stress will compound disease susceptibility.

Specific Aims

Specific Aim 1: To investigate changes in physiologic responses to acute and chronic stress and determine if variations in aerobic exercise volume influence these responses.

Specific Aim 2: To investigate changes in immunological function in response to acute and chronic stress and determine if variation in aerobic exercise volume influence these responses.

Chapter 3: Methods

Overview

Twenty-six student participants from WKU`s Exercise Science Program were
recruited for this study and evaluated at four different time points over the course of an
academic semester (design displayed in Figure 1). The participants completed weekly
electronic surveys documenting stress levels, aerobic exercise, and symptoms related to
upper respiratory tract infections. The participants were categorized into three groups
depending on their reported exercise levels (sedentary, moderate exercise, and vigorous
exercise) based on ACSM guidelines. At the baseline and baseline reassessment sessions,
the participants were asked to complete the aforementioned surveys and were evaluated
for body composition, heart rate (HR), and blood pressure. Participants also completed a
submaximal step test during the baseline assessment to estimate submaximal VO_2 values
and aerobic fitness. During a pre-determined mid-term exam and final exam, participants
wore an ambulatory blood pressure cuff to measure blood pressure and HR at 10 minute
intervals until exam completion. Upon completion of each exam, participants completed
a survey evaluating perceived exam difficulty and had their body composition and a final
HR and blood pressure reading determined. In-test blood pressure and HR analysis
occurred in the course specific classroom, while all other anthropometric measures
occurred in the exercise physiology laboratory.

Additionally, 10 mL blood samples were collected from each participant at
baseline, immediately post mid-term exam, a baseline reassessment that occurs
approximately halfway between the mid-term exam and final exam, and immediately post
final exam. Blood samples were used to measure blood glucose, cortisol, IL-6, and cd11b

levels. Blood glucose levels were measured using a glucose monitor immediately post blood sample collection. Remaining samples were centrifuged for plasma and Peripheral Blood Mononuclear Cell (PBMC) collection. Blood draws and blood analysis occurred in the exercise physiology and exercise biochemistry laboratories, respectively.

Isolated PBMC's and plasma were aliquoted into micro centrifuge tubes and stored at -80°C until biochemical analysis. Analysis of cortisol and IL-6 concentrations required ELISA kits for protein quantification in plasma samples. CD11b levels in PBMC samples were measured by Western Blot analysis. All biochemical analysis will take place in the WKU Exercise Science Biochemistry Lab.

Baseline	Mid Term	Reassessment	Final	Weekly
Resting BP	In test/post test BP	Resting BP	In test/post test BP	Stress Survey
Resting HR	In test/post test HR	Resting HR	In test/post test HR	Disease Survey
BF%	BF%	BF%	BF%	Activity Recall
Blood Markers	Blood Markers	Blood Markers	Blood Markers	
Submax VO2	Exam Perception Survey		Exam Perception Survey	
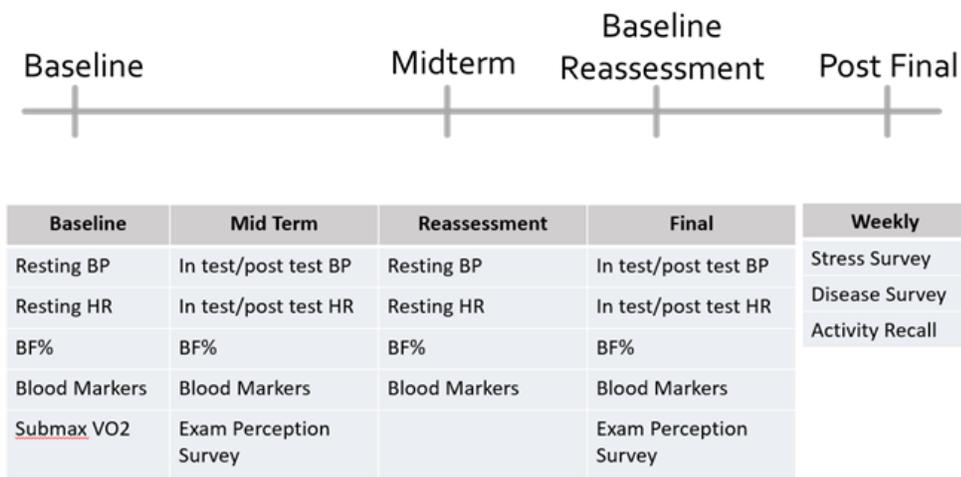
Figure 1. Data Collection Time Line

Step Test

Before the onset of the step test, a basic dietary log requesting food/water consumption data for the last 2-3 hours was administered to ensure the individuals health and safety during the step test (appendix A). In order to assess aerobic fitness, each participant completed the Åstrand-Rhyming Step Test. This involved stepping up-and-down on an aerobic step (The STEP; Sports Step Group) at a step height of four risers for

males and three risers for females at a step rate of 30 step-ups per minute (60 total step-up and step-down) for 6 minutes. HR was measured continuously using the Polar T31-coded heartrate monitor (Polar Electro), and aerobic capacity was predicted based on the HR response to the workload. A 5 minute rest period (or longer if needed by the participant) was allowed following the completion of this test.

Surveys

On-line surveys were distributed every Sunday using Qualtrics. The 3 online surveys questioned weekly disease symptoms, weekly exercise volume and intensity, and weekly stress levels (appendix C, D, and E). After the mid-term and final exam, participants completed a fourth survey questioning exam preparedness and difficulty (appendix F).

Height

Height in inches was measured at each of the four data collection points using a stadiometer (Seca). Shoes were removed for the measurement and the participant stood with his/her back against the post of the stadiometer and his/her heels touching the base of the stadiometer.

Weight

Weight in pounds was measured at each of the four data collection points using a DR-400 Detecto scale (Detecto Scales Inc.; New York). Shoes were removed for the measurement.

Body Composition

Body mass index and body fat percentage were measured using a Omron HBF-306C hand-held bio-electrical impedance analysis device (Omron Healthcare Inc.; Lake Forest, Illinois). Height (inches), weight (pounds), age (years), and gender were programmed into the device. The participant then stood quietly while holding the device at shoulder level with each hand on an electrode.

Blood Pressure

Base-line/Post-Exam

All measurements were made using a ASCuff Reusable Blood Pressure Cuff (American Diagnostic Corporation) and a 3M Littman Select stethoscope (Littman Quality). Participants sat quietly with their left arm at heart level. The blood pressure cuff was wrapped around the upper arm and aligned with the brachial artery. The stethoscope was placed below the antecubital space over the brachial artery, and the cuff was inflated to 20mmHg above the first Korotkoff sound. While slowly deflating the cuff, the first Korotkoff sound was recorded as systolic blood pressure and the disappearance of the Korotkoff sounds was recorded as diastolic blood pressure.

During Exam

SunTech Orbit blood pressure cuffs were worn by students throughout the duration of the final class exam. The blood pressure cuffs were attached to SunTech Oscar 2, 24 Hour Ambulatory Blood Pressure Monitors (SunTech Medical Inc.; Morrisville, North Carolina) which were programmed to inflate the

cuffs and record blood pressure and HR every 10 minutes for the duration of the exam. The cuffs were worn on the non-dominant arm.

Heart Rate

Heart was measured by palpating the radial artery in the left wrist for one minute while the participant sat quietly with the arm elevated to chest height.

Blood Glucose

The Relion Lancing Device (Bentonville, AR) was loaded with a Relion Ultra-Thin 30G Lancet. The end of the Lancing Device and the fingertip was cleaned with a Curity Alcohol Prep Wipe. The Relion Prime Blood Glucose Monitoring System was loaded with a Relion Prime Blood Glucose Test Strip. The Lancing Device was placed on the fingertip and, the lancet was triggered. The blood droplet was collected into the end of the test strip, and the blood glucose concentration was calculated and recorded. Gauze was applied with light pressure to the stick site until bleeding stopped.

Venipuncture

The blood samples were obtained using Universal Precautions and approximately 10ml of venous blood was drawn in order to measure the expression levels of growth factors in the blood. The blood was drawn from the participant's cubital or cephalic vein using the following protocol:

Vacutainer Safety-Lok Blood Collection Sets (BD; Franklin Lakes, New Jersey) were used for all blood draws. Before blood draw, the needle was attached to the Blood Collection Tube Holder (Dynarex; Orangeburg, New York) and a 10 mL K2 EDTA Blood Collection Tube (BD; Franklin Lakes, New Jersey) was selected. A tourniquet

(Market Lab; Caledonia, MI) was wrapped around the participant`s upper arm

approximately three inches above the elbow to stop returning blood flow. The selected

puncture site of the cubital or cephalic vein was sterilized with an alcohol prep wipe. The

needle was uncapped an inserted into the vein with the bevel up. The blood collection

tube was inserted into the tube holder at the end of the needle line, and the tube was filled

until the vacuum had reached outside barometric pressure. A gauze pad was placed over

the puncture site while withdrawing the needle. The tourniquet was removed to restore

blood flow, and firm pressure was applied to the puncture site using a gauze pad until

bleeding stopped. Additional gauze was secured with micropore tape to the puncture site

upon participant`s request.

Plasma Collection and Storage

10 mL blood collection tubes containing blood samples and the anticoagulant

EDTA were spun at 3.4 rpm x 1000 in a PowerSpin LX Centrifuge (Unico; Dayton, New

Jersey) for 15 minutes in order to separate the plasma from the solid contents of the blood

samples. 3 mL of plasma was removed from the blood sample and stored in 2, 1.5 mL

microcentrifuge tubes (Globe Scientific; Paramus, New Jersey) at -80°C.

Monocyte Isolation and Storage

3 mL of phosphate buffered saline (PBS) (Sigma-Aldrich Life Science; St. Louis,

Missouri) was added to the remaining blood sample. The sample was inverted three

times to mix and layered on top of 3 mL of Ficoll-1077 (GE Healthcare; St. Louis,

Missouri), which was contained in a 15 mL centrifuge tube, using a 2mL transfer pipette

(Samco Scientific). The 15 mL centrifuge tube centrifuged at 400xg for 20 minutes at

23°C. PBMC's were drawn from the samples using a 1ml pipette and placed in a 15 mL

centrifuge tube. 11 mL of PBS was added to the centrifuge tube. The tube was inverted

3 times and centrifuged at 100xg for 10 minutes at 23°C so that the PBMC's formed a

pellet at the bottom of the tube. The PBS and remaining PBMC's were transferred to

another 15 mL centrifuge tube and spun at 100xg for 10 minutes. The cell pellet was

resuspended by adding another 11mL of PBS and flicking the bottom of the tube. The

tube was then spun for 10 minutes at 100xg. The PBS was poured from both 15 mL

tubes into a glass beaker for waste disposal. Remaining PBS was pipetted off the top of

the pellets using a 200μL pipette. 100 μL of RIPA lysis buffer with protease inhibitor

(Santa Cruz Biotechnology; Santa Cruz, CA) was pipetted into each 15 mL tube. The

solution was pipetted up and down 3 times to suspend the PBMC's in the RIPA buffer

and induce cellular lysis. The solution was placed into a 1.5 mL microcentrifuge tube

and stored at -80°C.

ELISA Assays (enzyme-linked immunosorbent assay)

Cortisol concentrations from plasma samples was determined using a Cayman

Chemical ELISA Kit (Ann Arbor, Michigan). Prepared standards, samples, cortisol

acetylcholinesterase (AChE) tracer, and cortisol ELISA monoclonal antibody were

pipetted into a 96 well plate that was pre-coated with a goat polyclonal anti-mouse IgG.

The plate was incubated overnight. The plate was washed to remove unbound reagents

and then Ellman`s Reagent was added to the wells, reacting with Cortisol AChE to

initiate a color change. The color change was read at a wavelength of 412 nm using a

microplate spectrophotometer (BioTek; Winooski, Vermont). The color change of each

well was inversely proportional to the cortisol concentration in each sample.

Abcam`s IL-6 Human High-Sensitivity in vitro ELISA kit (Abcam; Cambridge, Massachusetts) was used to determine IL-6 concentrations in plasma samples. Prepared standards, samples, and a biotinylated monoclonal antibody were pipetted into a 96 well plate that was pre-coated with a monoclonal antibody specific for IL-6. After incubation and washing, the enzyme Streptavidin-HRP, was added to each well, which subsequently bound to the biotinylated antibody. After a second wash, the addition of a chromogen TMB substrate solution was added to initiate a color change reaction. The color change was read a wavelength of 450 nm and was directly proportional to the concentration of IL-6 in each sample.

Western Blot

Protein concentrations were determined via Bradford protein estimation assay to ensure equal protein loading, then samples were run on an SDS poly-acrylamide gel in Tris-glycine SDS buffer for proper protein separation and were then transferred electrophoretically onto a PVDF membrane overnight at 4 degrees. The membrane was blocked in a 5% milk TBST solution for 1 hour. All primary antibodies were diluted at a concentration of 1:1000 in TBST and membranes were incubated with a primary antibody solution overnight at 4 degrees. After primary incubation, the membranes were washed three times with TBST solution then incubated with a secondary HRP conjugated antibody solution for 1 hour at room temperature. Membranes were then washed three more times with TBST then developed using a chemiluminescent substrate in a FlourChem HD2 chemiluminescence imager (San Jose, CA). Band intensity was determined using BioRad ImageLab software. All proteins of interest were normalized to the values of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Due to issues with

the chemiluminescent substrate and the chemiluminescence imager, Western Blot results for this study were not obtainable.

Statistical Analysis

Statistical analyses employed a repeated measures ANOVA (exam HR, exam systolic blood pressure, and exam diastolic blood pressure) and mixed methods (cortisol, IL-6, blood glucose, body mass, body fat percentage, resting HR, resting systolic blood pressure, resting diastolic blood pressure, test anxiety, and 12 week stress) containing fixed and random effects were performed using SPSS version 25. An α value of 0.05 was selected for determination of statistical significance.

Chapter 4: Results

Physiologic Variables

A mixed methods analysis comparing differences between the sedentary, moderate, and vigorous exercise groups across the four time points revealed no significant main effects nor interaction effects for cortisol ($F_{3,36}=0.839$, $p=0.482$), IL-6 ($F_{3,18}=1.601$, $p=0.223$), blood glucose ($F_{3,36}=0.941$, $p=0.431$), body mass ($F_{3,15}=0.608$, $p=0.620$), body fat percentage ($F_{3,36}=0.416$, $p=0.742$), systolic blood pressure ($F_{3,36}=1.579$, $p=0.211$), diastolic blood pressure ($F_{3,35}=0.504$, $p=0.682$), or HR ($F_{3,39}=0.500$, $p=0.685$). This means that there were no significant changes within a single exercise group over time nor were there significant differences between the three exercise groups at any of the four time-points.
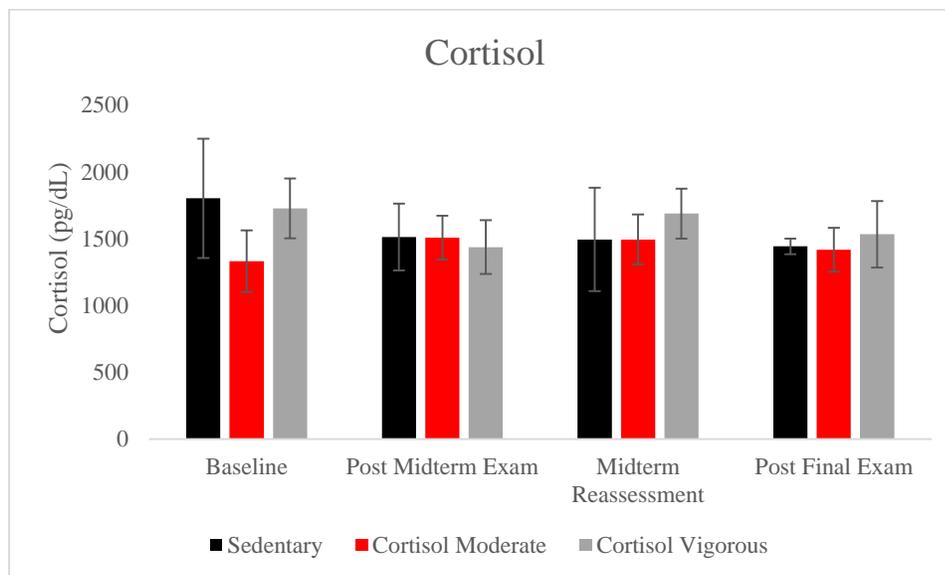

Figure 2. Cortisol. Comparison of mean cortisol concentrations (±SE) between three exercise groups at four time-points.
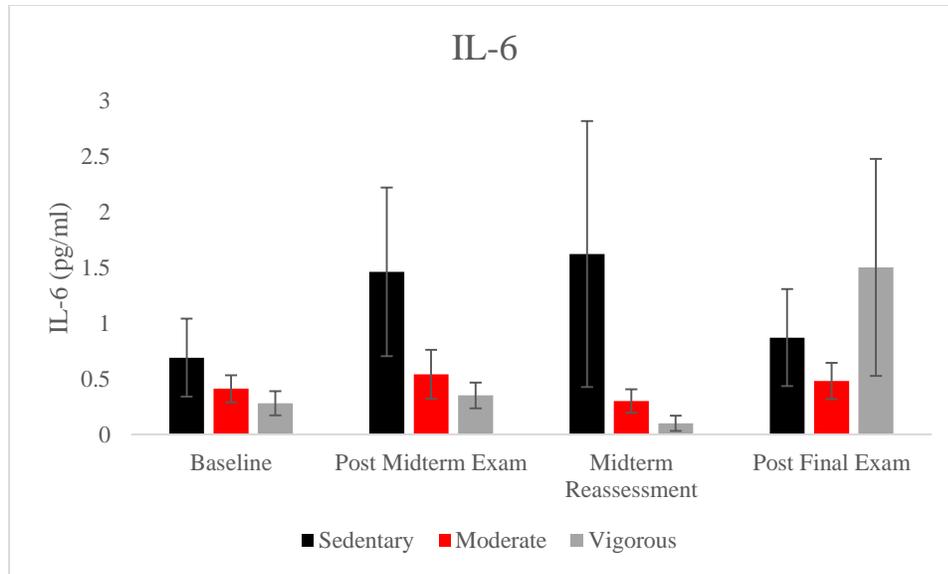
**Figure 3. IL-6.** Comparison of mean IL-6 concentrations (±SE) between three exercise groups at four time-points.
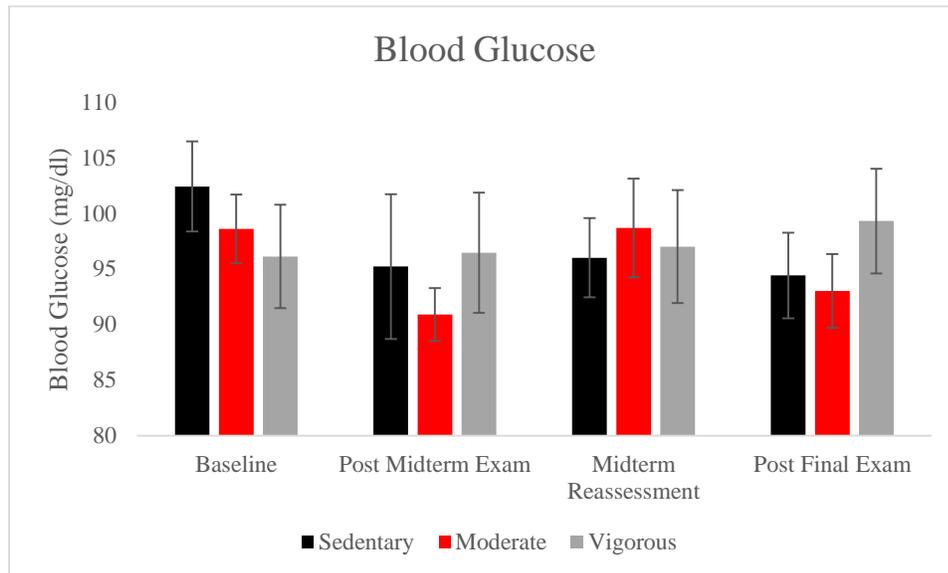
Figure 4. Blood Glucose. Comparison of mean blood glucose concentrations (±SE) between three exercise groups at four time-points.
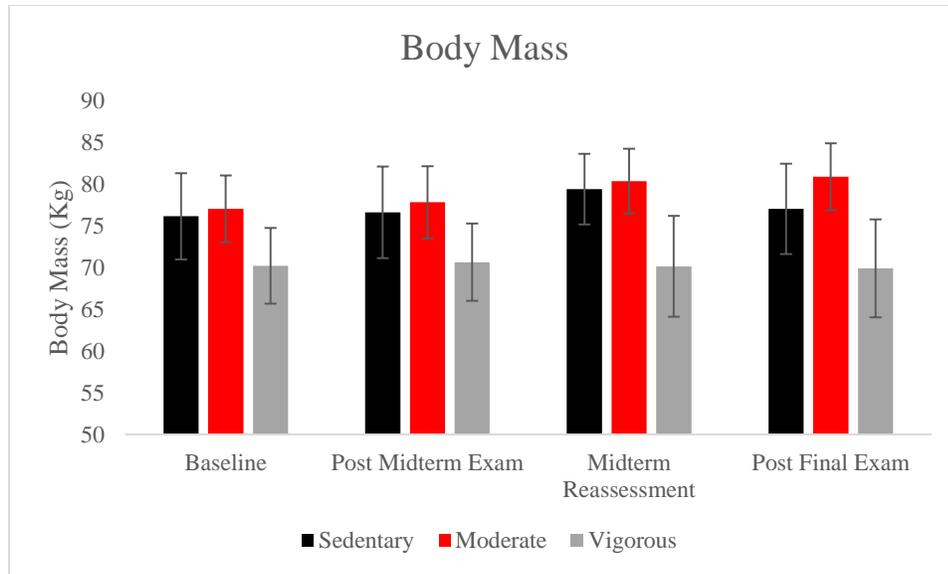
**Figure 5. Body Mass.** Comparison of mean body mass (±SE) between three exercise groups at four time-points.
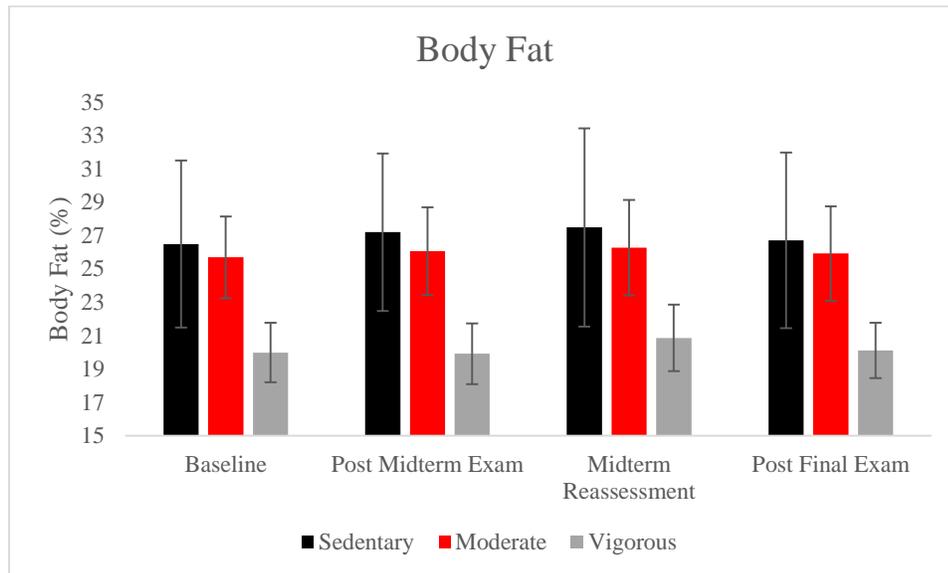
Figure 6. Body Fat. Comparison of mean body fat (±SE) between three exercise groups at four time-points.
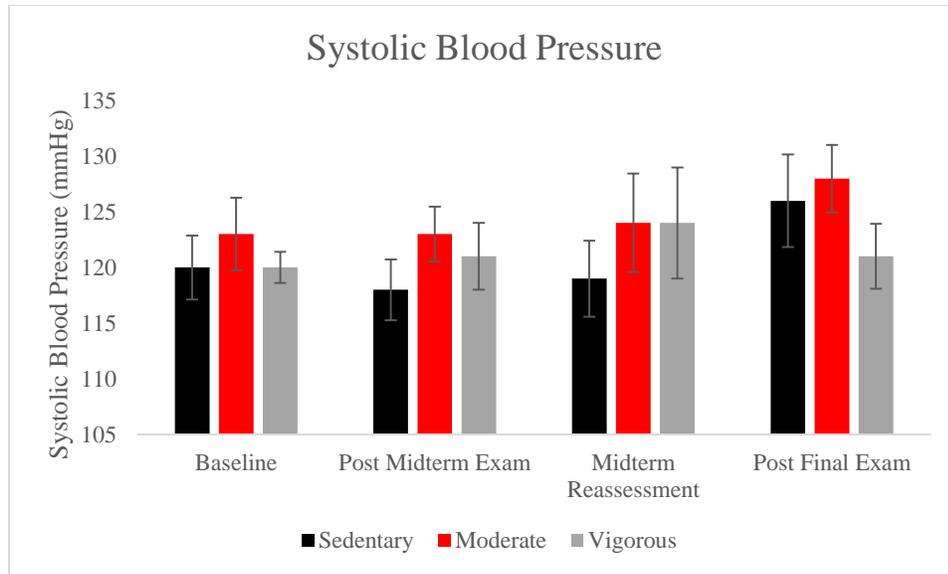
Figure 7. Systolic Blood Pressure. Comparison of mean systolic blood pressure (±SE) between three exercise groups at four time-points.
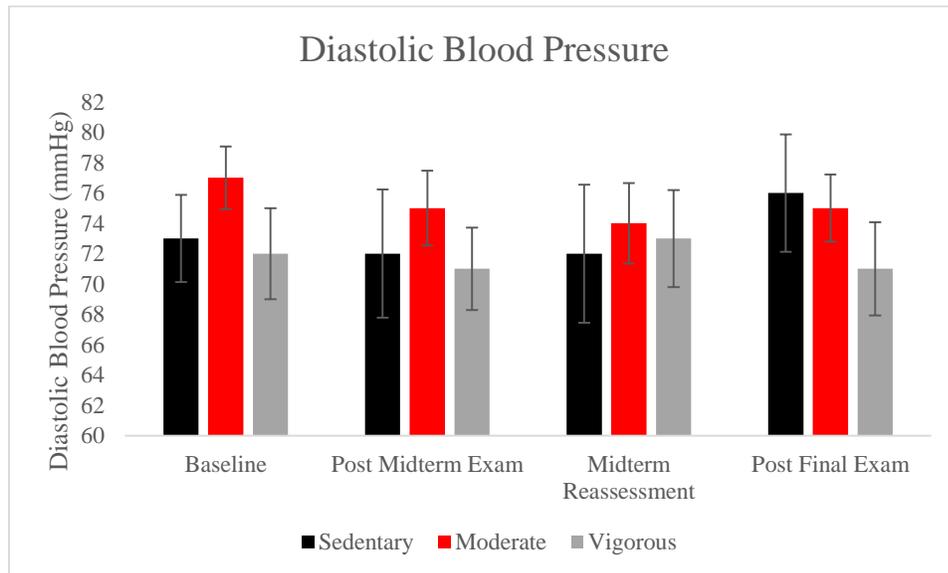

Figure 8. Diastolic Blood Pressure. Comparison of mean diastolic blood pressure (±SE) between three exercise groups at four time-points.
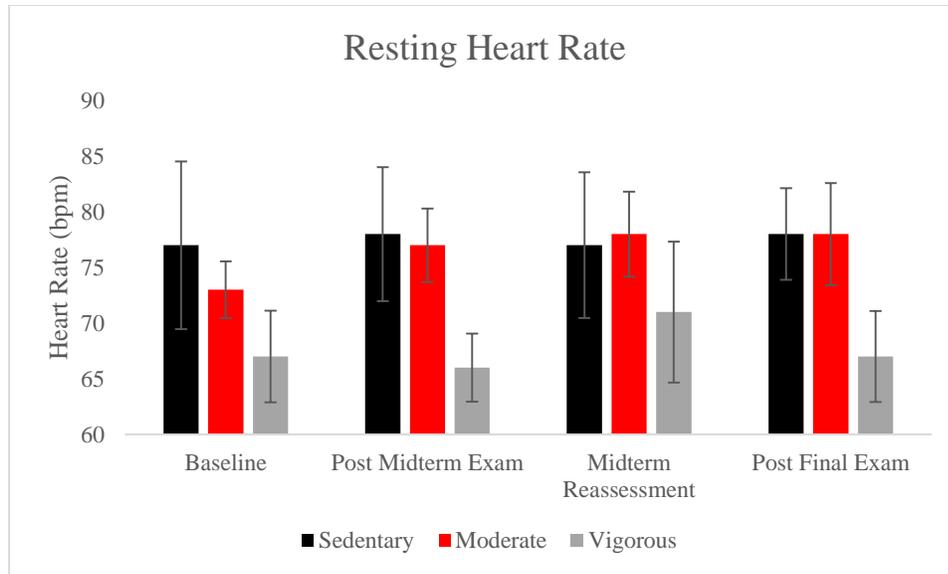
Figure 9. Resting HR. Comparison of mean HR (±SE) between three exercise groups at four time-points.

Exam HR and Blood Pressure

A repeated measures ANOVA comparing differences in systolic blood pressure at rest reported a significant main effect of time ($F_{3,15}=6.368$, $p=0.005$) and a non-significant interaction effect ($F_{6,15}=0.455$, $p=0.830$). A repeated measures ANOVA comparing differences in diastolic blood pressure at rest reported a significant main effect of time ($F_{3,15}=6.753$, $p=0.004$) and a non-significant interaction effect ($F_{6,15}=1.241$, $p=0.341$). There was a significant increase in systolic and diastolic blood pressures from rest to the exam. There was not a significant difference in systolic and diastolic blood pressures between exercise groups.
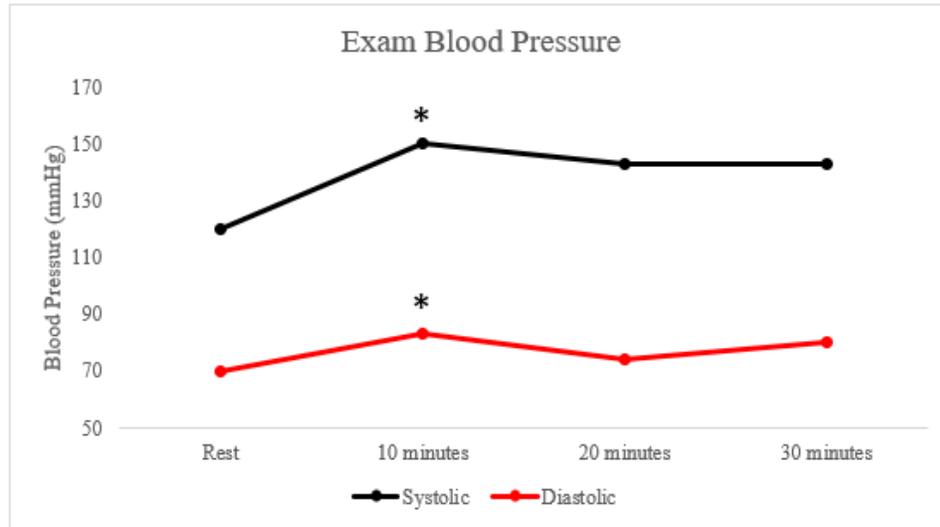
Figure 10. Exam Blood Pressure. Mean systolic and diastolic blood pressure (mmHg) recorded at rest and every 10 minutes for the first 30 minutes of a final exam for all students. *indicates significant difference from rest of p<0.05.

Exam HR

A mixed methods analysis comparing differences in HR at rest reported a non-significant main effect of time $F_{(3,15)}=3.005$, $p=0.063$ and a non-significant interaction effect $F_{(6,15)}=1.778$, $p=0.171$. There is not a significant difference in mean HR values between time-points, nor is there a significant difference between exercise groups.
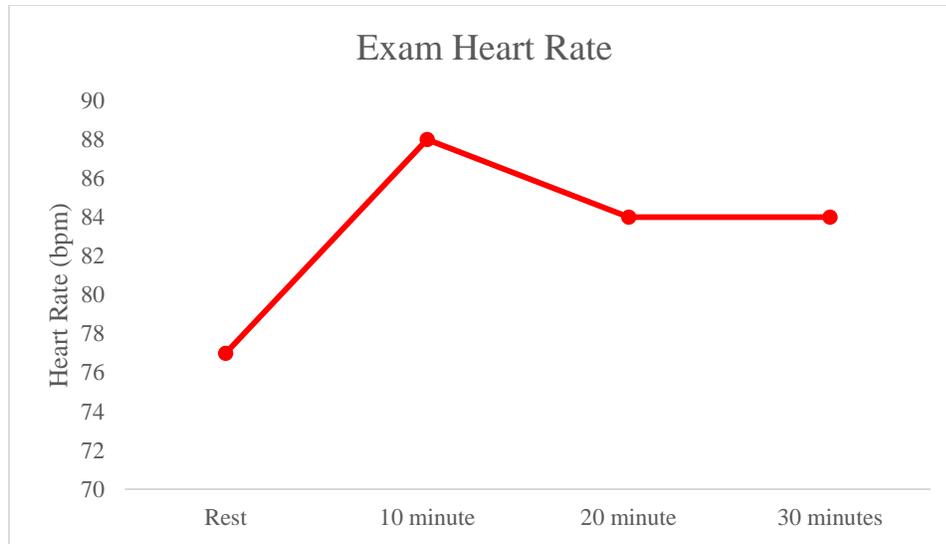
Figure 11. Exam HR. Mean HR (bpm) recorded at rest and every 10 minutes for the first 30 minutes of a final exam for all students.

Test Anxiety

A mixed methods analysis was employed to compare the stress/anxiety levels of the midterm exam to the stress levels of the final exam. Comparisons were made for each question of the Post Exam Evaluation Survey. There were no significant main effects nor interaction effects for questions 1, 2, 4, 5, 6, and 7 (table 1). There was a significant main effect for question 3 ($F_{1,18}$=6.290, p=0.022) but no interaction effect ($F_{2,18}$=0.122, p=0.886). This indicates that students as a whole felt more stressed/anxious during the midterm exam, but there was no significant difference in stress/anxiety between exercise groups.

Table 1. Statistical values of Post Exam Evaluation Survey

Survey Question	F-value	p-value
1. How prepared did you feel before beginning the exam?	$F_{1,18}=0.145$	0.708
2. How stressed/anxious did you feel *before* the exam?	$F_{1,18}=0.138$	0.714
3. How stressed/anxious did you feel *during* the exam?	$F_{1,18}=6.290$	0.022*
4. Did you perceive the exam to be easier, equal to, or harder than expected?	$F_{1,18}=0.061$	0.801
5. How stressed/anxious do you feel about receiving your exam grade?	$F_{1,17}=1.110$	0.307
6. Did you consume any stimulants (coffee, energy drink, etc.) the day of the exam?	$F_{1,18}=0.594$	0.451
7. Did you experience any changes in sleep patterns in the days leading up to the exam?	$F_{1,18}=0.982$	0.335

*indicates significant difference of $p<0.05$.
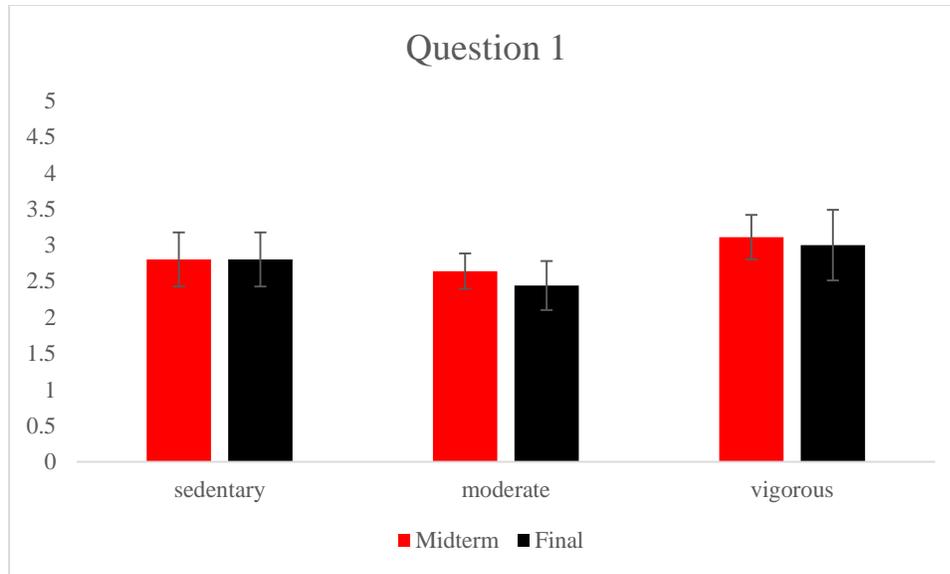
Figure 12. Question 1. After the midterm and final exam, students answered the question 'How prepared did you feel before beginning the exam?' on a scale of 1 to 5 with 1 indicating 'very prepared' and 5 indicating 'not prepared at all'.
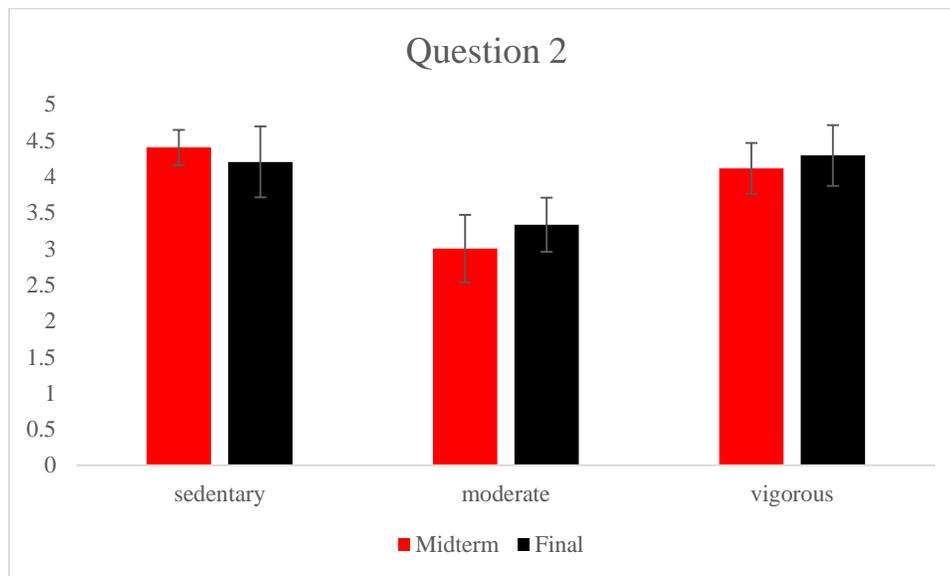
Figure 13. Question 2. After the midterm and final exam, students answered the question 'How stressed/anxious did you feel *before* the exam?' on a scale of 1 to 5 with 1 indicating 'no stress/anxiety' and 5 indicating 'high stress/anxiety'
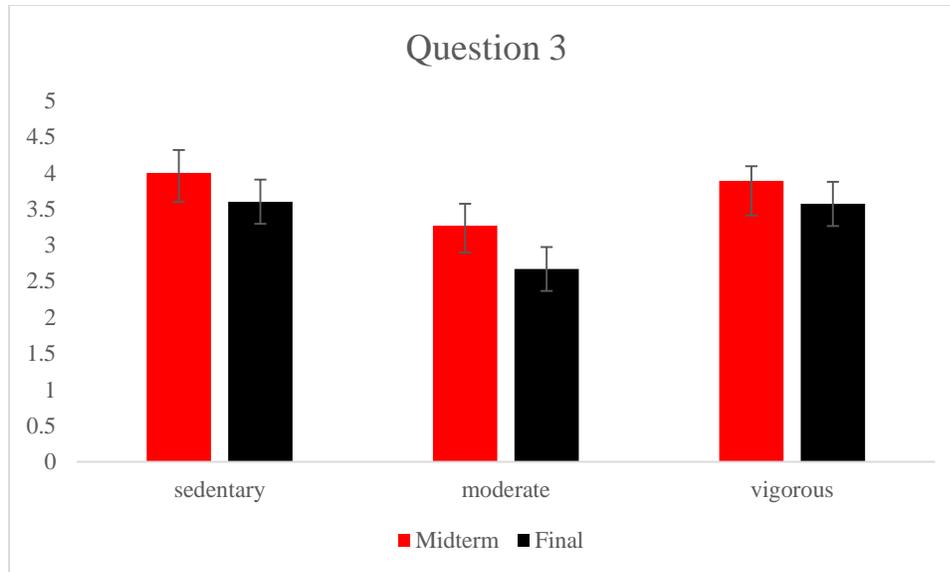
Figure 14. Question 3. After the midterm and final exam, students answered the question

'How stressed/anxious did you feel *during* the exam?' on a scale of 1 to 5 with 1

indicating 'no stress/anxiety' and 5 indicating 'high stress/anxiety'.
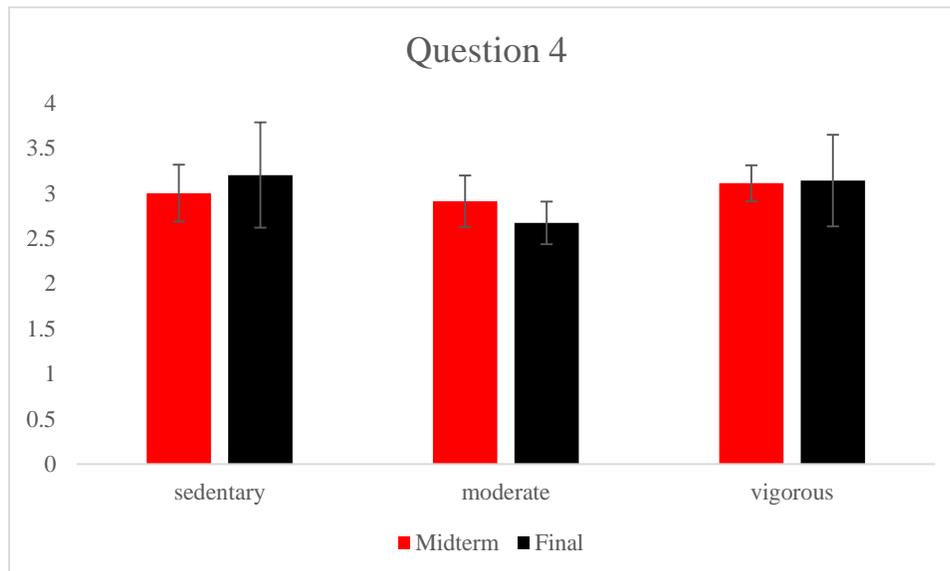
Figure 15. Question 4. After the midterm and final exam, students answered the question

'Did you perceive the exam to be easier, equal to, or harder than expected?' on a scale of

1 to 5 with 1 indicating 'much easier' and 5 indicating 'much harder'.
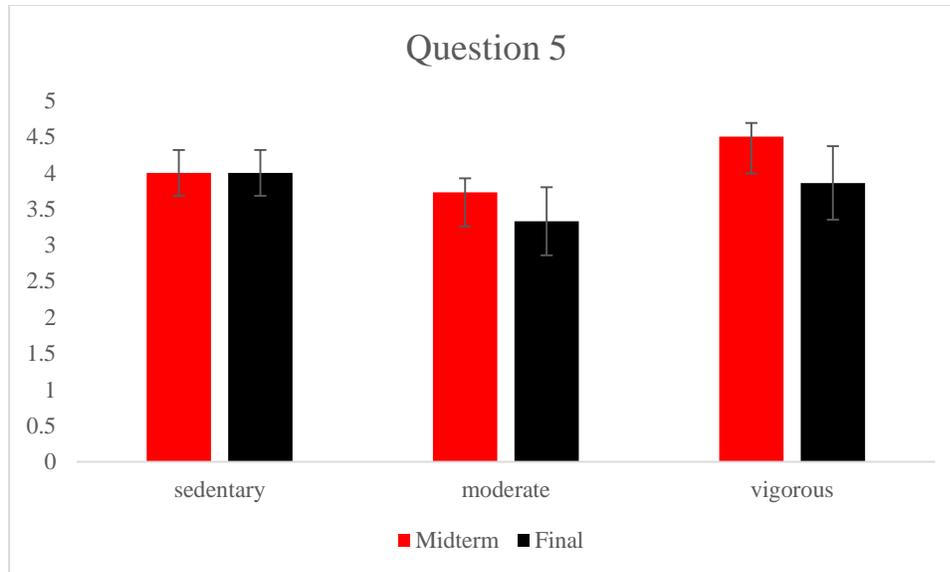
Figure 16. Question 5. After the midterm and final exam, students answered the question 'How stressed/anxious do you feel about receiving your exam grade?' on a scale of 1 to 5 with 1 indicating 'no stress/anxiety' and 5 indicating 'high stress/anxiety'.
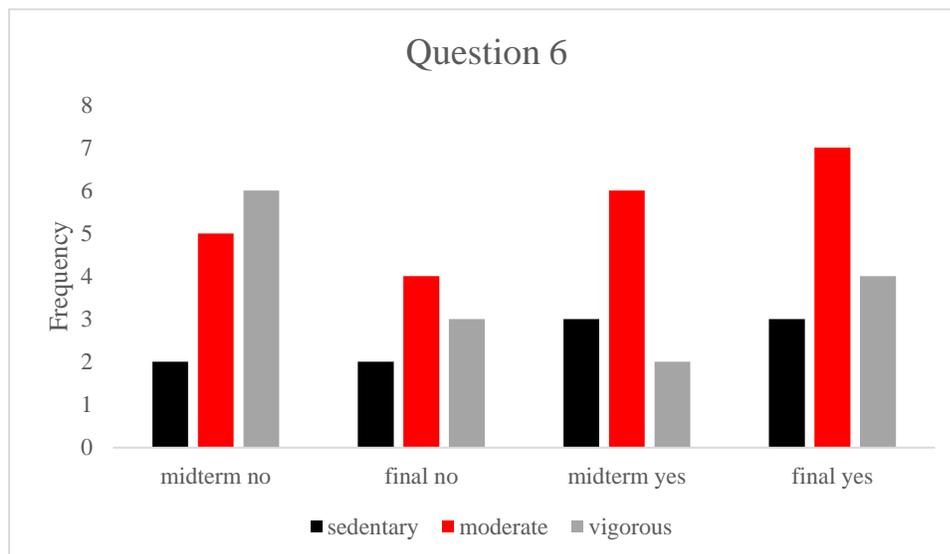
Figure 17. Question 6. After the midterm and final exam, students answered the question 'Did you consume any stimulants (coffee, energy drink, etc.) the day of the exam?'
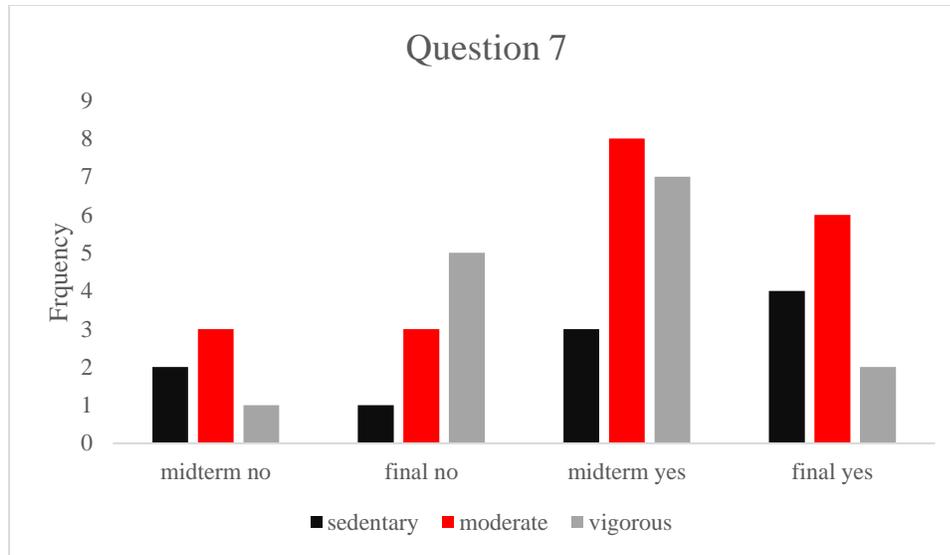
Figure 18. Question 7. After the midterm and final exam, students answered the question 'Did you experience any changes in sleep patterns in the days leading up to the exam?'

12 Week Stress

A mixed methods analysis comparing weekly stress levels between the sedentary, moderate, and vigorous exercise groups over 12 weeks revealed no significant main effects $F(2,27)=1.068$, $p=0.219$. This indicates that there was no significant difference in stress level between any of the weeks.
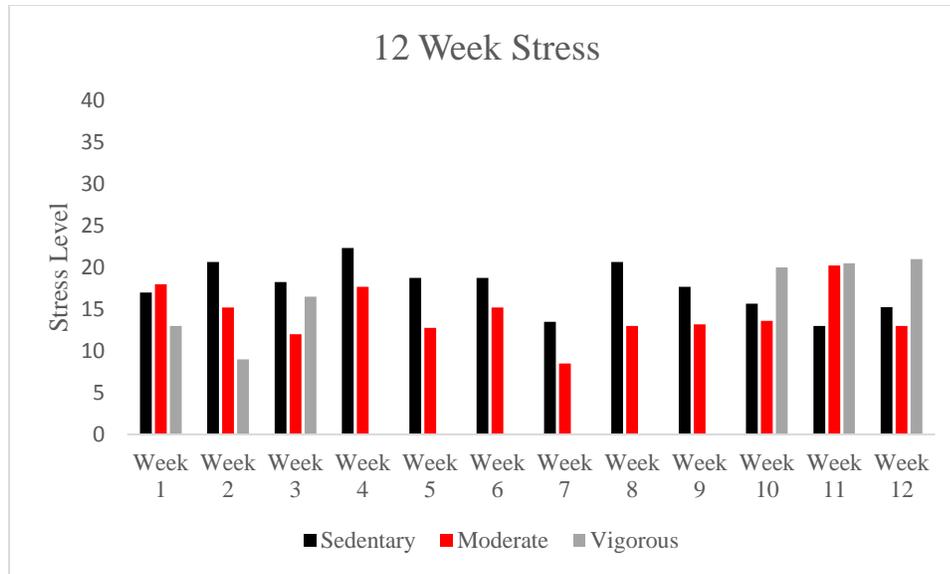
Figure 19. 12 Week Stress. Comparison of weekly stress levels between three exercise

groups. Stress levels were scaled from 0 to 40. Failure to identify surveys from the

vigorous exercise group resulted in an absence of data for weeks 4, 5, 6, 7, 8, and 9.

Chapter 5: Discussion

Introduction

The results of this study did not reveal an increase in chronic stress over the course of the semester as shown by a lack of significant differences in resting heartrate, resting blood pressure, IL-6 concentrations, weekly Perceived Stress Scale Surveys, and Disease Symptom Recall Surveys. Acute stress was detected by significant increases in heart rate and blood pressure during the final exam, but there were no significant differences in cortisol or blood glucose levels. A larger variation in activity level between participants is recommended for future studies in order to see the hypothesized results.

Chronic Stress

Results from this study failed to indicate a significant effect of time nor was there a significant difference between exercise groups for body mass or body fat percentage. The vigorous exercise group had a slightly lower, but not significant, mean body mass compared to the moderate and sedentary groups. This corresponds with the vigorous group also having the lowest body fat percentage, although the difference in body fat percentage was not significant. The slightly lower body fat percentage of the vigorous group corresponds with multiple studies that report lower body fat percentages with higher exercise levels (Medeirons, 2016; Sasaki, 1987). To see statistical differences in body fat percentage between exercise groups, it is recommended that future studies recruit participants with greater contrast in their exercise and sedentary habits. The participants recruited for this study were college students who walk across campus

between classes on a daily basis. With this in mind, even the participants in the sedentary group received exercise unintentionally. Additionally, the vigorous group was comprised of students exercising independently rather than the university athletes. Studies that do see changes in this variable recruit vigorous exercisers from the professional level (Gleeson, 2004).

Resting diastolic blood pressure, resting systolic blood pressure, and resting heart rate also all failed to be significantly different between exercise groups and across time-points. Lack of variation between groups is again thought to be attributed to similarities in the college lifestyle between participants. Because of a lack of statistical difference when comparing baseline blood pressure and heart rate to the following time-points, there is no physiologic evidence that students are physically affected by the stress of their workload over the course of a semester. The participants were third and fourth year students who were accustomed to college-level workloads. It is possible that a significant increase in these variables may have occurred throughout the semester if the students were less experienced first year students who had yet to develop coping strategies for dealing with the unfamiliar college environment. Additionally, resting heart rate and blood pressure were recorded immediately after the students turned in their exams. At this point, students stress levels had likely decreased, thereby lowering heart rate and blood pressure levels. To see a significant change from the baseline values as compared to the 'midterm' or 'final' time-point, recording these resting levels immediately before the students entered the exam may have recorded more of the physiologic change that occurs under acute stress.

The insignificant changes in physiologic variables over the semester is supported by the data from the weekly Perceived Stress Scale Surveys. The results from the current study failed to indicate a significant effect of time nor was a significant difference between exercise groups for the Perceived Stress Scale Surveys. There was not a reported increase in perceived stress as the semester progressed. While students may have had an increase in workload, they were confident in their abilities to manage their work, as indicated by the responses on the weekly surveys. When scoring the Perceived Stress Scale Surveys, the ability to manage stress negated the high scores of a busy week. Additionally, failure of participants to complete the surveys correctly resulted in an absence of data for the vigorous group during weeks 4, 5, 6, 7, 8, and, 9.

Furthermore, failure for stress to significantly increase over the semester also corresponds to the constantly low IL-6 levels, which were hypothesized to increase over time if stress levels were chronically high. Results from this study fail to indicate a significant effect of time nor was there a significant difference between exercise groups for IL-6. It is possible that college students do not encounter enough stress in their daily lives to increase IL-6 levels. It is also possible that, IL-6 being an indicator of chronic exposure to stress, the academic semester was not long enough to illicit a change in IL-6 concentrations. Measuring IL-6 over the course of a year or an entire college career may have been necessary to see changes in IL-6 concentrations caused by daily stressors.

Because there was no indication of increased stress levels throughout the semester as indicated by the above variables, it corresponds that there was not a difference in disease incidence throughout the semester as well. However, due to insufficient retention of participants for this particular variable, there was an inadequate amount of data to

conduct a between groups analysis for the weekly Disease Symptom Recall Survey. The figure displaying the collected data is located in appendix G. In addition to the lack of significant differences in stress levels, it is also likely that there was not enough variation between the exercise volumes in each group to be influential. Spence et al. (2007) and Gleeson et al. (2013) recruited elite athletes that completed 11 hours or more of aerobic exercise per week, which is greater than the approximately 6 hours per week that the vigorous group completed in the current study. Also, Nieman et al. (1990a) and Peters et al. (1983) followed participants that completed a marathon or ultramarathon, neither of which were completed by any participant during the current study. It can be reasonably inferred from the current study that the average college student that participated in the current study did not complete enough vigorous aerobic exercise to negatively influence disease susceptibility. Furthermore, it is proposed that several participants in the vigorous group reported severe 'persistent muscle soreness' and severe 'joint aches and pains' as symptoms of disease on the weekly Disease Symptom Recall Survey, when these symptoms were actually attributed to exercise. These symptoms were often reported on a routine number of days during the week, indicating that it followed an exercise plan. This could have artificially elevated the disease symptoms, indicating even lower scores from the vigorous exercise group.

Acute Stress

There was a significant increase in systolic and diastolic blood pressures during the exam when compared to resting values. It is regularly seen that HR and blood pressure increase before and during academic exams with the release of epinephrine (Curtis, 2002; Elwess, 2005; Florence, 2014; Padgett, 2003; Simic, 2011; Steptoe, 2011).

42

The current study supports these previous findings. Similar to blood pressure, HR increases with exposure to stress and epinephrine release. This was shown by the increase in HR during the exam compared to resting values; however, this increase was not considered significant.

Results from this study fail to indicate a significant increase in cortisol comparing resting levels to post exam levels. Johannsen et al. (2009) found that cortisol levels were significantly elevated during an exam compared to measurements taken four weeks post exam. However, the current study did not find significant differences in cortisol concentrations between any of the time-points or exercise groups. A confounding variable that was not controlled for that likely influenced the cortisol concentrations was the time of day each sample was taken. Cortisol levels naturally vary throughout the day (Adam, 2001), so efforts were made for each participant to visit the lab at the same time of day for each time-point. However, the exact appointment times varied within each participant due to the daily variation in class schedule. This may have influenced the overall outcome of the cortisol results. Additionally, Dickerson et al. (2004) found that academic exams with oral components provoked the highest degree of physiologic stress and cortisol release. It is possible that a significant increase in cortisol concentrations would have occurred if the students were giving oral presentations in front of an audience rather than a written exam.

Cortisol is responsible for regulating levels of glucose in the blood stream. Since blood glucose is dependent on cortisol release in times of stress, it makes sense that the lack of significant increase in cortisol levels did not cause a significant increase in blood glucose levels. Furthermore, eating a meal also causes an increase in blood glucose, and

all students ate at least one meal before data collection. It was advised that students eat approximately the same foods before each data collection so as not to influence blood glucose levels by dietary changes. However, it is difficult to ensure that the participants closely adhered to this request, further confounding the data. It would be interesting to see if blood glucose would have risen during the exam if students were in a fasted state; however, if they are not adjusted to performing in a fasted state, this could have negatively influenced the exam performance of the individual.

Results from this study failed to indicate significant differences when comparing the results of the Post Exam Evaluation Survey (appendix F) between the midterm and final exam except for Question 3. As a whole, students` stress score was (3.64 ± 0.172) during the midterm and (3.19 ± 0.255) during the final. It is possible that the midterm may have been the first exam in some classes, so students would not know what to expect during this exam. During the final exam, students were already familiar with the test format used in that class, thereby potentially lowering their anxiety levels (Kling, 2005; Yamin, 1989).

The survey also asked if there was a change in caffeine consumption or sleep patterns before the exams. There was not a significant change in caffeine consumption, indicating that any minor changes in HR or blood pressure were not attributed to increased caffeine intake. Also, sleep cycles influence cortisol levels (Kumari, 2009), but there was no significant change in sleep disturbances before the exams, so minor changes in cortisol were likely not attributed to sleep disturbances.

Conclusion

The results of this study conclude that students are not negatively impacted by academic stress nor by academic stress in combination with vigorous aerobic exercise volumes. This is indicated by the lack of variation between exercise groups across time-points for the majority of the investigated variables. While the participants were grouped by exercise volume, the exercise volume of the vigorous group was not high enough to see the hypothesized differences in disease incidents or inflammatory markers. Furthermore, all participants were college students in the Exercise Science Department that walked from class to class across campus and participated in weekly exercise science laboratory experiences that unavoidable involved some type of physical activity. With this in mind, even the sedentary group received some physical activity despite lack of intention. A future study may see the hypothesized results if there was a greater difference between activity habits between the sedentary and vigorous exercise groups, such as between Division I cross-country runners and sedentary office workers. Furthermore, difficulties with participant retention over the 12 week data collection and failure to answer all questions on the online surveys resulted in limited data analysis. Online survey data collection can be improved by inhibiting the submission of the survey until all questions are completed.

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APPENDIX A

24 Hour Dietary/Water Consumption Recall

Participant: _____

Visit #: _____

1. Have you consumed a meal today? ☐Yes ☐No
    If yes, how many? _____
    How long ago was your most recent meal? _____

2. Would you say that your food consumption over the past 24 hours has been typical of your normal daily habits? ☐Yes ☐No

3. Have you consumed any water today? ☐Yes ☐No
    If yes, how much? _____
    How long ago did you most recently consume water? _____

4. Would you say that your water consumption over the past 24 hours has been typical of your normal daily habits? ☐Yes ☐No

5. Have you consumed any caffeinated foods or beverages today? ☐Yes ☐No
    If yes, how recent was your most recent consumption? _____

6. Have you consumed any beverages containing alcohol today? ☐Yes ☐No
    If yes, how recent was your most recent consumption? _____

7. Have you used any form of tobacco (either cigarette or smokeless forms) today? ☐Yes ☐ No
    If yes, how recent was your most recent use of a tobacco product? _____

Date _____

Interaction between Academic Stress, Aerobic Exercise, and Immune Function

Participant Initials _____   **Participant Age _____**

Height (inches) _____   **Weight (lbs) _____**

Body Fat % _____   **BMI _____**

Blood Pressure _____   **RHR _____**

Resting Blood Glucose _____

Step Test

1 Min HR _____

2 Min HR _____

3 Min HR _____

4 Min HR _____

5 Min HR _____

6 Min HR _____   **5th/6th AVG _____**

APPENDIX C

Disease Symptom Recall Survey

Participant initials (first, middle, last) _____

In the past week, what has been your symptom severity? If selecting an option other than "none" how many days were the symptoms present?

1. Sore throat (none, light, moderate, severe) (number of days 1,2,3,4,5,6,7)
2. Mucus in throat (none, light, moderate, severe) (number of days 1,2,3,4,5,6,7)
3. Runny nose (none, light, moderate, severe) (number of days 1,2,3,4,5,6,7)
4. Cough (none, light, moderate, severe) (number of days 1,2,3,4,5,6,7)
5. Repetitive sneezing (none, light, moderate, severe) (number of days 1,2,3,4,5,6,7)
6. Fever (none, light, moderate, severe) (number of days 1,2,3,4,5,6,7)
7. Persistent muscle soreness (none, light, moderate, severe) (number of days 1,2,3,4,5,6,7)
8. Joint aches and pains (none, light, moderate, severe) (number of days 1,2,3,4,5,6,7)
9. Weakness (none, light, moderate, severe) (number of days 1,2,3,4,5,6,7)
10. Headache (none, light, moderate, severe) (number of days 1,2,3,4,5,6,7)

Gleeson, M. et al (2013). "Influence of training load on upper respiratory tract infection incidence and antigen-stimulated cytokine production. Scandinavian Journal of Medicine and Science in Sports, 23 (4), 451-457.

Perceived Stress Scale

Participant Initials (First, Middle, Last) _____

0=Never   1=Almost Never   2=Sometimes   3=Fairly Often   4=Very Often

1. In the last week, how often have you felt that you were upset because of something that happened unexpectedly? 0   1   2   3   4

2. In the last week, how often have you felt that you were unable to control the important things in your life? 0   1   2   3   4

3. In the last week, how often have you felt nervous and "stressed"? 0   1   2   3   4

4. In the last week, how often have you felt confident about your ability to handle your personal problems? 0   1   2   3   4

5. In the last week, how often have you felt that things were going your way? 0   1   2   3   4

6. In the last week, how often have you found that you could not cope with all the things that you had to do? 0   1   2   3   4

7. In the last week, how often have you been able to control irritations in your life? 0   1   2   3   4

8. In the last week, how often have you felt that you were on top of things? 0   1   2   3   4

9. In the last week, how often have you been angered because of things that were outside of your control? 0   1   2   3   4

10. In the last week, how often have you felt difficulties were piling up so high that you could not overcome them? 0   1   2   3   4

Cohen, S. et. al (1983). A global measure of perceived stress. Journal of Health and Social Behavior, 24, 386-396.

APPENDIX E

Seven Day Physical Activity Recall

Participant initials (first, middle, last) _____

1. Please record the number of minutes of light, moderate, and vigorous aerobic exercise completed each day during the past week.

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Light (RPE 9-11)							
Moderate (RPE 12-13)							
Vigorous (RPE14-17)							

2. Please record the number of minutes of strength training completed each day during the past week.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday

3. Compared to your physical activity over the past three months, was last week`s physical activity more, less, or about the same?
   a) More
   b) Less
   c) About the same

4. Were there any special circumstances concerning this week`s physical activity recall?
   a) None
   b) Injury all week
   c) Injury part of week
   d) Illness all week
   e) Illness part of week
   f) Other: _____

Sallis, JF, Haskell, WL, & Wood, PD. (1985). Physical activity assessment methodology in the five-city project. *American Journal of Epidemiology, 121*, 91-106.

APPENDIX F

Post Exam Evaluation

Participant Initials (First, Middle, Last)_____

1. How prepared did you feel before beginning the exam?
   Very prepared 1   2   3   4   5 Not prepared at all

2. How stressed/anxious did you feel *before* the exam?
   No stress/anxiety 1   2   3   4   5 High stress/anxiety

3. How stressed/anxious did you feel *during* the exam?
   No stress/anxiety 1   2   3   4   5 High stress/ anxiety

4. Did you perceive the exam to be easier, equal to, or harder than expected?
   Much easier 1   2   3   4   5 Much harder

5. How stressed/anxious do you feel about receiving your exam grade?
   No stress/anxiety 1   2   3   4   5 High stress/anxiety

6. Did you consume any stimulants (coffee, energy drink, etc.) the day of the exam?
   Yes   No

7. Did you experience any changes in sleep patterns in the days leading up to the exam?
   Yes   No

8. Please list any large academic stressors for all courses (exams, large project deadlines, presentations, etc.) that have already or will occur this week.

   _____

   _____

   _____

   _____

   _____

Disease Symptoms

A mixed methods analysis comparing disease symptoms between the sedentary, moderate, and vigorous exercise groups over 12 weeks was attempted. Collected data was too sporadic to make statistical comparisons between groups.
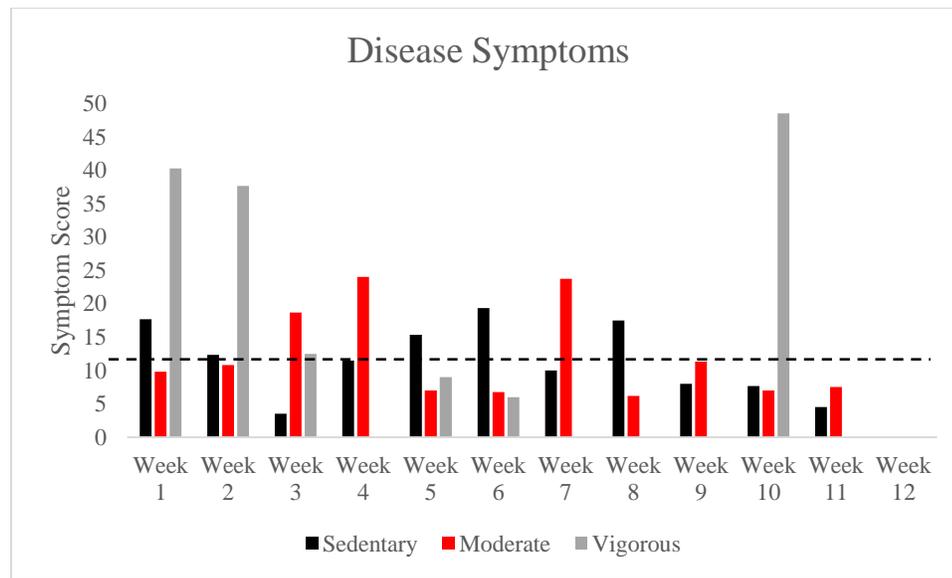
Figure 20. Disease Symptoms. Comparison of weekly disease symptoms between three exercise groups. A score of ≥ 12 indicated an upper respiratory tract infection. The dashed line indicates a score of 12. No data for week 12.