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LEUKOCYTE RESPONSIVENESS TO EXERCISE IN HCMV+ INDIVIDUALS

By

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Bachelor of Science in Kinesiology

University of Nevada, Las Vegas

2013

A thesis submitted in partial fulfillment of the requirements for the

Master of Science – Exercise Physiology

Department of Kinesiology and Nutrition Sciences

School of Allied Health Sciences

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May 2015

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We recommend the thesis prepared under our supervision by

Jared Nicholas Wilson

entitled

Leukocyte Responsiveness to Exercise in HCMV+ Individuals

is approved in partial fulfillment of the requirements for the degree of

Master of Science - Exercise Physiology
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May 2015

Abstract

Introduction: Emerging research suggests that the lymphocyte immune response during exercise is amplified in individuals who are positive for human cytomegalovirus (HCMV+). However, the responses of monocytes and neutrophils in HCMV+ individuals are unknown. HCMV, a type of herpes virus, infects 50% or more of the adult population in the United States. This virus can become a cause for concern in individuals who have a compromised immune system, which has been shown to occur after high-intensity exercise.

Purpose: The purpose of this study was to characterize the lymphocyte, monocyte, and neutrophil responses to exercise in HCMV+ individuals.

Methods: Participants were male (n = 7) and female (n = 9), between the ages of 18 and 44 (26.38 ± 8.94) years old. Participants were either positive (HCMV+) or negative (HCMV-) for HCMV. Participants visited the Exercise Physiology laboratory on three separate occasions: (1) HCMV screening, (2) 100% VO_{2max} test, (3) 80% VO_{2max} run for 20 minutes. Four blood samples were taken during the third visit: (1) Pre-exercise, (2) Post-exercise, (3) 30 minutes post-exercise, and (4) 60 minutes post-exercise. 2 (virus status) x 4 (sampling condition) mixed-model factorial ANOVA procedures with repeated measures on sampling condition were performed on absolute and relative circulating lymphocytes, monocytes, and neutrophils.

Results: No interactions of absolute or relative values for HCMV status and time were found for any of the three leukocyte subsets. Significant main effects for time for both absolute (neutrophils: $p < .001$; monocytes: $p < .001$; lymphocytes: $p < .001$) and relative (neutrophils: $p < .001$; monocytes: $p < .001$; lymphocytes: $p < .001$) values were seen for all leukocyte

subsets regardless of virus status. Significant differences for absolute and relative values were seen between sampling conditions for all leukocyte subsets.

Discussion: The effects of high-intensity exercise on circulating monocyte and neutrophil volumes in the post-exercise period were the main findings of this study. We report for the first time that HCMV status does not affect circulating neutrophil responses to high-intensity exercise, though exercise-induced neutrocytosis (a significant increase in neutrophil volume) is seen during the post-exercise and 60 minutes post-exercise sampling conditions, regardless of HCMV status. There is no HCMV effect on circulating monocyte responses to exercise, though exercise-induced monocytosis was seen during the post-exercise sampling condition regardless of HCMV status.

Acknowledgments

I wish to express my sincere thanks to my advisor, Dr. James Navalta, for his mentorship throughout this research project.

Dedication

I dedicate this document to my late father, Clarence Foster Wilson.

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Chapter 1

Introduction

Exercise induces acute physiological changes, especially in cells of the immune system. Emerging research suggests that the lymphocyte immune response during exercise is significantly increased in individuals who are positive for human cytomegalovirus (HCMV+). [1] Specifically, certain lymphocyte subsets (namely, CD8+ T cells) have been shown to increase in circulating volume, termed lymphocytosis, as exercise intensity increases, and undergo a significant drop in cell volume for up to two hours in the post-exercise period, termed lymphocytopenia. [2-4] However, characterization of the circulating monocyte response to exercise is unknown and there are conflicting findings in the literature regarding circulating neutrophil responses in the post-exercise period. [5, 6] In order to determine the consequences of high-intensity exercise on circulating leukocytes, characterizing the effects of exercise on the aforementioned leukocyte subsets is necessary.

Approximately 50-80% of adults in the United States have been infected with human cytomegalovirus (HCMV), depending on the population studied. [7] Human cytomegalovirus, also known as human herpes virus-5, is a common virus that is related to other herpes viruses, including herpes simplex-1 and -2, varicella-zoster, and Epstein-Barr. [7] Although the virus remains dormant in healthy individuals, when the immune system is compromised, HCMV may be a cause for concern. To date, there is no vaccine against this infection, which costs the American health care system $1.86 billion annually. [8]

With regard to the obesity epidemic in the United States, regular exercise represents an attractive mode of prevention. Because of this, one of the goals of Healthy People 2020 is to increase the proportion of adults who meet current Federal physical activity guidelines for

aerobic physical activity of moderate intensity from 43.5% to 47.9% of the population. However, emerging evidence suggests that individuals with HCMV (HCMV+) display a significantly amplified lymphocyte response following bouts of exercise compared with individuals who are negative for HCMV (HCMV-). [1] The response of monocytes and neutrophils in the post-exercise period in HCMV+ individuals is yet to be investigated. Because of the possibility for individuals to be immunocompromised in the post-exercise period, increasing the physical activity in individuals with HCMV may be problematic. While the post-exercise response in HCMV+ individuals has not been studied, it is reasonable to postulate that a greater decrease in immune cell volume (indicating an immunocompromised state) would be present, temporarily placing them at risk until circulating immune cell volume can return to a homeostatic state. Therefore, during and post-exercise, HCMV+ individuals risk an immune challenge that could compromise their health.

Understanding the functions and roles of lymphocytes, monocytes, and neutrophils is necessary in order to characterize the possible risks associated with volume fluctuations within these leukocyte phenotypes.

Lymphocytes

Lymphocytes are responsible for mediating and carrying out responses of the adaptive immune system. Subtypes of lymphocytes include natural killer cells (function in cell-mediated, innate immunity), T cells (function in cell-mediated, adaptive immunity), and B cells (function in anti-body-mediated, adaptive immunity).

Monocytes

Monocytes are part of the innate immune system. These cells fulfill two distinct roles: 1) replenish sentinel macrophages under homeostatic states and 2) respond to inflammation

signals at sites of infection/damage within tissues and coordinate the immune response from these sites.

Neutrophils

Neutrophils form an essential part of innate immunity. They respond to inflammatory signals and are involved in phagocytosis of invading pathogens and damaged/apoptotic cells.

Purpose

In HCMV+ individuals, characterizing the leukocyte response is necessary when attempting to explain how one may be at greater risk immediately following a bout of exercise. The purpose of this study was to characterize the responses of total circulating lymphocytes, monocytes, and neutrophils to exercise in HCMV+ individuals. The study outcomes will improve scientific knowledge and practice, not only in terms of exercise training, but also potentially in medical practice and therapeutic settings.

Hypotheses

Null Hypothesis 1: There will be no significant change in lymphocyte response in the post-exercise period in HCMV+ individuals when compared to the pre-exercise sampling condition.

Alternate Hypothesis 1: There will be a significant change in the lymphocyte response in the post-exercise period in HCMV+ individuals when compared to the pre-exercise sampling condition.

Null Hypothesis 2: There will be no significant change in lymphocyte response in the post-exercise period regardless of HCMV status when compared to the pre-exercise sampling condition.

Alternate Hypothesis 2: There will be a significant change in the lymphocyte response in the post-exercise period regardless of HCMV status when compared to the pre-exercise sampling condition.

Null Hypothesis 3: There will be no significant change in the monocyte response in the post-exercise period in HCMV+ individuals when compared to the pre-exercise sampling condition.

Alternate Hypothesis 3: There will be a significant change in the monocyte response in the post-exercise period in HCMV+ individuals when compared to the pre-exercise sampling condition.

Null Hypothesis 4: There will be no significant change in the monocyte response in the post-exercise period regardless of HCMV status when compared to the pre-exercise sampling condition.

Alternate Hypothesis 4: There will be a significant change in the monocyte response in the post-exercise period regardless of HCMV status when compared to the pre-exercise sampling condition.

Null Hypothesis 5: There will be no significant change in the neutrophil response in the post-exercise period in HCMV+ individuals when compared to the pre-exercise sampling condition.

Alternate Hypothesis 5: There will be a significant change in the neutrophil response in the post-exercise period in HCMV+ individuals when compared to the pre-exercise sampling condition.

Null Hypothesis 6: There will be no significant change in the neutrophil response in the post-exercise period regardless of HCMV status when compared to the pre-exercise sampling condition.

Alternate Hypothesis 6: There will be a significant change in the neutrophil response in the post-exercise period regardless of HCMV status when compared to the pre-exercise sampling condition.

Delimitations

- Participants were male and female.
- Male participants were between the ages of 18 and 44.
- Female participants were between the ages of 18 and 43.
- Participants filled out the American College of Sports Medicine (ACSM) pre-participation screening questionnaire and were required to be in the "Low Risk" category for cardiovascular disease according to the questionnaire.
    - The participants were required to be able to answer "None of the above" on the questionnaire, check nothing in the first section (history, symptoms, other health issues), or check no more than one cardiovascular risk factor, and are a male younger than 45 years of age or a female younger than 55 years of age, to be considered "Low Risk" according to the ACSM algorithm.
- Participants were excluded from the study based on the following criteria: (a) they were pregnant, (b) had implantable devices such as a pacemaker or automatic implantable cardioverter defibrillators (AICD), (c) orthopedic (acute or chronic musculoskeletal injury), cardiovascular (coronary artery disease), respiratory (chronic obstructive pulmonary disease or asthma), and metabolic conditions (diabetes), and

(d) currently smokes or quit smoking less than six months prior to filling out of the questionnaire.

- Participants were either positive for HCMV (HCMV+) or negative for HCMV (HCMV-).
- Eight participants positive for HCMV (HCMV+) and eight participants negative for HCMV (HCMV-) were used in the study.
- Participants performed a VO_{2max} test.
- Participants performed at 80% of their VO_{2max} for 20 minutes.
- A total of five blood draws per participant were completed over the course of the study.
    - One blood draw for initial screening of HCMV.
    - Four blood draws (at rest prior to exercise, immediately post-exercise, 30 minutes post-exercise, and 60 minutes post-exercise) during the visit in which participants ran at 80% VO_{2max} for a total of four blood draws.

Definition of Terms

- Human cytomegalovirus (HCMV): A type of herpes virus that usually produces very mild symptoms in an infected person but may cause severe neurological damage in people with weakened immune systems and in newborns.
- Leukocytes: The cells of the immune system are responsible for the identification and elimination of pathogens. The five kinds of leukocytes that play a role in these processes are monocytes, neutrophils, eosinophils, basophils, and lymphocytes.
- Lymphocyte: A type of leukocyte that is responsible for the mediation and actions of the adaptive immune system. The adaptive immune system is responsible for

remembering and identifying antigens whose corresponding pathogens have already been introduced into the immune system.

- Exercise-Induced Lymphocytopenia: The condition of having low concentrations of lymphocytes in the blood following exercise.
- Exercise-Induced Lymphocytosis: The condition of having elevated levels of lymphocytes in the blood during exercise.
- Monocyte: A type of leukocyte, which is part of the innate immune system, that are responsible for responding to inflammatory responses and phagocytizing dying or infected cells. Once monocytes enter a tissue, they become sentinel macrophages that remain within that tissue.
- Neutrophil: A type of leukocyte, which is part of the innate immune system, that is responsible for mediating the earliest phases of inflammatory reactions.

Chapter 2

Literature Review

Human cytomegalovirus, also known as human herpes virus-5, is a common virus that is related to other herpes viruses, including herpes simplex-1 and -2, varicella-zoster, and Epstein-Barr.[7] Approximately 50-80% of adults in the United States have been infected with human cytomegalovirus (HCMV), depending on the population studied. [7] When the immune system becomes suppressed in individuals with HCMV (HCMV+), there is an increased likelihood to develop symptoms such as fever, hepatitis, pneumonia, seizures, and coma. [7] Due to the prevalence of infection in the adult population and the severity of symptoms in an immunosuppressed state, it is imperative to identify the mechanisms responsible for immunosuppression in HCMV+ individuals.

Characterization of the effects that high-intensity exercise has on specific cells of the immune system is necessary in order to identify the mechanisms involved in the fluctuations of immune cell volume in both HCMV+ and HCMV- individuals. As mentioned previously, there is currently no literature regarding circulating monocyte responses to exercise and there is a conflict in the literature with respect to neutrophil responses to high-intensity exercise, leading this literature review to focus primarily on how circulating lymphocytes are affected by exercise.

Lymphocytes are the cells of the adaptive immune system that are responsible for the identification of antigens and coordination of the proceeding immune response. The two types of lymphocytes are T cells and B cells. The two types of T cells discussed in detail within this literature review will be CD4+ (helper) and CD8+ (cytotoxic) T cells. CD8 lymphocytes can be further divided into phenotypes: naïve, central memory, effector

memory, and terminally differentiated cells. Naïve cells are T lymphocytes that have not yet been exposed to an antigen, central and effector memory cells are T lymphocytes that have been exposed to an antigen and are the primary defense against antigens, and terminally differentiated cells are T lymphocytes that are nearing their programmed cell death.

Lymphocytopenia occurs in one of two ways: cellular migration, which is the movement of cells out of the vasculature and into the target tissue(s), and apoptosis, which is the process of cell death that is programmed/controlled by intracellular mechanisms. The aforementioned lymphocyte classifications and cellular processes, as well as HCMV and its effects, will be discussed relative to exercise-induced immunosuppression.

Lymphocytosis

Exercise has been shown to cause an immediate mobilization of lymphocytes in the blood, a process known as lymphocytosis. [9-12] Lymphocytosis has been shown to be greatest among those lymphocytes with a cytotoxic phenotype, namely CD8+ T lymphocytes. [13, 14] Though CD8+ T lymphocytes elicit the greatest response to exercise, an overall increase in lymphocyte concentration is likely due to the mobilization of many additional lymphocyte populations into the vasculature, such as CD4+ T cells, CD19+ B cells, CD 16+ natural killer cells, and CD56+ natural killer cells. During exercise, the ratio of CD4-to-CD8 T lymphocytes decreases, demonstrating a greater increase in CD8+ T lymphocytes than CD4+ T lymphocytes. [13] Immediately following both resistance and endurance exercise protocols, a significant decrease in lymphocytes within the vasculature, termed lymphocytopenia, has been a prevalent finding within the literature. [1], [3, 4], [10-13], [15-23]

Apoptosis

Apoptosis is an integral process within cellular physiology. It plays an essential role in healthy organ and cellular development, as well as the maintaining of homeostasis. [24] Apoptosis fulfills this role via deletion of cells that are nonfunctional, harmful, misplaced, and abnormal. [25] Apoptosis typically affects individual cells, rather than clusters of cells or organs, which fall under the aforementioned categories. [24] This process initially begins via cell shrinkage, which is when the cytoplasm condenses and the nucleus becomes fragmented. After this process occurs, the cell breaks up into several vesicles containing entirely intact organelles and fragments of the nucleus. [26] These vesicles are then absorbed by proximal cells or macrophages. [27] When apoptosis is interrupted or impeded by the disturbance of signaling pathways, cancers, infectious diseases, autoimmune diseases, and neurodegenerative diseases have been seen to be outcomes of such disturbances. [28] Since both resistance [22] and endurance [14, 15], [29], [30, 31] exercise have been shown to induce immunosuppression in humans, understanding the effects exercise has on apoptosis specifically is necessary when characterizing the immunosuppressive response.

A common finding in the literature is that all leukocyte subsets increase with exhaustive exercise. [32, 33] Upon cessation of exercise, leukocyte subsets do not respond uniformly as they do during exercise. For example, granulocytes (neutrophils, eosinophils, and basophils) can remain increased for many hours post-exercise, whereas lymphocyte levels in the blood decrease quickly to levels at or below pre-exercise levels. [33, 34] Characterizing post-exercise lymphocytopenia is a focus of many investigators to date.

Exercise and Lymphocyte Apoptosis

Currently, the literature presents conflicting results with regard to apoptosis and post-exercise lymphocytopenia. Steensberg et al. (2002) conducted a study in which eleven healthy male participants ran on a treadmill for 2.5 hours at 75% of their VO_{2max} and blood samples were taken in order to test for apoptotic lymphocytes post-exercise. The blood samples were extremely comprehensive, consisting of nine total blood draws: before the initiation of the protocol, 30 minutes and 90 minutes into the exercise protocol, immediately post-exercise, and one, two, four, eight, and 24 hours post-exercise. Though the percentage of early apoptotic lymphocytes in the circulation was increased two hours after exercise, the total number of early apoptotic cells were not affected with regard to exercise. This finding is consistent with other studies in which apoptosis was determined to not be affected in the post-exercise period. [35-37] Despite this, other investigators have shown apoptosis to be a factor in post-exercise lymphocytopenia. [4], [17], [29], [38]

A study conducted by Navalta, Sedlock, and Park (2007) found that exercise intensity does affect exercise-induced lymphocyte apoptosis. This study found that lymphocyte apoptosis begins to occur between 40% and 60% of an individual's VO_{2max}. [17] Another study conducted by Navalta et al. (2013) found that absolute changes in cell count for CD4+ and CD8+ T lymphocytes did not change significantly at 76% and 87% of VO_{2max}, but there was a significant change in absolute cell count of both subsets at exhaustion (100% VO_{2max}). However, when absolute changes from baseline were calculated, there was a significant change in CD4+ lymphocyte apoptosis at 76% and 87% of VO_{2max}. CD8+ cells saw a significant change when compared to baseline values at 76% of VO_{2max}, but not at 87% of

VO_{2max}.[4] These studies illustrate that exercise elicits significant differences in lymphocyte apoptosis, assuming an intensity threshold is met.

Exercise and Neutrophil Apoptosis

Studies investigating the effects of exercise on neutrophil apoptosis have conflicting findings. Syu et al. (2011) showed that acute incremental exercise induced an increased magnitude of neutrophil apoptisis. [6] This same group conducted a similar study in which participants performed repeated bouts of moderate exercise for 30 minutes per day, 5 days per week at 60% VO_{2max} and this protocol delayed neutrophil apoptosis, as opposed to accelerating it. [5] Mooren et al. (2012) found evidence that supported the latter study in which neutrophil apoptosis was delayed after a marathon run, intensive exercise, downhill running, and resistance exercise. [39]

Lymphocyte Apoptosis Markers

Within the literature, various biochemical markers and methods are used for the purpose of identifying lymphocytes undergoing apoptosis. Though many biochemical markers are used to identify the three types of lymphocytes (T cells, B cells, and natural killer cells), Annexin V+ is a marker that is predominantly used in recently published studies to identify apoptosis in T lymphocytes. [3, 4], [18], [22, 23], [29], [32], [35, 36] In addition to using Annexin V+ to identify apoptosis in T lymphocytes, morphological techniques [17], [19], [20], [29], [38], [40] and identification of mitochondrial transmembrane potential (MTP) [41] have been used in order to assess lymphocyte apoptosis.

Navalta et al. (2009) utilized morphological techniques in order to identify lymphocyte apoptosis, both before and immediately after exercise in a group of endurance-trained athletes. [16] The authors state that methods that utilize biochemical markers to

identify lymphocyte apoptosis report significantly lower values of apoptosis following exercise when compared to the utilization of morphological techniques. It is stated that this may be due to the fact that biological markers assess apoptosis during a single stage of the cell-death process (usually Annexin V+ in the early stage of the process), creating a possibility that these techniques may not be sensitive enough to detect an apoptotic response to exercise. To illustrate this further, morphological techniques have reported apoptotic indexes (the number of apoptotic lymphocytes divided by the total number of lymphocytes counted) [16] between 19.1% and 51.5%, [17], [38] whereas techniques utilizing biochemical markers have reported apoptotic index values between 2.2% and 2.6%. [32], [35] Though the morphological techniques clearly elicit a higher observed apoptotic index scores, the nature of the process itself is a cause for concern.

Two characteristics associated with morphological analysis have been pointed out in the literature as short-comings, and they have actually been used to justify the utilization of biochemical markers in place of morphology. Navalta, Mohamed, El-Baz et al. (2010) stated that the morphological process may not provide accurate measurements due to the fact that the time necessary to prepare the samples for assessment can result in inaccurate measurements. [40] Navalta et al. (2009) measured the time it took to identify at least 100 lymphocytes per slide for both pre- and post-exercise blood smears.[16] The average time it took for the identification process for the pre-exercise blood smears was just over 40 minutes, and the average time it took for the post-exercise blood smears was around 30 minutes. Since exercise-induced immunosuppression is time-sensitive, the 30-40 minutes needed to quantify the apoptotic response can cause findings to be inaccurate. The second limiting characteristic of morphological analysis is the inherent subjective nature of the

process, meaning that the identification of the apoptotic lymphocytes is entirely dependent on the abilities and reliability of the investigator/technician counting the cells. These two limitations of morphological techniques are in opposition to the characteristics of biochemical techniques.

Annexin V+ is a type of phospholipid-binding protein that binds to phosphatidylserine, which is a protein complex that is flipped from the inner monolayer of the cell to the outer monolayer, where it serves as a marker for phagocytosis by macrophages and neighboring cells. Annexin V+ is a popular biochemical marker used to identify apoptotic cells in the post-exercise period. [3, 4], [18], [22, 23], [29], [32], [35, 36] Unlike morphological techniques, the utilization of Annexin V+ to identify apoptotic T lymphocytes bypasses lengthy identification times and the possibility of human error.

Lymphocyte Migration

Cellular migration is a process in which individual cells move in and out of the vasculature by way of cellular adhesion to the endothelium. This migration of cells to and from lymphoid pools is for the purpose of maintaining immunity. [3]

Exercise and Lymphocyte Migration

It has been established that apoptosis is responsible for the immunosuppression that is seen in the post-exercise period, but recent studies have provided evidence that apoptosis is not the only mechanism contributing to post-exercise lymphocytopenia. A study conducted by Simpson et al. (2007) concluded that cellular migration was the primary mechanism responsible for the post-exercise lymphocytopenia seen after bouts of level and downhill running. [42] This finding led future investigations to begin quantifying both apoptosis and

migration in the post-exercise period, as opposed to looking at these mechanisms in isolation. [3, 4], [18], [22, 23]

Investigations employing anaerobic, [3] aerobic, [4], [18] and resistance training [22, 23] have measured both apoptosis and cellular migration as causes of post-exercise immunosuppression. Friedmen et al. (2012) investigated the effects of intermittent anaerobic Wingate cycling on post-exercise lymphocytopenia. It was found that migration significantly increased in the post-exercise period in cytotoxic CD8+ T cells and all other T cells measured. In addition, little evidence was found that the particular anaerobic protocol used in this study had any effect on apoptosis in any of the T lymphocyte subsets measured. [3] Further studies utilizing anaerobic exercise protocols are needed in order to further investigate this phenomenon that seems contrary to findings in studies employing aerobic exercise protocols.

Cellular migration during aerobic exercise has been studied more extensively than during anaerobic exercise. Navalta et al. (2013) investigated the effects of both moderate-intensity (76% VO_{2max}) and high-intensity (87% and 100% VO_{2max}) aerobic exercise on apoptosis and migration in CD4+, CD8+, and CD19+ T lymphocyte subsets. [4] It was found that absolute changes for apoptosis of CD4+ and CD8+ T lymphocytes significantly increased at 76% VO_{2max}, but not at 87% or 100% VO_{2max}. Conversely, significant increases were seen for cellular migration in CD4+ T cells at 87% VO_{2max} and in CD8+ T cells at 87% and 100% VO_{2max}. These results show CD4+ and CD8+ T cells to be prone to apoptosis with moderate exercise, and as exercise intensity increases, cellular migration becomes the primary mechanism of post-exercise immunosuppression.

A study conducted by Navalta et al. (2014) investigated the effects of high-intensity interval runs to exhaustion over consecutive days on lymphocyte subset apoptosis and cellular migration. [18] Participants performed three consecutive days of intermittent running protocols at 100% and 50% of VO_{2max} for 30 seconds each. The results indicate that CD4+ T cells responded the least to this type of exercise protocol with significant results only occurring on the third day of the protocol. CD8+ T cells responded to a greater extent than CD4+ T cells over the course of the protocol, and CD8+ T cells were more prone to cellular migration than apoptosis due to the high intensity of the exercise protocol. These studies provide evidence for typical trends that T lymphocyte subsets seem to follow immediately following aerobic activity. CD4+ cells respond in an immunosuppressive fashion to a greater degree at moderate exercise intensities than at higher exercise intensities and are more prone to undergo apoptosis rather than cellular migration in the post-exercise period. Conversely, CD8+ cells respond more to higher intensity exercise and typically undergo cellular migration to a greater extent than apoptosis in the post-exercise period.

Prestes et al. (2014) and Pereira et al. (2012) investigated the effects of resistance training on post-exercise apoptosis and migration. [22, 23] Both studies were conducted by the same research group, so the protocols were fairly similar. Prestes et al. (2014) focused on the effects of different types of resistance training on both immunosuppressing mechanisms. Participants performed a hypertrophy protocol that consisted of performing exercises at their respective 10 repetition maximums and performed an endurance protocol, which was defined as performing exercises at 60% of their respective 10 repetition maximums. Both protocols utilized a one-minute rest interval between sets. Pereira et al. (2012) focused on the effects of different lengths of rest intervals (one minute and three minutes) between sets of exercises

mirroring the hypertrophy protocol of Prestes et al. (2014). Both studies found that neither exercise protocol, [23] nor rest interval length, [22] had significantly different effects on CD8+ T cells in the post-exercise period. Prestes et al. (2014) reported that apoptosis was significantly greater in CD4+ T cells in the hypertrophy protocol than in the endurance protocol. Additionally, cellular migration was greater immediately following exercise in the endurance protocol than in the hypertrophy protocol. Pereira et al. (2012) reported that CD4+ T cells underwent greater amounts of apoptosis and cellular migration immediately following exercise during the modality utilizing one-minute rest intervals versus the modality utilizing three-minute rest intervals. These two investigations provide evidence that, with respect to resistance training, responses of CD8+ T cells in the post-exercise period are less dependent on protocol characteristics than CD4+ T cells.

Lymphocyte Migration Marker

Cellular migration is typically measured via the utilization of the biochemical marker CX_3CR1 within the literature investigating mechanisms of post-exercise immunosuppression. CX_3CR1, otherwise known as the fractalkine receptor, is a transmembrane protein and chemokine involved in the adhesion and migration of leukocytes. The technique utilizing this protein to identify cellular migration has been used in studies investigating the effects of anaerobic, [3] aerobic, [4], [18] and resistance training on cellular migration out of the vasculature in the post-exercise period. [22, 23] Throughout the aforementioned literature, this marker has proven to be a reliable and accurate method of assessing cellular migration in the post-exercise period.

Human Cytomegalovirus

Human cytomegalovirus (HCMV) is one of five herpes viruses found in humans (the others being herpes simplex 1, herpes simplex 2, varicella zoster, and Epstein-Barr). [7] HCMV persists following primary infection and maintains the possibility to reactivate throughout life. It infects anywhere from 50% to 80% of adults, depending on the population being investigated. [7] HCMV has been a research focus in recent years due to its ability to cause severe symptoms and even death in an immunosuppressed state. Though exercise can lead to a significant amount of immunosuppression in individuals with HCMV, immunologists have also focused on the effects HCMV can have on bone marrow transplant recipients and those with acquired immunodeficiency syndrome (AIDS). [7] Though the nature of immunosuppression is not unique among viruses, the prevalence and ability to produce clinical problems in infected populations have made it a topic of interest among researchers in varying fields.

In 1999, the Institute of Medicine ranked a vaccine to prevent HCMV at the highest priority due to the economic costs that could be avoided and the years of life and disability that would be saved by the development of a vaccine. [8] HCMV is a cause of mononucleosis and fatal infections in immunosuppressed individuals, as well as deafness and neurological diseases of fetuses and infants. The United States, countries in Europe, and other developed countries have reported that HCMV is the leading infectious cause of damage in the developing fetus in utero. [8]

Regarding exercise, HCMV has been shown to cause increased immunosuppression with regard to specific T cell subsets when compared with individuals who are not infected with the virus. [1] Turner et al. (2010) conducted a study in which half of the subjects were

HCMV+ and half were HCMV-. The investigators had them run for 60 minutes at 80% of their VO_{2max} and collected blood samples before, one minute prior to cessation of exercise, 15 minutes, and 60 minutes post-exercise. Specific phenotypes of CD4+ and CD8+ T cells were analyzed (naïve, central memory, effector memory, and terminally differentiated phenotypes). HCMV+ individuals exhibited nearly twice the mobilization during exercise and lymphocytopenia post-exercise of CD8+ T cells than HCMV- individuals. [1] CD4+ T cells did not exhibit any significant differences during or after exercise between HCMV+ and HCMV- groups. With regard to the characterization of the response of the CD8+ phenotypes, effector memory and terminally differentiated cells showed the greatest amounts of increases during exercise and egress post-exercise. Post-hoc analysis of these findings showed that exercise intensity, age, BMI, physical activity, fitness level, and heart rate were not responsible for the differences between HCMV+ and HCMV- groups, indicating that infection history was a determinant of the immunological response to exercise. Additionally, the lack of CD4+ responses between groups was of no surprise since CD4+ T cells typically respond to exercise to a lesser degree than CD8+ T cells. Although this study did examine the responses of CD4+ and CD8+ T cell phenotypes, it did not analyze the specific contributions of apoptosis and migration to immunosuppression in the post-exercise period.

Protocol and Participant Delimitations

Exercise Intensity

A key factor influencing exercise-induced lymphocytopenia is exercise intensity. Since exercise can induce significant immunosuppression post-exercise, it is logical to conclude that there must be a threshold during a bout of exercise at which a significant increase in immunosuppression will occur post-exercise. Navalta, Sedlock, & Park conducted a study to estimate this threshold. In this study, participants completed an incremental maximal effort exercise test (VO_{2amz}). [17] Exercise intensity was increased by increasing the grade of inclination in 2% increments. The bouts of increasing intensity were separated by rest periods in order to obtain blood samples for analysis of lymphocyte apoptosis.

Using morphological techniques, the threshold to induce a significant change in lymphocyte apoptosis was estimated to occur between 40% and 60% VO_{2max}. In addition to this finding, it was observed that apoptosis is directly correlated with exercise intensity after this threshold is passed. This additional finding is further supported by other studies in which increasing exercise intensity elicits an increased exercise-induced immunosuppressive response. [2], [4], [32], [38] In addition to exercise intensity, training status is a determining factor with the exercise-induced immunosuppressive response.

Training Status

A study conducted by Mooren, Lechtermann, and Volker sought to determine if training status of an individual was a determining factor in exercise-induced immunosuppression. [29] In this study, there were two separate groups of subjects. One group participated in a marathon race and the second group participated in a treadmill protocol. The marathon group consisted of 38 male subjects who performed a VO_{2max} test and were

grouped into the high- or low-trained group. Subjects with a VO_{2max} of more than 60mL·min^{-1}·kg^{-1} were classified as "highly trained," and individuals with a VO_{2max} of less than 55mL·min^{-1}·kg^{-1} were classified as "badly trained." After this initial testing, the athletes participated in the 2002 Munster marathon. The treadmill group consisted of 10 subjects who also performed a VO_{2max} test, then performed an "exhaustive treadmill exercise test" corresponding to 80% of their VO_{2max}. About two weeks later, five of the original 10 participants performed a "low intensity treadmill exercise test" at 60% of their VO_{2max} (five were not able to participate due to injuries).

With regard to the treadmill group, the observed apoptosis response further supports the previously discussed findings that exercise intensity does have a direct relationship with immunosuppressive responses. The exhaustive protocol elicited higher post-exercise apoptosis measurements when compared to the post-exercise measurements of the low-intensity protocol.

With regard to the marathon group, the subjects that were classified as "highly trained" had a significantly higher rate of basal lymphocyte apoptosis prior to the marathon. After the marathon, post-exercise blood draws showed an increase in apoptosis for the "badly trained" group, whereas a decline in apoptosis was observed in the "highly trained" group. This means that the lymphocytes of the "highly trained" athletes underwent less immunosuppression (apoptosis) than those from the "badly trained" athletes. The investigators pointed out that although training status is shown to influence exercise-induced apoptosis, age has been shown to be related to apoptosis as well. [43] Schindowski et al. aimed to investigate this phenomenon and concluded that basal lymphocyte apoptosis is directly correlated with an individual's age. Though, in the study conducted by Schindowski et al.,

the age difference was greater than 25 years, and the study conducted by Mooren et al. (2004) had an age difference of only 11.2 years. In addition, Schindowski et al. reported higher surface expression of Fas receptors (a type of death receptor on the surface of cells that leads to apoptosis) from the elderly, which was not observed in the groups in the study conducted by Mooren et al. These factors suggest that the age difference in the current study was likely not responsible for the difference of exercise-induced apoptosis between the "badly trained" and "highly trained" groups. Though this may be the case, it is necessary to take into account that a large difference in age among the participants could be problematic when drawing conclusions on a study investigating post-exercise immunosuppression.

Exercise Frequency

Time in between exercise bouts can also have an effect on apoptosis yield immediately following exercise. Hsu et al. (2001) conducted a study in which twelve trained, male participants performed aerobic activity for three consecutive days within three separate conditions (35%, 60%, and 85% of VO_{2max}). [41] Each subject performed a VO_{2max} test one week prior to the initiation of the first protocol. The three days of each condition had the same exercise protocol: run on a treadmill for 30 minutes at one of the three percentages of the previously obtained VO_{2max}. Each condition had a four-week rest interval from the cessation of the previous condition to the initiation of the next condition. Blood samples were taken for each condition as follows: immediately before and after exercise on the first day, immediately before and after exercise on the third day, once on the fifth day, and once on the seventh day. This study measured apoptosis via mitochondrial transmembrane potential (MTP) in blood lymphocytes. This method was used due to the fact that MTP is a driving force for ATP formation, and since manipulation of ATP formation is a precursor to

apoptosis, MTP is directly correlated with apoptotic potential. [44] With regard to lymphocyte apoptosis, only a significant difference on day five was seen at 35% of measured VO_{2max}, which is consistent with the study conducted by Navalta, Sedlock, and Park (2007) where it was determined that in order for a significant change in apoptosis to occur, individuals must exercise at a threshold of 40 to 60% of their VO_{2max}. [17] Conversely, significant changes were seen before and after exercise on day three and on day five at 60% of measured VO_{2max} and after exercise on day one and before and after exercise on day three at 85% of measured VO_{2max}. These findings show that consecutive days of strenuous (60% of VO_{2max} or greater) exercise elicit a significantly greater occurrence of apoptosis in circulating blood lymphocytes. Because of this, it can be concluded that adequate rest between exercise bouts is needed in order to limit confounding variables and more accurately measure blood lymphocytes apoptosis caused by exercise and not chronic fatigue.

Gender

Navalta et al. (2007) investigated the influences of both gender and menstrual cycle phase on lymphocyte apoptosis. [17] Seven untrained men and seven untrained women underwent two VO_{2max} tests. The women completed one of the tests during the follicular phase of their menstrual cycle and completed the other test during the mid-luteal phase of their menstrual cycle. The men completed their maximal exertion tests with a similar period of time in between each test as the female subjects. Two blood samples were taken for each VO_{2max} test, one sample immediately before and one sample immediately after the test. The percent of exercise-induced lymphocyte apoptosis was similar between the female subjects and male subjects, indicating there are no gender differences with regard to exercise-induced apoptosis. In addition, lymphocyte apoptosis had no significant differences between the two

menstrual cycle phases that were tested. Though females have been participants in numerous studies investigating exercise-induced lymphocyte apoptosis, the effects of gender and menstrual cycles on lymphocyte apoptosis were not reported in the literature before this study. [17]

Summary

Research investigating the effects of exercise on circulating lymphocytes is abundant, while investigations regarding the responses of circulating neutrophils and monocytes show conflicting findings and are nonexistent, respectively. As exercise intensity increases, lymphocyte apoptosis has been shown to increase, though it is important to keep in mind that CD8+ T cells are responsible for a majority of the lymphocytopenia seen at higher intensities when compared to CD4+ T cells.

HCMV research is necessary due to its prevalence and severity of symptoms in immunosuppressed individuals. To date, literature has only shown a significance difference in circulating CD8+ T cells in HCMV+ when compared to HCMV- individuals, while no literature has investigated the effect HCMV has on circulating neutrophils and monocytes.

Exercise intensity, exercise frequency, and age have a direct relationship with lymphocyte apoptosis. Training status has been shown to have a negative relationship with lymphocyte apoptosis and gender has no effects on circulating lymphocytes in the post-exercise period.

Chapter 3

Methods

Participants: Participants in the HCMV- group (n = 8) were 18 to 43 years of age (23.88 ± 8.04 years old) and had an average BMI of 22.86 ± 3.41. Participants in the HCMV+ group (n = 8) were 18 to 44 years of age (28.88 ± 9.60 years old) and had an average BMI of 23.53 ± 3.56. All participants completed the American College of Sports Medicine (ACSM) pre-participation screening questionnaire and were in the "Low Risk" category for cardiovascular disease according to the ACSM health risk questionnaire.

Participants were recruited from the University of Nevada, Las Vegas student population and the greater Las Vegas community through word of mouth. Participants were not monetarily compensated for their time; however, they were able to receive their data from the metabolic and immune testing.

At the time of recruitment and prior to participation in any experimental protocol, each subject completed the American College of Sports Medicine (ACSM) pre-participation screening questionnaire, and signed an informed consent document approved by the institutional review board for the protection of human subjects at the University of Nevada, Las Vegas. Subjects were required to be in a "Low Risk" category for cardiovascular disease according to the ACSM health risk questionnaire. If the participants were able to answer "None of the above" on the questionnaire, check nothing in the first section (history, symptoms, other health issues), or check no more than one cardiovascular risk factor, and were a male younger than 45 years of age or a female younger than 55 years of age, they were considered "Low Risk" according to the ACSM algorithm, and were eligible to participate in the investigation. The ACSM criteria for low risk includes men younger than

45 years of age and women younger than 55 years of age who are asymptomatic and meet no more than one risk factor threshold for coronary artery disease risk. Subjects were excluded from the study based on the following criteria: (a) pregnant; (b) have implantable devices such as pacemaker or automatic implantable cardioverter defibrillators (AICD); (c) orthopedic (acute or chronic musculoskeletal injury), cardiovascular (coronary artery disease), respiratory (chronic obstructive pulmonary disease or asthma), and metabolic conditions (diabetes); and (d) currently smokes or quit smoking less than six months previous.

Protocol: Participants were recruited and initially screened for the presence of HCMV. A finger-stick blood sample was obtained (300 µL of whole blood) into a capillary tube coated with an anti-coagulant, and centrifuged at 1,160 rpm for 5 minutes. Upon completion of the centrifuging process, the plasma was withdrawn, diluted in a 1:21 ratio in sample diluent (10 µL sample into 200 µL diluent) and stored at -50° C until samples were analyzed through a commercially available ELISA kit (CMV IgG ELISA, GWB-B9FF92; Genway Biotech, Inc.; San Diego, CA). To perform the assay, all samples and controls were brought to room temperature, and 100 µL of diluted sample, reagent blank, calibrator, or controls were added to appropriate wells of a 96-well microliter plate. 1:21 dilution of test samples was prepared by adding 10 µL of the sample to 200 µL of sample diluent and then diluted samples were mixed. 100 µL of diluted sera, calibrator, or control were dispensed into appropriate wells and mixed. The plate was allowed to incubate for 20 minutes at room temperature. The liquid was then removed from all wells and the wells were washed three times with 300 µL of 1X wash buffer. 100 µL of enzyme conjugate was then dispensed into appropriate wells and the plate was allowed to incubate for 20 minutes at room temperature.

The enzyme conjugate was then removed from all wells. The wells were washed three times with 300 µL of 1X wash buffer. 100 µL of tetramethylbenzidine (TMB) substrate was dispensed into appropriate wells and allowed to incubate for 10 minutes at room temperature. Lastly, 100 µL of stop solution was added to appropriate wells. The plate was read at an optical density of 450nm. Individuals were considered HCMV+ if the antibody index was greater than 1.2. The antibody index is calculated as the sample optical density divided by the cut-off value, and the cut-off value is determined as the product of the mean calibrator optical density and the supplied calibrator factor.

Subjects were instructed to wear loose fitting clothing (i.e., shorts, t-shirt, and running shoes) and to report to the Exercise Physiology Laboratory for testing. In addition, they were instructed to be well hydrated, to have consumed their last meal at least two hours prior to testing, refrain from caffeinated beverages for two hours prior to testing, and to refrain from alcoholic beverages for at least six hours prior to the test. Participants were also asked to refrain from strenuous physical exercise during the day prior to testing. Prior to the graded exercise test, subject's body weight, height, and age were measured. The subjects were given instructions for ratings of perceived exertion and the graded exercise test protocol was also explained.

Participants were asked to report to the laboratory on two separate occasions. The first visit lasted approximately 30 minutes and the second visit lasted approximately 90 minutes. On the first visit, participants completed an incremental test to exhaustion (VO_{2max}) on a motor-driven treadmill. This test began at 3mph (80.47m/min) at a 0% grade, progressed to 5mph (134.1 m/min), and then a self-selected running speed. Following the stage in which running speed is selected, the speed was held constant and grade was elevated 3% with each

successive stage until termination of the exercise test. This protocol was utilized in order to allow participants to reach their so-called "maximal level of exertion" within 8-12 minutes of actual exercise. To be termed a maximal test, participants displayed at least two of the following criteria: obtained a heart rate that is within 10 beats per min of their age estimated maximum (estimated HR_{max} = 220 – age), had a respiratory exchange ratio of 1.10 or greater, terminated the test due to volitional fatigue, and displayed a rating of perceived exertion of 19 or greater on the Borg scale. Each stage was two minutes in length. The greatest amount of oxygen consumed during any given one-minute average was considered the maximal oxygen consumption (VO_{2max}). This value was used to calculate the intensity (i.e., speed and grade combination) that corresponded to 80% VO_{2max}.

To test the effect of prolonged exercise at moderate and high intensities, at least one week following the maximum exertion test, participants were asked to return to the laboratory for a 20 minute treadmill run at 80% (HCMV- = 82.58% $\pm$ 5.05%; HCMV+ = 80.50% $\pm$ 4.41%) of VO_{2max}. Workload (grade) adjustments were made during the run for participants who reached steady-state oxygen consumption values greater than 85% of their VO_{2max}. A 100 µL whole blood sample was obtained via finger-stick at rest prior to exercise, in the recovery period immediately following exercise, and at 30 minutes and 60 minutes post-exercise.

To test the volume of circulating leukocytes prior to exercise, in the recovery period immediately following exercise, and at 30 minutes and 60 minutes post-exercise, a hematology analyzer (ABX Micros 60 CS; serial number: 411CS76373; HORIBA Instruments Incorporation; Irvine, CA) was utilized immediately following each individual blood draw. The ABX Micros 60 utilizes 10µL of whole blood per analysis in order to

determine the volume of circulating leukocytes. The hematology analyzer was calibrated at the beginning of each testing day according to manufacturer instructions.

Statistical Analysis: A total of six 2 (virus status: HCMV-, HCMV+) x 4 (time: pre-exercise, post-exercise, 30 minutes post-exercise, 60 minutes post-exercise) mixed-model factorial analysis of variance (ANOVA) procedures were performed on absolute and relative values for circulating lymphocytes, monocytes, and neutrophils. Absolute circulating leukocytes were expressed as 10^3/ mm^3 and relative circulating leukocytes were expressed as a percent change from baseline (pre-exercise = 0%). Typical ranges for the leukocyte subsets investigated are: lymphocytes – 1.2 to 3.2 10^3/ mm^3, monocytes – 0.3 to 0.8 10^3/ mm^3 , and neutrophils – 1.2 to 6.8 10^3/ mm^3. Simple main effects analyses were performed if a significant interaction was found between virus status and time.

Chapter 4

Results

Absolute and Relative Circulating Neutrophils

Results revealed no significant interaction between sampling condition and HCMV status for absolute circulating neutrophils ($F = .255$, $p = .506$) or relative circulating neutrophils ($F = .660$, $p = .575$). The main effect for sampling conditions was significant for both absolute ($F = 24.040$, $p < .001$) and relative ($F = 22.596$, $p < .001$) circulating neutrophil volume.

Sidak pairwise comparisons revealed the pre-exercise mean was significantly lower than the post-exercise and 60 minutes post-exercise means ($p = .001$). Additionally, the 30 minutes post-exercise mean was significantly lower than the post-exercise and 60 minutes post-exercise means ($p < .001$) for absolute circulating neutrophil volume (See **Figure 1**).

Sidak pairwise comparisons revealed the post-exercise mean was significantly higher than the pre-exercise and 30 minutes post-exercise means ($p < .001$). Additionally, results revealed the 60 minutes post-exercise mean was significantly higher than the pre-exercise and 30 minutes post-exercise means for relative circulating neutrophil volume ($p < .001$) (See **Figure 2**).

Individual absolute circulating neutrophil responses can be seen in **Figure 3a** for HCMV+ individuals and in **Figure 3b** for HCMV- individuals.

Figure 1: Absolute Circulating Neutrophil Volume

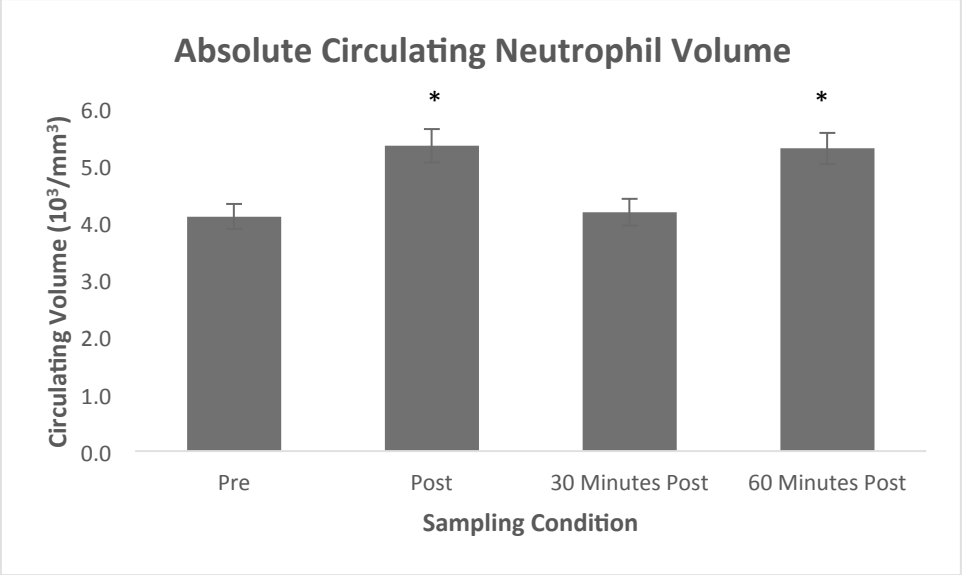

Figure 1: Absolute circulating neutrophil responses for all participants (n = 16), regardless of HCMV status. The Post and 60 Minutes Post conditions are significantly (*) higher when compared to the Pre and 30 Minutes Post conditions. The typical range for circulating neutrophils is 1.2 to 6.8 10^3/ mm^3.

Figure 2: Relative Circulating Neutrophil Volume

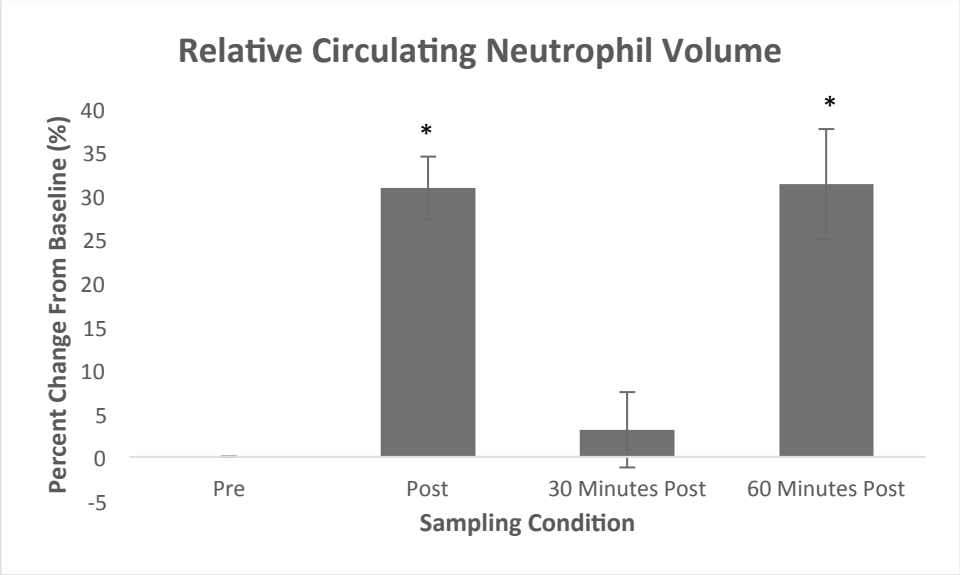

Figure 2: Relative circulating neutrophil responses for all participants (n = 16), regardless of HCMV status. The Post and 60 Minutes Post conditions are significantly (*) higher when compared to the Pre and 30 Minutes Post conditions.

Figure 3a: Absolute Circulating Neutrophils for HCMV+ Individuals

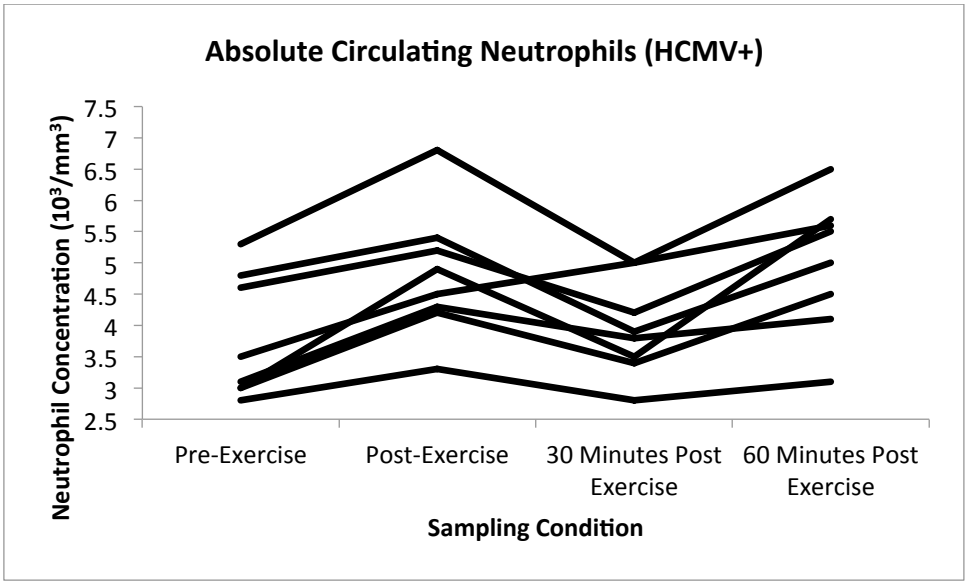

Figure 3a: Individual absolute circulating neutrophil responses for HCMV+ individuals.

Figure 3b: Absolute Circulating Neutrophils for HCMV- Individuals

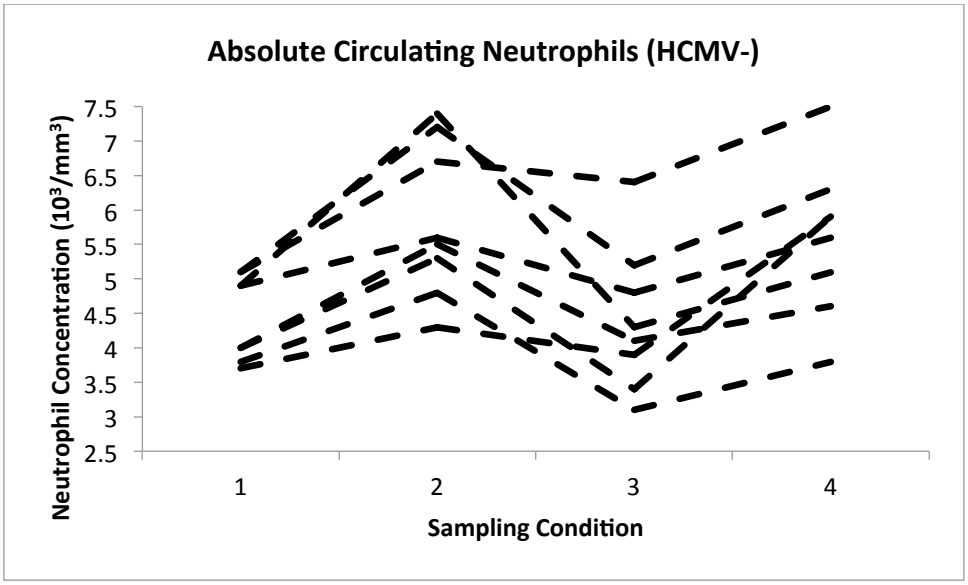

Figure 3b: Individual absolute circulating neutrophil responses for HCMV- individuals.

Absolute and Relative Circulating Monocytes

No significant interaction was found between sampling condition and HCMV status for absolute circulating monocytes ($F = .089$, $p = .952$) or relative circulating monocytes ($F = .746$, $p = .499$). The main effect for sampling conditions was significant for both absolute ($F = 23.704$, $p < .001$) and relative ($F = 29.758$, $p < .001$) circulating monocyte volume.

Sidak pairwise comparisons revealed the post-exercise mean was significantly higher than the pre-exercise ($p = .002$), 30 minutes post-exercise ($p < .001$), and 60 minutes post-exercise ($p < .001$) condition means for absolute circulating monocytes (See **Figure 4**).

Sidak pairwise comparisons revealed the post-exercise mean was significantly higher than the pre-exercise ($p = .001$), 30 minutes post-exercise ($p < .001$), and 60 minutes post-exercise ($p < .001$) condition means for relative circulating monocytes (See **Figure 5**).

Individual absolute circulating neutrophil responses can be seen in **Figure 6a** for HCMV+ individuals and in **Figure 6b** for HCMV- individuals.

Figure 4: Absolute Circulating Monocyte Volume

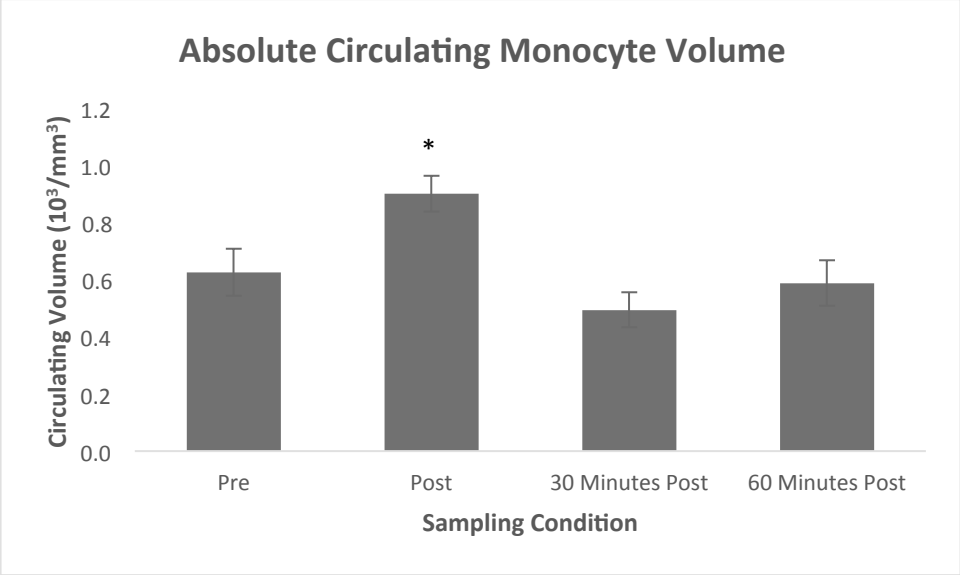

Figure 4: Absolute circulating monocyte responses for all participants (n = 16), regardless of HCMV status. The Post condition is significantly (*) higher when compared to the Pre, 30 Minutes Post, and 60 Minutes Post conditions. The typical range for circulating monocytes is 0.3 to 0.8 10^3/ mm^3.

Figure 5: Relative Circulating Monocyte Volume

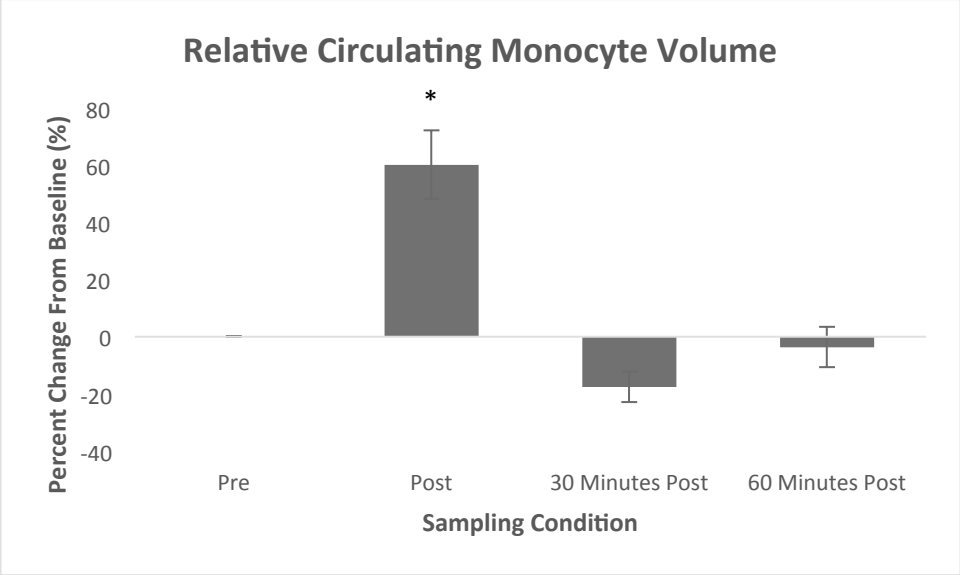

Figure 5: Relative circulating monocyte responses for all participants (n = 16), regardless of HCMV status. The Post condition is significantly (*) higher when compared to the Pre, 30 Minutes Post, and 60 Minutes Post conditions.

Figure 6a: Absolute Circulating Monocytes for HCMV+ Individuals

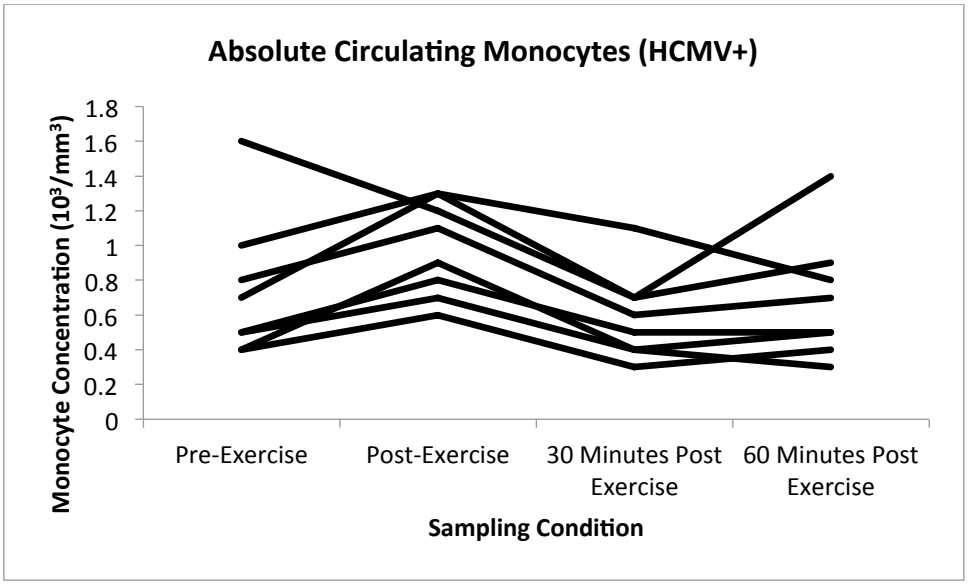

Figure 6a: Individual absolute circulating monocyte responses for HCMV+ individuals.

Figure 6b: Absolute Circulating Monocytes for HCMV- Individuals

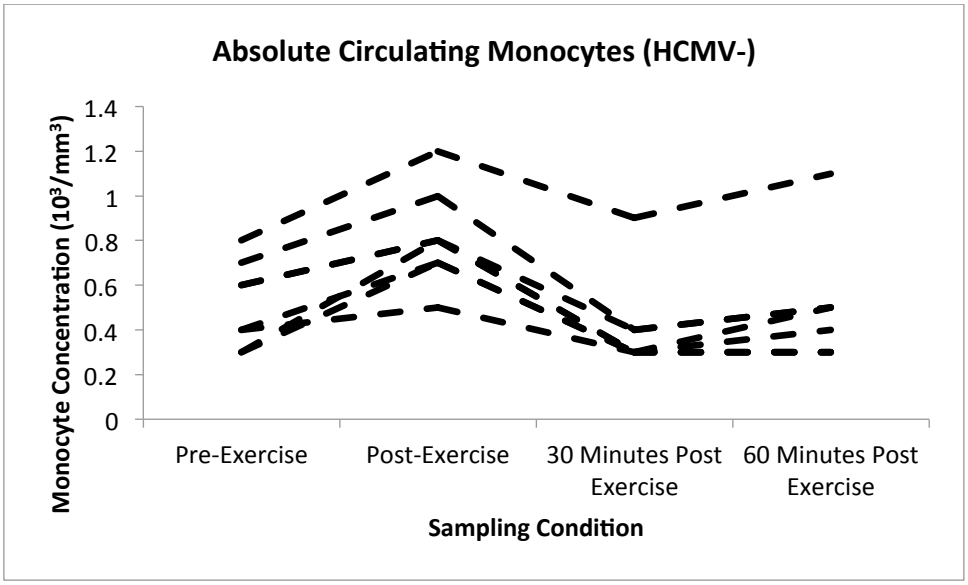

Figure 6b: Individual absolute circulating monocyte responses for HCMV- individuals.

Absolute and Relative Circulating Lymphocytes

Significant interactions were not seen between sampling condition and HCMV status for absolute circulating lymphocytes ($F = .22$, $p = .746$) or relative circulating lymphocytes ($F = .314$, $p = .648$). The main effect for sampling conditions was significant for absolute ($F = 55.175$, $p < .001$) and relative ($F = 56.620$, $p < .001$) conditions.

Sidak pairwise comparisons revealed the post-exercise condition mean was significantly higher than the pre-exercise ($p < .001$), 30 minutes post-exercise ($p < .001$), and 60 minutes post-exercise ($p < .001$) condition means for absolute circulating lymphocytes (See **Figure 7**).

Sidak pairwise comparisons revealed the post-exercise condition mean was significantly higher than the pre-exercise ($p < .001$), 30 minutes post-exercise ($p < .001$), and 60 minutes post-exercise ($p < .001$) condition means for relative circulating lymphocytes (See **Figure 8**).

Individual absolute circulating neutrophil responses can be seen in **Figure 6a** for HCMV+ individuals and in **Figure 6b** for HCMV- individuals.

Figure 7: Absolute Circulating Lymphocyte Volume

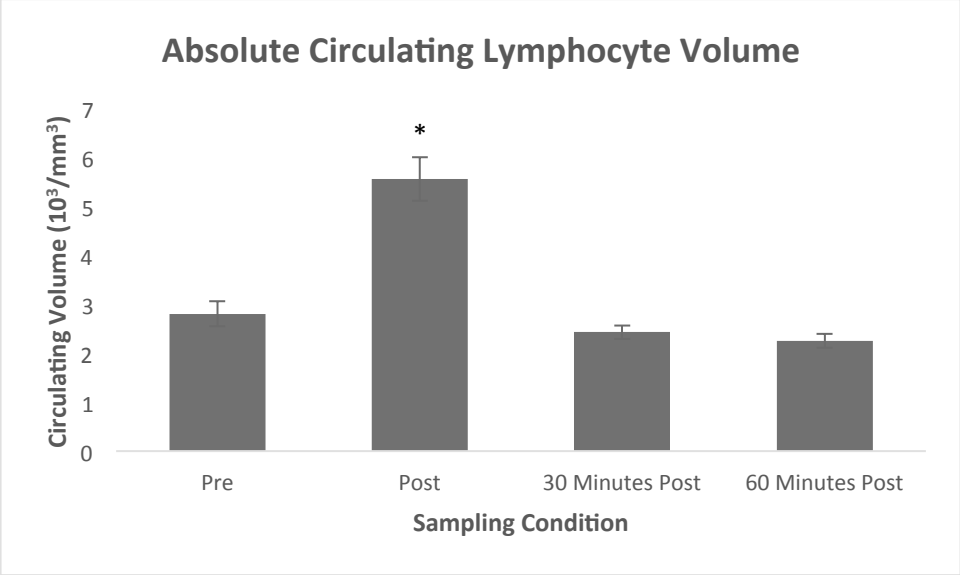

Figure 7: Absolute circulating lymphocyte responses for all participants (n = 16), regardless of HCMV status. The Post condition is significantly (*) higher when compared to the Pre, 30 Minutes Post, and 60 Minutes Post conditions. The typical range for circulating lymphocytes is 1.2 to 6.8 10^3/ mm^3.

Figure 8: Relative Circulating Lymphocyte Volume

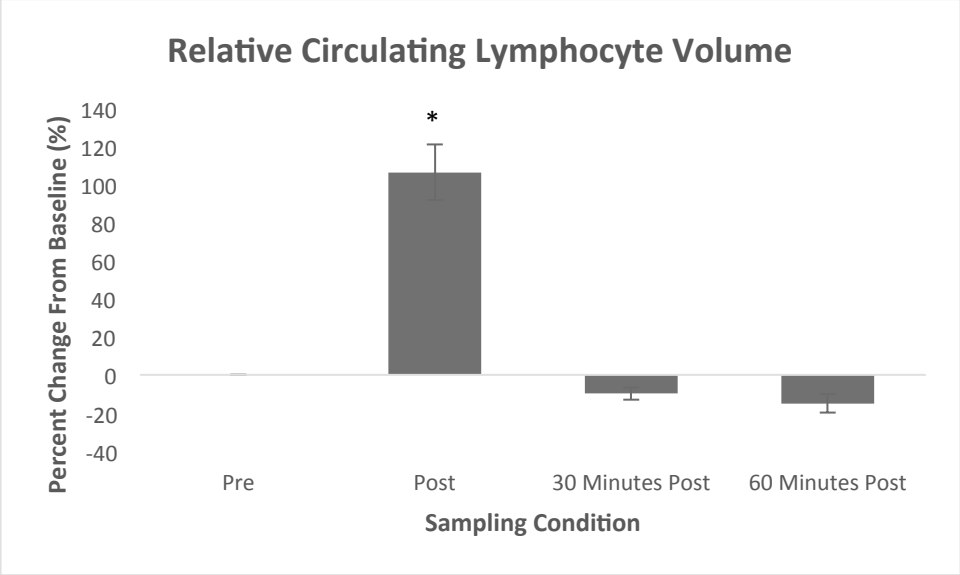

Figure 8: Absolute circulating lymphocyte responses for all participants (n = 16), regardless of HCMV status. The Post condition is significantly (*) higher when compared to the Pre, 30 Minutes Post, and 60 Minutes Post conditions.

Figure 9a: Absolute Circulating Lymphocytes for HCMV+ Individuals

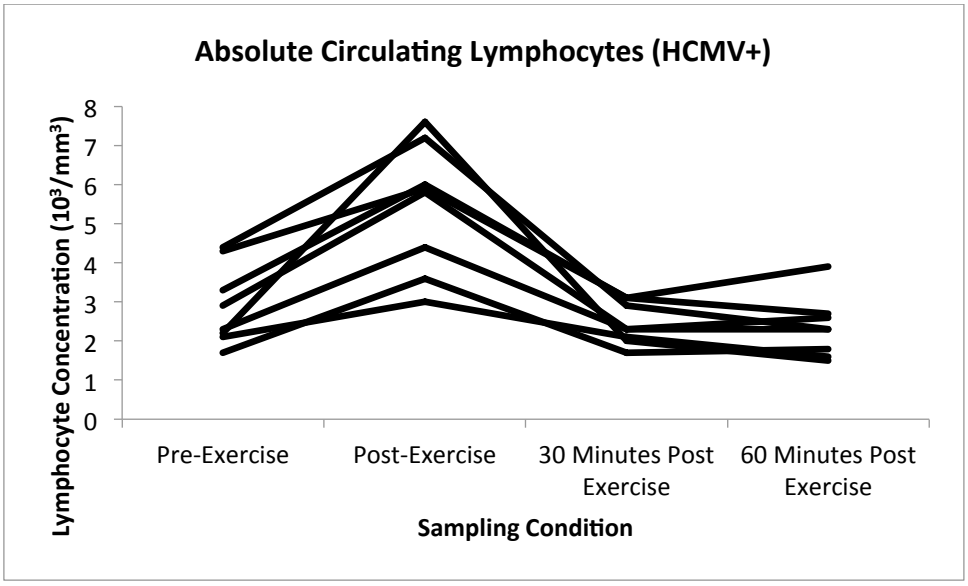

Figure 9a: Individual absolute circulating lymphocyte responses for HCMV+ individuals.

Figure 9b: Absolute Circulating Lymphocytes for HCMV- Individuals

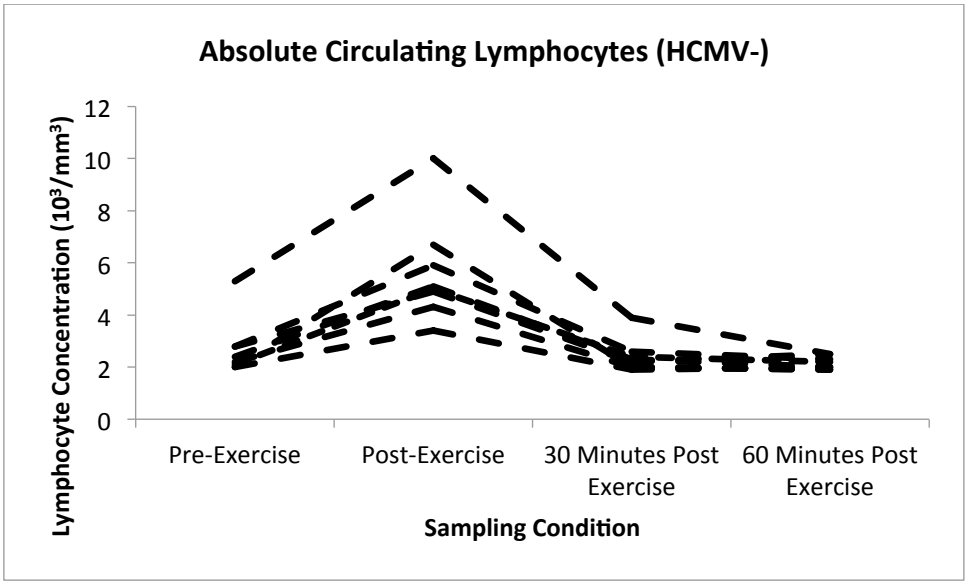

Figure 9b: Individual absolute circulating lymphocyte responses for HCMV+ individuals.

Chapter 5

Discussion

The purpose of this study was to characterize the lymphocyte, monocyte, and neutrophil responses to exercise in HCMV+ individuals. The main findings of this study are the effects of high-intensity exercise on circulating monocyte and neutrophil volume in the post-exercise period. We accept the second, fourth, and sixth alternate hypotheses that state that a significant change in circulating lymphocytes, neutrophils, and monocytes will be seen in the post-exercise period regardless of HCMV status.

We report for the first time that HCMV status does not affect circulating neutrophil responses to exercise, though there is a significant increase in neutrophils, or "neutrocytosis," during the post-exercise and 60 minutes post-exercise sampling conditions, regardless of HCMV status, as seen in **Figure 1** and **Figure 2**, which illustrate a two-phase response of circulating neutrophils to exercise. Neutrophils can be classified as either immature or mature. [45] Immature neutrophils have had little to no exposure to antigens and are relatively new cells, whereas mature neutrophils have had exposure to antigens and have been active for a longer period of time. [45] Mature neutrophils have a quicker inflammatory response time than immature neutrophils. This would explain the response in the present study where the initial peak in circulating neutrophils during the post-exercise sampling condition would represent an influx of mature neutrophils into the circulation, and the secondary peak seen at the 60 minutes post-exercise sampling condition would represent an influx of immature neutrophils into the circulation. Future studies should aim to differentiate between immature and mature neutrophils and their responses in the post-exercise period, as well as investigate

the contributions of apoptosis and migration to the characteristic circulating volume fluctuations seen here.

As with circulating monocytes, we report for the first time that there is no HCMV effect on circulating monocyte responses to exercise, though an initial increase in monocytes, or "monocytosis," was seen during the post-exercise sampling condition regardless of HCMV status as seen in **Figure 3** and **Figure 4**. This is the first study done investigating the responses of circulating monocytes to high-intensity running in the post-exercise period.

Although not measured in the present investigation, previous studies have shown lymphocyte cell count to be influenced by changes in catecholamine levels. [46] Lymphocytopenia (a significant decrease in circulating lymphocytes) was not seen in this study as it has been seen in previous studies. This could possibly be due to the fact that previous studies that saw this effect found the significance exclusively in CD8 T cells, whereas we analyzed whole lymphocytes (CD4, CD8, T helper, B cells, B helper cells). A synthesis of these two findings may suggest a significant increase in one or more types of circulating lymphocytes in the post-exercise period, which would result in the effects seen here. The drop in circulating lymphocytes at 30 and 60 minutes post-exercise when compared to the pre-exercise sampling condition was likely due to both apoptosis and cellular migration. It is reasonable to postulate that due to the high-intensity nature of the exercise protocol and previous findings in the literature regarding exercise intensity, [4] cellular migration may have had a greater contribution than apoptosis to the aforementioned drop in circulating lymphocytes at 30 and 60 minutes post-exercise when compared to the pre-exercise sampling condition.

No significant differences in circulating absolute and relative neutrophils, monocytes, or lymphocytes were seen between HCMV- and HCMV+ groups. Future studies should control for age and training status in both HCMV- and HCMV+ groups more strictly in order to possibly see a significant effect between the two groups. Although no significant differences were found between groups, the responses of circulating neutrophils and monocytes provide insight into the effects of high-intensity exercise on immune status. Future studies should aim to investigate the effects of chronic overtraining on immune status since athletic populations are more likely to engage in high-intensity exercise and be prone to overtraining after successive bouts of high-intensity exercise. The characterization of the neutrophil response is the most interesting finding due to the nature of the two-phase response. The responses and effects of immature neutrophils are less effective than the responses of mature neutrophils, [45] implying that for up to 60 minutes after high-intensity exercise, an individual's inflammatory and phagocytic responses are limited.

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Appendix

Absolute Circulating Neutrophils

Descriptive Statistics

	Virus	Mean	Std. Deviation	N
Time1	HCMV-	4.438	.6140	8
	HCMV+	3.762	.9812	8
	Total	4.100	.8641	16
Time2	HCMV-	5.850	1.1301	8
	HCMV+	4.825	1.0334	8
	Total	5.338	1.1724	16
Time3	HCMV-	4.400	1.0583	8
	HCMV+	3.950	.7672	8
	Total	4.175	.9227	16
Time4	HCMV-	5.588	1.1192	8
	HCMV+	5.000	1.0704	8
	Total	5.294	1.1006	16

Tests of Within-Subjects Effects

Source		df	F	Sig.	Partial Eta Squared
Time	Sphericity Assumed	3	24.040	.000	.632
	Greenhouse-Geisser	2.214	24.040	.000	.632
	Huynh-Feldt	2.835	24.040	.000	.632
	Lower-bound	1.000	24.040	.000	.632
Time * Virus	Sphericity Assumed	3	.779	.512	.053
	Greenhouse-Geisser	2.214	.779	.479	.053
	Huynh-Feldt	2.835	.779	.506	.053
	Lower-bound	1.000	.779	.392	.053
Error (Time)	Sphericity Assumed	42			
	Greenhouse-Geisser	30.992			
	Huynh-Feldt	39.696			
	Lower-bound	14.000			

a. Computed using alpha = .05

Pairwise Comparisons

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-1.238*	.145	.000	-1.682	-.793
	3	-.075	.175	.999	-.611	.461
	4	-1.194*	.222	.001	-1.873	-.515
2	1	1.238*	.145	.000	.793	1.682
	3	1.163*	.210	.000	.521	1.804
	4	.044	.241	1.000	-.694	.781
3	1	.075	.175	.999	-.461	.611
	2	-1.163*	.210	.000	-1.804	-.521
	4	-1.119*	.168	.000	-1.633	-.605
4	1	1.194*	.222	.001	.515	1.873
	2	-.044	.241	1.000	-.781	.694
	3	1.119*	.168	.000	.605	1.633

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Sidak.

Relative Circulating Neutrophils

Descriptive Statistics

	Virus	Mean	Std. Deviation	N
Time1	HCMV-	.000	.0000	8
	HCMV+	.000	.0000	8
	Total	.000	.0000	16
Time2	HCMV-	31.300	12.3511	8
	HCMV+	30.289	17.0390	8
	Total	30.794	14.3857	16
Time3	HCMV-	-1.542	14.0832	8
	HCMV+	7.791	19.9144	8
	Total	3.124	17.3453	16
Time4	HCMV-	26.3648	22.18412	8
	HCMV+	36.1688	28.79218	8
	Total	31.2668	25.34080	16

Tests of Within-Subjects Effects

Source		df	F	Sig.	Partial Eta Squared
Time	Sphericity Assumed	3	22.596	.000	.617
	Greenhouse-Geisser	2.237	22.596	.000	.617
	Huynh-Feldt	2.873	22.596	.000	.617
	Lower-bound	1.000	22.596	.000	.617
Time * Virus	Sphericity Assumed	3	.660	.581	.045
	Greenhouse-Geisser	2.237	.660	.540	.045
	Huynh-Feldt	2.873	.660	.575	.045
	Lower-bound	1.000	.660	.430	.045
Error (Time)	Sphericity Assumed	42			
	Greenhouse-Geisser	31.321			
	Huynh-Feldt	40.223			
	Lower-bound	14.000			

a. Computed using alpha = .05

Pairwise Comparisons

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-30.794*	3.720	.000	-42.171	-19.418
	3	-3.124	4.312	.980	-16.310	10.061
	4	-31.267*	6.425	.001	-50.916	-11.618
2	1	30.794*	3.720	.000	19.418	42.171
	3	27.670*	4.742	.000	13.169	42.171
	4	-.472	5.832	1.000	-18.307	17.363
3	1	3.124	4.312	.980	-10.061	16.310
	2	-27.670*	4.742	.000	-42.171	-13.169
	4	-28.142*	4.939	.000	-43.245	-13.040
4	1	31.267*	6.425	.001	11.618	50.916
	2	.472	5.832	1.000	-17.363	18.307
	3	28.142*	4.939	.000	13.040	43.245

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Sidak.

Absolute Circulating Monocytes

Descriptive Statistics

	Virus	Mean	Std. Deviation	N
Time1	HCMV-	.513	.1885	8
	HCMV+	.738	.4069	8
	Total	.625	.3276	16
Time2	HCMV-	.813	.2100	8
	HCMV+	.988	.2748	8
	Total	.900	.2530	16
Time3	HCMV-	.400	.2070	8
	HCMV+	.587	.2532	8
	Total	.494	.2435	16
Time4	HCMV-	.488	.2642	8
	HCMV+	.687	.3523	8
	Total	.588	.3181	16

Tests of Within-Subjects Effects

Source		df	F	Sig.	Partial Eta Squared
Time	Sphericity Assumed	3	23.704	.000	.629
	Greenhouse-Geisser	2.081	23.704	.000	.629
	Huynh-Feldt	2.626	23.704	.000	.629
	Lower-bound	1.000	23.704	.000	.629
Time * Virus	Sphericity Assumed	3	.089	.966	.006
	Greenhouse-Geisser	2.081	.089	.922	.006
	Huynh-Feldt	2.626	.089	.952	.006
	Lower-bound	1.000	.089	.770	.006
Error (Time)	Sphericity Assumed	42			
	Greenhouse-Geisser	29.136			
	Huynh-Feldt	36.764			
	Lower-bound	14.000			

a. Computed using alpha = .05

Pairwise Comparisons

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-.275*	.057	.002	-.451	-.099
	3	.131	.062	.270	-.057	.319
	4	.037	.042	.947	-.091	.166
2	1	.275*	.057	.002	.099	.451
	3	.406*	.033	.000	.304	.508
	4	.312*	.052	.000	.155	.470
3	1	-.131	.062	.270	-.319	.057
	2	-.406*	.033	.000	-.508	-.304
	4	-.094	.053	.463	-.256	.068
4	1	-.037	.042	.947	-.166	.091
	2	-.312*	.052	.000	-.470	-.155
	3	.094	.053	.463	-.068	.256

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Sidak.

Relative Circulating Monocytes

Descriptive Statistics

	Virus	Mean	Std. Deviation	N
Time1	HCMV-	.000	.0000	8
	HCMV+	.000	.0000	8
	Total	.000	.0000	16
Time2	HCMV-	69.940	52.4155	8
	HCMV+	50.401	43.5565	8
	Total	60.171	47.6368	16
Time3	HCMV-	-20.461	22.3761	8
	HCMV+	-14.531	21.4636	8
	Total	-17.496	21.4014	16
Time4	HCMV-	-2.009	37.8682	8
	HCMV+	-5.179	16.6441	8
	Total	-3.594	28.3047	16

Tests of Within-Subjects Effects

Source		df	F	Sig.	Partial Eta Squared
Time	Sphericity Assumed	3	29.758	.000	.680
	Greenhouse-Geisser	1.861	29.758	.000	.680
	Huynh-Feldt	2.287	29.758	.000	.680
	Lower-bound	1.000	29.758	.000	.680
Time * Virus	Sphericity Assumed	3	.746	.531	.051
	Greenhouse-Geisser	1.861	.746	.475	.051
	Huynh-Feldt	2.287	.746	.499	.051
	Lower-bound	1.000	.746	.402	.051
Error (Time)	Sphericity Assumed	42			
	Greenhouse-Geisser	26.047			
	Huynh-Feldt	32.024			
	Lower-bound	14.000			

a. Computed using alpha = .05

Pairwise Comparisons

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-60.171*	12.048	.001	-97.013	-23.329
	3	17.496*	5.481	.039	.735	34.258
	4	3.594	7.312	.997	-18.768	25.955
2	1	60.171*	12.048	.001	23.329	97.013
	3	77.667*	9.489	.000	48.650	106.684
	4	63.764*	11.089	.000	29.853	97.676
3	1	-17.496*	5.481	.039	-34.258	-.735
	2	-77.667*	9.489	.000	-106.684	-48.650
	4	-13.903	6.047	.204	-32.393	4.588
4	1	-3.594	7.312	.997	-25.955	18.768
	2	-63.764*	11.089	.000	-97.676	-29.853
	3	13.903	6.047	.204	-4.588	32.393

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Sidak.

Absolute Circulating Lymphocytes

Descriptive Statistics

	Virus	Mean	Std. Deviation	N
Time1	HCMV-	2.7000	1.09935	8
	HCMV+	2.9000	1.02120	8
	Total	2.8000	1.03021	16
Time2	HCMV-	5.6625	2.00922	8
	HCMV+	5.4375	1.64050	8
	Total	5.5500	1.77576	16
Time3	HCMV-	2.4125	.64462	8
	HCMV+	2.4375	.53168	8
	Total	2.4250	.57096	16
Time4	HCMV-	2.1625	.25036	8
	HCMV+	2.3375	.77263	8
	Total	2.2500	.56214	16

Tests of Within-Subjects Effects

Source		df	F	Sig.	Partial Eta Squared
Time	Sphericity Assumed	3	55.175	.000	.798
	Greenhouse-Geisser	1.337	55.175	.000	.798
	Huynh-Feldt	1.531	55.175	.000	.798
	Lower-bound	1.000	55.175	.000	.798
Time * Virus	Sphericity Assumed	3	.220	.882	.015
	Greenhouse-Geisser	1.337	.220	.715	.015
	Huynh-Feldt	1.531	.220	.746	.015
	Lower-bound	1.000	.220	.647	.015
Error (Time)	Sphericity Assumed	42			
	Greenhouse-Geisser	18.715			
	Huynh-Feldt	21.435			
	Lower-bound	14.000			

a. Computed using alpha = .05

Pairwise Comparisons

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-2.750*	.318	.000	-3.722	-1.778
	3	.375	.138	.096	-.047	.797
	4	.550	.229	.172	-.151	1.251
2	1	2.750*	.318	.000	1.778	3.722
	3	3.125*	.368	.000	2.000	4.250
	4	3.300*	.443	.000	1.944	4.656
3	1	-.375	.138	.096	-.797	.047
	2	-3.125*	.368	.000	-4.250	-2.000
	4	.175	.125	.701	-.206	.556
4	1	-.550	.229	.172	-1.251	.151
	2	-3.300*	.443	.000	-4.656	-1.944
	3	-.175	.125	.701	-.556	.206

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Sidak.

Relative Circulating Lymphocytes

Descriptive Statistics

	Virus	Mean	Std. Deviation	N
Time1	HCMV-	.000	.0000	8
	HCMV+	.000	.0000	8
	Total	.000	.0000	16
Time2	HCMV-	115.680	52.9886	8
	HCMV+	96.755	65.6240	8
	Total	106.218	58.4424	16
Time3	HCMV-	-7.370	12.5523	8
	HCMV+	-12.230	13.5854	8
	Total	-9.800	12.8824	16
Time4	HCMV-	-13.993	17.6055	8
	HCMV+	-15.856	22.9994	8
	Total	-14.925	19.8097	16

Tests of Within-Subjects Effects

Source		df	F	Sig.	Partial Eta Squared
Time	Sphericity Assumed	3	56.620	.000	.802
	Greenhouse-Geisser	1.204	56.620	.000	.802
	Huynh-Feldt	1.349	56.620	.000	.802
	Lower-bound	1.000	56.620	.000	.802
Time * Virus	Sphericity Assumed	3	.314	.815	.022
	Greenhouse-Geisser	1.204	.314	.624	.022
	Huynh-Feldt	1.349	.314	.648	.022
	Lower-bound	1.000	.314	.584	.022
Error (Time)	Sphericity Assumed	42			
	Greenhouse-Geisser	16.851			
	Huynh-Feldt	18.881			
	Lower-bound	14.000			

a. Computed using alpha = .05

Pairwise Comparisons

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-106.218*	14.910	.000	-151.815	-60.620
	3	9.800	3.270	.056	-.199	19.799
	4	14.925	5.120	.066	-.733	30.582
2	1	106.218*	14.910	.000	60.620	151.815
	3	116.018*	14.101	.000	72.897	159.138
	4	121.142*	15.211	.000	74.625	167.660
3	1	-9.800	3.270	.056	-19.799	.199
	2	-116.018*	14.101	.000	-159.138	-72.897
	4	5.124	3.582	.684	-5.830	16.079
4	1	-14.925	5.120	.066	-30.582	.733
	2	-121.142*	15.211	.000	-167.660	-74.625
	3	-5.124	3.582	.684	-16.079	5.830

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Sidak.

Curriculum Vitae

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Grants

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2013	**Wilson, J.N.**, Nordin, A.D. & Dufek, J.S. Landing Strategy Patterns at Selected Bodyweight Percentages. Nevada UROP-INBRE, $5,000.

Presentations

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