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Impact of Psychological and Physical Stressors on the Exercise-Induced Immune Response in Collegiate Swimmers

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IMPACT OF PSYCHOLOGICAL AND PHYSICAL STRESSORS ON THE EXERCISE-INDUCED IMMUNE RESPONSE IN COLLEGIATE SWIMMERS

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List of Terms

Cortisol – a hormone released by the adrenal cortex of the suprarenal gland during hypothalamic pituitary adrenocortical axis activation resulting in systemic physiological responses; physical and psychological stressors can induce its release during the "fight-or-flight" response

Cell Surface Markers – detectable proteins on the surface of cells that allow identification of specific cell types

Cytomegalovirus (CMV) – a typically asymptomatic viral infection that remains in an infected individual for life when viral reactivation can occur

Cytotoxic (CD8+) T-cells – aka cytotoxic T-lymphocytes are a type of white blood cell that can identify and kill virally-infected cells and cells with DNA damage; become activated by helper (CD4+) T-cells

Exercise – physical activity that results in activation of the hypothalamic pituitary adrenocortical axis; a type of stressor that can be characterized by form (e.g. running vs. swimming vs. biking), duration, and intensity

Helper (CD4+) T-cells – aka helper T-lymphocytes are a type of white blood cell that can activate (and suppress) other types of immune cells, such as cytotoxic T-cells to kill target cells and B-cells to secrete antibodies; important for immune system activation during an infection

Immune Risk Profile – a cluster of unfavorable changes to immune cell parameters, including inverted (<1.00) CD4+:CD8+ ratio, persistent viral (e.g. CMV) infection, and accumulation dysfunctional senescent T-cells; predictive of mortality in old and very-old adults (Wikby et al. 2002; Wikby et al. 1998; Pawelec et al. 2001)

Immunological space – a concept that a finite amount of physical space within secondary lymphoid tissues (e.g. lymph nodes, spleen, etc.) limits the number and type of immune cells in a person

Inflamm-aging – the concurrent age-associated decline in immune function and chronic low-grade inflammation

Lymphocytosis – the mobilization of lymphocytes (T-cells, B-cells, and natural-killer cells) in response to hypothalamic pituitary adrenocortical axis activation (i.e. exercise and/or psychological stressors), infection, or injury

Psychological Stress – cognitive, emotional, and physiological changes in response to a stimulus or threat (actual or perceived) greater than that which can be tolerated by an individual's coping capabilities

Senescent Phenotype – a classification of T-cells that describes characteristic dysfunction and unique identifiable surface markers

Serostatus – presence or absence of antibodies in blood serum; seropositive individuals have levels of antibodies specific to a particular antigen (e.g. CMV) that are above a certain threshold, while seronegative individuals have levels of antibodies below said threshold

Stressor – an actual or perceived threat to an organism that results in a response (i.e. "stress response") by the organism; stressors can be physical (e.g. exercise) or psychological (e.g. public speaking, interpersonal conflict) and acute (seconds to minutes) or chronic (days to weeks and longer)

Stress Response – an adaptive, protective physiological change that occurs as a result of a stressor; occurs through hypothalamic pituitary adrenocortical axis activation

Swimming – a form of exercise; a specific type of physical stressor

T-cells – type of white blood cell called lymphocytes with broad immune function; can be classified as either helper (CD4+) T-cells or cytotoxic (CD8+) T-cells

URTI – upper respiratory tract infection is an acute illness of the upper airways often observed in athletes during periods heavy training

Latent viral reactivation – in an already infected host, a period of increased viral replication and proliferation not caused by re-infection by the virus in question

Abstract

Evidence suggests high-intensity exercise training increases incidence of upper respiratory infection in young-adult athletes (Spence et al. 2007). Collegiate athletes experience chronic stress, which has been shown to result in increased proportions of late-differentiated CD8+ T-cells of a dysfunctional phenotype (Bosch et al. 2009). However, many studies fail to consider possible moderators such as psychological stress and cytomegalovirus (CMV) infection. **Purpose**: To examine the impact of psychological stress, CMV infection, and exercise on proportions of early- and late-differentiated CD4+ and CD8+ T-cells in collegiate swimmers over a 7-month training period. **Methods**: Data were collected from NCAA Division 1 swimmers (13 M, 12 F: 19.7 ± 0.8 yrs) at 3 timepoints: early-season (October, *n*=18), immediate post-season (April, *n*=23), and off-season (June, *n*=10). Participants performed an in-water anaerobic capacity power test, consisting several 25-yard swims with increasing resistance. Early-morning serum samples were taken before (resting) and immediately following exercise. Flow cytometry after monoclonal antibody staining determined proportions of CD4+ and CD8+ T-cells categorized as early- (CD57-/KLRG1-) or late- (CD57+/KLRG1+) differentiated. ELISA determined resting serum cortisol concentration and CMV serostatus (CMV+ or CMV-). Data presented as mean ± SE. **Results**: Proportions of resting early- and late-differentiated CD4+ ($p=0.025$ and $p=0.001$, respectively) and late-differentiated CD8+ ($p=0.006$) T-cells differed between April and October timepoints. In April, exercise induced a preferential mobilization (% change) of late- (111.77 ± 32.5 %) compared to early- (-5.72 ± 1.6 %) differentiated CD4+ T-cells and late- (54.75 ± 7.9 %) compared to early- (-25.35 ± 3.2 %) differentiated CD8+ T-cells. Similar mobilization patterns occurred at October and June timepoints. Serum cortisol concentration had no effect on immune cell proportions. CMV+ had lower resting proportions of early-differentiated CD4+ ($p=0.007$) and CD8+ ($p=0.045$) T-cells compared to CMV-.

Conclusions: Training status partially regulates T-cell proportions over time. CMV infection results in unfavorable changes in the CD4+ T-cell repertoire, which may compromise immune protection against novel pathogens.

Chapter 1. Introduction

1.1. General Introduction

Stressors elicit physiological and immunological responses. These responses maintain homeostasis and prevent infection but have the potential to become detrimental if poorly regulated. Regular moderate-intensity exercise is known to positively impact immune function. However, athletes, as a result of consecutive training sessions and inadequate recovery, may experience impaired immunity. Acute intense exercise and endurance training can result in suppression of immune function and/or exacerbate pro-inflammatory responses (Pedersen & Nieman 1998). Intense exercise, in particular, appears to be detrimental to immune protection in the context of competitive sporting events (Walsh et al. 2011). Similarly, chronic psychological stress is associated a blunted immune response (Dhabhar & McEwen 1997) and poor regulation of inflammation (Miller et al. 2009; Vitlic et al. 2014). This leads to a pro-inflammatory state comparable to the chronic low-grade inflammation observed in older individuals, a phenomenon coined *inflamm-aging* (Franceschi et al. 2007). There is no current indication that the immunosuppressive effects of intense exercise training are harmful later in life or linked to inflamm-aging. However, these immunosuppressive effects do resemble characteristics associated with increased mortality risk and poorer disease outcomes.

Inflamm-aging is associated with increased risk of morbidity and mortality (De Martinis et al. 2005; Lencel & Magne 2011). Patients affected by inflamm-aging experience impaired vaccine responsiveness, increased circulating pro-inflammatory cytokines, and latent viral reactivation (Kiecolt-Glaser et al. 2002; Poland et al. 2014). Additionally, inflamm-aging is an integral part of the Immune Risk Profile (IRP), an umbrella term grouping changes in immune

cell parameters associated with impaired immune function and 4-year all-cause mortality in older adults (Pawelec et al. 2001). Other hallmark features of the IRP include inverted (<1.00) CD4+:CD8+ ratio, persistent viral infection, and expansion of [senescent] T-cells (Ndumbi et al. 2015; Boren and Gershwin 2004). Functional characteristics of senescent T-cells include diminished replicative capacity and responsiveness to apoptotic signals. As a result of these dysfunctions, senescent T-cells impair immune cell responsiveness to novel pathogens and increase risk of infection (Tu & Rao 2016). Similar to individuals exhibiting IRP characteristics, young athletes are also prone to inverted CD4+:CD8+ ratio after acute bouts of high-intensity exercise (Mackinnon et al. 1997; Lee et al. 2012).

Research attempting to determine causes of IRP characteristics suggests that both physical and psychological stressors contribute to the development of T-cell replicative senescence (Epel 2009) along with characteristics of the IRP. While an abundance of research describes immunological responses to acute and chronic stressors, there is a paucity of data investigating IRP characteristics within younger populations (< 30 yrs). Research is needed to better understand immune dysfunction, changes in susceptibility to infection, and premature immune aging in collegiate athletes, a population exposed to both risk factors and protective factors.

This research may elucidate a clearer relationship between various stressors and immunocompromised populations. Furthermore, it may allow for identification and prediction of individuals with an increased risk for developing immune dysfunction and immunosuppression.

Since collegiate athletes are subjected to daily exercise training in addition to psychological stressors (e.g. academics, relationships, etc.), they may be at an increased risk of negative immunological consequences of training. Research surrounding the subject, however, remains discordant. Clow and Hucklebridge (2001) suggested exercise training and

psychological stress may have a negative synergistic effect on athlete immunity and health. Evidence suggests high-intensity exercise training increases incidence of upper respiratory-tract infection (URTI) in athletes (Spence et al. 2007). Similarly, (Nieman et al. 1990) used a J-shaped model to describe the relationship between exercise training volume and URTI risk: Individuals who trained at moderate volume exercise workloads experience the lowest URTI risk, compared to those who trained at low and high volume workloads experience moderate and high URTI risk, respectively. An S-shaped curve (Malm 2006) elaborates on the Nieman's J-shaped model by suggesting *elite* athletes actually experience a reduced infection odds ratio compared to less elite athletes exercising at high volume exercise workloads. These findings are relevant for all exercising individuals but may be particularly important for collegiate athletes.

Student-athlete well-being remains pivotal in collegiate athletics, as sickness may contribute to subpar performance in practice and competition. Unfortunately, monitoring athlete stress remains difficult. Because of technical and financial constraints, team physicians and athletic training staff often rely on subjective measures of stress and well-being. These subjective measures of well-being, such as self-reported sleep quality and URTI symptoms, may lack the sensitivity necessary to detect changes in immune function. Furthermore, infection by a novel pathogen and latent viral reactivation may share symptomatic similarities. Finally, most studies examining UTRI in athletes fail to account for possible moderating factors, such as psychological stress and persistent viral infection. Doing so may elucidate a clearer relationship between URTI incidence and exercise in athletes. Consequently, it is paramount to use objective biomarkers of stress and changes in immune cell populations in order to adequately characterize the impact of prolonged training on the health of athletes. The present study aimed to consider the aforementioned oversights and difficulties by analyzing the impact of psychological and physical stressors on the immune function of collegiate student-athletes.

1.2. Specific Aims

The overarching aim of this project (Specific Aim #1) was to explore the impact of psychological and training-induced stressors on the immune function of NCAA-level collegiate swimmers. Data have been collected in athletes at rest to examine subjective and objective measures of stress throughout the competition season. Hypothesis #1: Higher resting serum cortisol concentrations would be associated with subjective measures of stress along with greater proportions of late-differentiated and dysfunctional senescent T-cells. Additionally, we examined the impact of acute exercise bouts on the immune response of collegiate swimmers throughout the season (Specific Aim #2). Hypothesis #2: Degree and composition of lymphocytosis induced by acute exercise would be affected by resting serum cortisol concentration and overall well-being of the athletes.

1.3. Overview of the Immune System

The human immune system consists of the innate immune system and the adaptive immune system. The innate immune system serves as the first line of defense against infection; it recognizes and destroys pathogens quickly and non-specifically in a semi-isolated manner (Janeway & Medzhitov 2002; Medzhitov & Janeway 2002). The adaptive immune system confers prolonged protection against pathogens identical or very similar to those previously encountered. By recognizing and remembering specific molecular structures, adaptive immunity confers specificity and memory in antigen recognition. Antigen presentation allows rapid communication between the two to identify and prevent uncontrolled growth of infected host

cells and non-self cells. The innate and adaptive arms of the immune system are complimentary. They communicate to elicit a response proportional to the pathogenic threat.

Conserved through evolution, the innate immune system defends against common pathogens through localized, non-specific attack. Innate immune cells (e.g. macrophages, natural killer cells, basophils, and dendritic cells) reside along physical barriers that separate human tissue from the outside world. Beneath skin and mucous membranes, innate immune cells act as a second line of defense. For instance, macrophages exist beneath the skin and in alveoli of the lungs, two barriers susceptible to infiltration by pathogens. Innate effector cells identify non-self cells by recognizing common molecular patterns. For example, Toll-like receptor 5 (TLR-5) binds flagellin, a protein composing flagella on bacteria. As a result of TLR-5 binding flagellin, macrophages become activated, ultimately presenting a portion of the flagellin protein for other immune cells to detect. Insoluble proteins cause inflammation, which recruits innate effector cells and localizes their effect. Phagocytes and natural killer (NK) cells destroy pathogens after complement system proteins tag them. Dysregulation of inflammatory proteins can lead to a chronic pro-inflammatory state, which can be harmful. Innate immunity may be insufficient and, thus, require specialized cells of the adaptive immune system to neutralize pathogenic infection.

Adaptive immune cells such as mature memory B-cells and memory T-cells deftly recognize previously encountered pathogens and work with innate immune cells to suppress pathogenic proliferation. The ability of adaptive immune cells to remember antigens and to respond robustly describes immunological memory (Janeway et al. 2001). Normal aging and pathological immune aging negatively impact T-cells more so than B-cells; therefore, T-cell population changes are the primary focus of the present study.

T-cells can replicate from few to many in a process called clonal expansion, a fundamental aspect of the adaptive immune system. By retaining low counts of antigen-specific

immune cells until threat of infection requires a response, limited *immunological space* remains available within lymphoid organs. The adaptive immune response occurs during the late stages of a primary exposure to an antigen and provides long-term protection against that specific antigen. The robustness of the immune response is dependent on various factors, such as stress-induced modulation.

1.4. T-Cells

T-cells mediate the adaptive immune response. Specifically, they control cell-mediated immunity, which monitors and eliminates intracellular pathogens. T-cells categorically and uniquely express CD3 surface glycoproteins, which enables their identification amongst other leukocytes. Two distinct types of T-cells exist: helper T-cells and cytotoxic T-cells. Helper T-cells secrete cytokines to communicate with other immune cells and stimulate cytotoxic T-cells, which become alert and active during infection. Cytotoxic T-cells destroy cells infected by viruses. T-cells can be identified by unique combinations of surface proteins at various points throughout their lifetime.

T-cells have two characteristic receptors: a protein complex called CD3 along with either CD4 or CD8 co-receptor. CD3 facilitates communication between extracellular and intracellular environments, because its 6 protein subunits span the cell membrane. T-cell receptors (TCR), CD3, and a co-receptor form a complex consisting of various light and heavy chains. Expression of both CD4 and CD8 co-receptors occurs in newly maturing T-cells found in the thymus. Over time, only one co-receptor, either CD4 or CD8, will be expressed by the cell; this expression of CD4 or CD8 is nearly always mutually exclusive (Koretzky 2010; Gallagher et al. 1989). A pathogen, identified as non-self, is presented to the naive T-cell by specialized antigen-

presenting cells (APC), thus inducing the naive T-cells to differentiate into an effector cell, capable of clearing the pathogen.

1.5. T-Cell Differentiation

Over a T-cell's lifespan, it expresses a variable profile of surface markers. The presence or absence and level of expression of certain surface markers is indicative of that particular cell's stage of differentiation. T-cell differentiation/senescence may be sufficiently described by five putative markers: CD45, CD28, CD27, CD57, and killer cell lectin-like receptor G-1 (KLRG1) (Sallusto et al. 2004; Voehringer et al. 2002; Tarazona et al. 2000). Among others, these functional and molecular markers have been studied and can provide additional information about T-cells at various stages of differentiation (Table 1).

Table 1. Summary of surface markers on T-cells at various stages of differentiation.

CCR7	+	+	–	–
CD45RA	+	–	–	+
CD28	++	+++	+/–	–
CD27	++	+++	+/–	–
CD57	–	–	+	++
KLRG1	–	+/–	+	++

-: no/very low expression; +: unit of expression (Larbi & Fulop 2014).

A majority of research describes T-cell differentiation as a chronological sequence. More precisely, the field recognizes a linear model describing a sequential progression of discrete T-

cell phenotypes. While this method is useful for discussion and scientific consensus, the steps are more likely continuous and not unidirectional (Larbi & Fulop 2014). Four established sub-populations of T-cells are discussed in the current literature and are presented least-to-most differentiated: naïve, central memory (CM), effector memory (EM), and terminally-differentiated effector memory cells re-expressing CD45RA (Xu and Larbi 2017).

Two markers commonly utilized to describe level of T-cell differentiation include C-C motif chemokine receptor CCR7 and CD45RA. Using only these two markers, T-cell are categorized into four sub-populations: CD45RA+CCR7+ naive, CD45RA−CCR7+ CM, CD45RA−CCR7− EM, and CD45RA+CCR7- TEMRA T-cells (Sallusto et al. 2004). CCR7 induces morphological polarization in naïve T-cells (Bardi et al. 2003), which is important for antigen recognition and communication with other immune cells. CCR7 also allows naïve T-cells to travel to secondary lymphoid organs by modulating T-cell migration speed (Kaiser et al. 2005). CD45 is a receptor-linked protein tyrosine phosphatase (Desai et al. 1994). The intracellular domain of CD45 regulates TCR signal transduction during activation of the T-cell. The extracellular domain of CD45 exists as several isoforms. Alternative splicing allows for expression of different combinations of exons, CD45RA, CD45RB, and CD45R0 (Hermiston et al. 2003). Larbi & Fulop (2014) suggested that the most important isoforms are CD45RA and CD45RO, as they undergo isoform switching which contributes to T-cell differentiation and exhaustion. Differential expression of CD45RA, particularly, was used to describe stage of T-cell differentiation. Naïve T-cells express CD45RA, which is down-regulated with subsequent differentiation to CM and EM T-cells. Interestingly, CD45RA appears to again become re-expressed in terminally-differentiated T-cells, giving them their name terminally-differentiated effector memory cells re-expressing CD45RA (TEMRA) (Larbi & Fulop 2014).

Two other markers, CD28 and CD27, have been used to describe cellular history. Surface glycoproteins CD28 and CD27 act as co-receptors that ligate complimentary receptors on the surface of APCs and strengthen the connection of the complex. CD28 binds its B7 co-receptor and CD27 binds CD70. Co-stimulation by CD28 and CD27 is important to overcome the crosslink TCR requirement, ultimately leading to cellular proliferation. Thus, CD28 and CD27 are indicative of a cell's proliferative history. As naïve T-cells differentiate to become CM T-cells, CD28 and CD27 expression is up-regulated; however, with further differentiation, expression of these markers decreases markedly. Naïve T-cells express both CD28 and CD27, CM T-cells tend to express higher levels, EM T-cells exhibit decreased expression, and TEMRA T-cells express no or very low levels of expression. IL-15 and IL-21 and signaling molecules of the JAK/STAT pathway partially mediate CD28 expression in CD8+ memory T-cells (Nguyen & Weng 2010). Generally, increased differentiation is associated with progressive loss of surface markers, CCR7, CD45, CD28, and CD27, which corresponds to loss of function (Reinke et al. 2013; Monteiro et al. 2007). However, expression of some markers does increase with differentiation.

Highly-differentiated TEMRA/senescent T-cells express CD57 and KLRG1. Palmer et al. (2005) examined the proliferative capacity of CD4+CD57+ T-cells. Despite strong mitogen stimulation, CD4+CD57+ T-cells exhibited significantly reduced proliferation compared to CD4+CD57- T-cells. This finding supports other research that, too, suggests cells expressing CD57 exhibit impaired proliferative capacity, even after stimulation (Merino et al. 1998; Vivar et al. 2008). Interestingly, Chong et al. (2008) found $CD8^{hi}$CD57+ T-cells to be highly proliferative. The authors suggest this direct contrast is due to previous studies utilizing carboxyfluorescein succinimidyl ester (CFSE), a staining dye used in fluorescence-based

proliferation assays. Here, CFSE was found to be cytotoxic to $CD8^{hi}$CD57+ T-cells. CD57 alone may not be sufficient to label as highly-differentiated.

KLRG1 is another marker that is present on cells with impaired proliferative capacity and replicative senescence. KLRG1 is associated with cells that have undergone a large number of cell divisions, thus indicating their high level of differentiation (Ibegbu et al. 2005; Voehringer et al. 2001). This association appears true irrespective of increased age of the individual. Henson et al. (2009) found that KLRG1 expression significantly increased from the early-differentiated (CD8+CD28+CD27+) subset to the intermediate-differentiated (CD8+CD28−CD27+) subset and again from the intermediate-differentiated (CD8+CD28−CD27+) subset to the late-stage-differentiated (CD8+CD28−CD27−) subset. Furthermore, when KLRG1 signaling was blocked during stimulation of CD8+CD28-CD27- T-cells, proliferative capacity was spontaneously induced in these cells. This finding suggests that KLRG1 is an inhibitor of cell proliferation. Together, KLRG1 and CD57 appear sufficient to describe a T-cell existing in a state of replicative senescence. Use of the CD57+KLRG1+ phenotype is common in the recent pool of research (Larbi and Fulop 2014; Stubbe et al. 2006).

Using CD45, CD28, CD27, CD57, and KLRG1 together allows for an accurate description of relative levels of differentiation within a population of T-cells in circulation. These, among other markers, are useful in describing the characteristics, function, and implications of cells that exhibit a senescent phenotype.

1.6. Senescent T-Cell Characteristics

T-cell replicative senescence is a state of immune cell dysfunction characterized by an accumulation of T-cells with a senescent phenotype (Simpson 2011). Hayflick & Moorhead

(1961) first described senescent cells with their *in vitro* studies of human lung fibroblasts. They noted that senescent cells, while viable, exist in a state of arrested cell cycling and become unresponsive to apoptotic signaling. T-cell replicative senescence contributes to impaired immune function in the elderly, because senescent T-cells are dysfunctional (Ostan et al. 2008). They occupy a large portion of immunological space, exhibit diminished proliferative capabilities, demonstrate a blunted responsiveness to apoptotic signals, and increased pro-inflammatory cytokine production.

Cells of the senescent phenotype can be characterized by expression and non-expression of specific surface glycoproteins. Diminished expression of CD28 concomitant to presence of other surface proteins (KLRG1 and/or CD57+) identifies T-cells as senescent (Brenchley et al. 2003; Simpson et al. 2007). Upon activation, CD28 acts as co-receptor to regulate functions like transcription of IL-2, cellular adhesion, and apoptosis among others (Thompson et al. 1989).

By occupying a large part of the finite immunological space, less physical space is available for naïve T-cells to exist in secondary lymphoid tissues. Thus, protection against novel pathogens is reduced (Deeks 2011). While senescent T-cells remain viable in protecting against the antigen to which they bind, they do not retain proliferative capacity upon antigen stimulation, rendering them incapable of clonal expansion. Consequently, the less numerable naïve T-cells cannot defend against novel pathogens, resulting in a blunted adaptive immune response.

Senescent T-cells found in aging populations exhibit reduced proliferative capacity and response to apoptotic signals. Voehringer et al. (2002) described differentiated populations of T-cells (and NK cells, too) expressing KLRG1 that cannot proliferate subsequent to mitogenic stimulation, thus limiting their ability to clonally expand. During their lifespan, immune cells experience persistent regulation to ensure proper function. Pro-apoptotic signals mediate this maintenance of the immune cell repertoire. The cell surface death receptor CD95 (Fas/Apo-1)

and its ligand (CD95L) can result in apoptosis through DNA fragmentation by caspase-8 (Simpson 2011). Other pro-apoptotic signals contribute to this regulation. Such signals are notable during exercise. These include increased levels of inflammatory cytokines, catecholamines, cortisol, and reactive oxygen species (Simpson 2011). Interestingly, Spaulding et al. (1999) found CD28-CD57+ T-cells to robustly resist apoptosis when exposed to these apoptotic signals. Finally, the senescent phenotype is associated with increased pro-inflammatory cytokine production (Chou & Effros 2013; Coppé et al. 2010).

Cells of a senescent phenotype appear harmful both in terms of immunological parameters and disease outcomes. Presence of the senescent T-cell phenotype in individuals is associated with inverted CD4+:CD8+ ratio and more aggressive clinical disease course (Nunes et al. 2012). Characteristics of dysfunction in senescent T-cells are also present normal and pathological aging populations.

1.7. Inflamm-Aging and the Immune Risk Profile

Aging populations experience a decline in immune function whereby experiencing increased infection and higher incidence of disease and mortality. *Inflamm-aging* describes the age-associated decline in immune function and the concurrent increased pro-inflammatory state (Pawelec et al. 1995). It is suggested that increased concentrations of pro-inflammatory cytokines are a mechanism contributing to increased mortality risk in elderly populations (Franceschi et al. 2007).

Alterations in immunoendocrine biomarkers occur with inflamm-aging. Presence of particular phenotypic and serologic parameters, called the IRP, can predict mortality in aging populations. The IRP consists of a cluster of biomarkers including low or inverted CD4+:CD8+

T-cell ratio, CMV seropositivity, and unfavorable changes to immune cell populations (Barron et al. 2015) similar to those discussed above. Previously, the IRP was associated with increased mortality risk in octogenarians and nonagenarians (Wikby et al. 1998; Wikby et al. 2002). Recent research suggested the IRP is predictive of increased 2-year mortality in hexagenarians, too (Strindhall et al. 2013). Research continues to identify a relationship between the IRP and increased mortality risk in younger cohorts.

Similarities exist between inflammaging, some IRP parameters, and immune responses to chronic stress. Increased concentrations pro-inflammatory cytokines (IL-1β, IL-6, TNFα, and CRP) are associated with development of certain cancers, neurodegenerative diseases, stroke, and metabolic dysfunction (Krabbe et al. 2004; Goto 2008). Inflammation associated with chronic stress results from excessive pro-inflammatory cytokines along with subsequent increases in cellular adhesion molecules and chemokines (Tian et al. 2014). Furthermore, chronic job stress is associated with a reduced CD4+:CD8+ T-cell ratio and higher percentage of senescent T-cells (Bosch et al. 2009). Physical and psychological stressors appear to trigger similar immune responses and may have a cumulative negative synergistic effect (Clow & Hucklebridge 2001). This becomes particularly pertinent when considering the stressors affecting collegiate student-athletes.

1.8. The Immune Response

Stressors elicit physiological responses, including cardiovascular, hormonal, and immunological changes. Stressors can be short acting or long acting and can be physical or psychological. In fact, physical and psychological stressors appear to elicit similar physiological responses via hypothalamic pituitary adrenocortical (HPA) axis activation and the fight-or-flight response

(Gleeson et al. 2011; Gleeson et al. 2013). Furthermore, immunological responses appear non-specific to the type of stressor. That is, psychological stressors and physical stressors elicit a similar immune response of similar magnitude and duration (Clow & Hucklebridge 2001). However, acute versus chronic stressors generate paradoxical immune responses. Acute stressors may be protective and even advantageous for performance and survival. Conversely, chronic stress can be detrimental to overall health and immune function.

1.9. Acute Physical and Psychological Stressors

During exercise, particularly intense exercise, ventilatory volume increases favoring oral breathing, a shift from nasal breathing at rest (Bennett et al. 2003). Increased gut permeability during prolonged exercise may increase endotoxin release into the circulation (de Punder & Pruimboom 2015). These changes during exercise may increase an individual's exposure to pathogens and ultimately risk of infection, however, it becomes important to examine body's immune response to acute exercise.

Hormonal changes occur during acute stress. The sympathetic adrenal medulla (SAM) and HPA become activated in response to some stressor. Within the SAM, the hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary to secrete adrenocorticotropic hormone (ACTH) (Majzoub 2006). ACTH travels through circulation to the adrenal medulla, which is stimulated to release epinephrine (E) and norepinephrine (NE). E and NE travel the bloodstream and cause systemic fight-or-flight effects like increased heart rate, respiration rate, and blood pressure. Complementary to the SAM is the HPA. The HPA functions nearly identically to the SAM until ACTH stimulates the adrenal cortex to release corticosteroids (i.e. cortisol). In the context of acute stress, cortisol is anti-

inflammatory, promotes healing, and mobilizes energy. An important soluble protein co-stored and co-released with catecholamines is chromogranin A (CgA) (D'amico et al. 2014). CgA can be cleaved into several bioactive peptides that regulate metabolism by exerting broad effects throughout the body, including on the immune system.

Generally, acute stress mimics the immune response during an infection. That is, lymphocytes, including T-cells, become mobilized into peripheral circulation. This process is referred to as lymphocytosis. Just as the catecholamine response to acute physical stress (exercise) appears intensity-dependent (Miyazaki et al. 2001), so does the degree of lymphocytosis. Given the presence of β-adrenergic receptors on all lymphocytes (Sanders & Straub 2002), the catecholamine response to exercise is a crucial determinant to the degree of lymphocytosis (Benschop et al. 1996). Acute exercise and pharmacological infusion appear equally effective and dose-responsive in inducing lymphocytosis. Anane et al. (2009) examined specific lymphocyte mobilization in response to three different stress tasks including a psychological public speaking stress test, acute exercise, and pharmacological infusion of a β-agonist (isoproterenol). γδ T-cells, CD8+ T-cells, and NK cells were mobilized in a dose-dependent manner during all three tasks. This mobilization of lymphocytes results from stimulation by catecholamines and cortisol (McCarthy & Dale 1988).

Interestingly, acute exercise has been shown to mobilize late-differentiated and senescent T-cells more favorably than naïve T-cells (Simpson et al. 2007; Campbell et al. 2009). This finding could have implications for freeing up immunological space. After acute exercise when senescent T-cells are mobilized into circulation, they may be exposed to greater amounts of catecholamines, cortisol, and reactive oxygen species. Exposure to pro-apoptotic stimuli may facilitate their clearance. Similarly, acute exercise mobilizes late-differentiated NK cells more so than it does their naïve counterparts (Bigley et al. 2014). The cost of adaptation, known as

allostatic load, results in wear-and-tear on the body and brain (Baum et al. 2012). As the duration of a stressor increases, the protective immune response becomes less capable of defending against novel pathogens. Brief naturalistic stressors, such as academic final examinations, are slightly longer in duration and begin to suppress immune function (Segerstrom & Miller 2004). And as stressors become longer yet, the immune response becomes even more dysfunctional. Specifically, chronic heart rate and blood pressure elevations can lead to immunosuppression and a pro-inflammatory state.

1.10. Chronic Physical and Psychological Stress

Chronic stress can be harmful to immune function and overall health. Impaired immune function secondary to chronic stress includes impaired vaccine responsiveness, latent viral reactivation, a pro-inflammatory state, and changes in immune cell populations.

This poor vaccine responsiveness is seen in chronically stressed individuals. Poorer cytokine responses, specifically influenza virus-specific IL-2 responses, are associated with increased risk of influenza infection (McElhaney et al. 1994; Kiecolt-Glaser et al. 1996). Kiecolt-Glaser et al. (1996) examined influenza-antibody response following vaccination in caregivers looking after a spouse with progressive dementia (stressed) versus controls. Stressed caregivers had a poorer virus-specific T-cell response, which was evidenced by lower *in vitro* influenza-stimulated production of interleukin-2 (IL-2) and interleukin-1β (IL-1β) compared to controls. A possible mechanism behind this impairment relates to reduced interferon (IFN)-γ production (Frick et al. 2009). IFN's utilize ribonuclease and protein kinase to degrade viral mRNA and inhibit viral protein synthesis, respectively. IFN-γ is associated with MHC-antigen presentation.

Therefore, reduced IFN-γ production inhibits MHC-antigen presentation and subsequent recognition.

Another consequence of chronic stress is an increased rate of latent viral reactivation. That is, chronically-stressed individuals exhibit elevated viral titers. Rector et al. (2014) examined associations of psychological stress and cytomegalovirus (CMV) reactivation. CMV-immunoglobulin G (IgG) numbers increased in a dose-response manner to various mental health variables, such as sleep problems, anxiety, and depression (Rector et al. 2014). Interestingly, these associations were stronger in individuals of low socioeconomic status, alluding to a moderating effect of social factors. Finally, neither C-reactive protein (CRP) nor glucocorticoids were associated with CMV-IgG titers, indicating viral reactivation occurred independently of inflammation and HPAC activity.

Finally, chronic stress induces changes in immune cell populations. T-cells appear to shift away from a naïve to a senescent phenotype. This is shift is detrimental, since senescent T-cells are terminally-differentiated and have reduced proliferative capacity (Müller and Pawelec 2014). Furthermore, senescent T-cells exhibit impaired responsiveness to novel pathogens and increased pro-inflammatory cytokine production (Orjalo et al. 2009; Krabbe et al. 2004; Graham et al. 2006).

Chronic stress is an unsurprising consequence for collegiate student-athletes. Typically, exercise training sessions occur at last once daily and can surpass four sessions during competitions. Further, student-athletes experience academic and interpersonal stressors not unique from those experienced by their non-athlete classmates. Inadequate recovery in between training sessions can compromise performance and well-being.

1.11. Clinical and Performance Implications: URTI in Athletes

Chronic exercise training negatively affects immune function. It becomes crucial to consider athletes undergo consecutive bouts of acute exercise for years on end. Walsh et al. (2011) describe the "open window" theory where opportunistic infections may take advantage of an athlete's impaired immune function during the 3-72 hours after high-intensity exercise. These windows can become additive, further suppressing immune function with inadequate recovery.

A general consensus within the field suggests moderate exercise enhances immune function, while strenuous exercise has a suppressive effect (Matthews et al. 2002; Walsh et al. 2011). Similarly, training load appears to influence immune function. The current pool of literature suggests a phase shift of the dose-response immune protection conferred by exercise. When a condition of intensity or duration is surpassed, immunosuppression occurs. This immunosuppression may compromise an individual to opportunistic infection.

Evidence suggests high-intensity exercise *training* increases incidence of URTI in athletes (Spence et al. 2007). Similarly, Nieman et al. (1990) use a J-shaped model to describe the relationship between exercise volume and URTI risk. Individuals exercising at moderate workloads experience the lowest URTI risk, whereas those exercising at low and high workloads experience moderate and high URTI risk, respectively. An S-shaped curve (Malm 2006) elaborates on the J-shaped model, suggesting *elite* athletes, surprisingly, experience a reduced infection odds ratio compared to less elite athletes exercising at high workloads. This finding implies elite athletes may exhibit improved ability to adapt to high-workload training. This improved adaptation may confer protection against pathogenic infection.

Immunosuppression, if significant, has potential to manifest clinically significant URTI symptoms as a result of novel infection. Other times, the degree of immunosuppression may be

less than that required for infection, thus the athlete remains asymptomatic. Regardless of clinical symptoms, chronic stress does impair the adaptive immune response and induce disadvantageous changes in immune cell populations (Epel 2009) and other characteristics of the IRP. While much research exists to describe immunological responses to acute and chronic stressors, there is a paucity of data investigating IRP characteristics within younger populations. Research is needed to understand premature immune aging and immune dysfunction in collegiate athletes. This research may elucidate a clearer relationship between stressors and immunocompromised populations.

Student-athlete well-being is paramount to athlete success. Sickness may contribute to subpar performance in practice and competition. Therefore, coaches and athletic training staff attempt to monitor athletes' stress, however, this remains difficult. Nearly all of the aforementioned studies fail to account for psychological stress, which may elucidate a clearer relationship between URTI incidence and exercise in athletes.

Since psychological and physical stressors evoke similar physiological and immune responses, serum cortisol concentration can be used to monitor total HPA axis activation. However, due to practical and financial constraints, coaches and athletic training staff may instead rely on subjective measures of well-being. These subjective measures, such as self-reported sleep quality and URTI symptoms, may lack sensitivity necessary to detect changes in immune function. Consequently, it is paramount to use objective biomarkers of stress and changes in immune cell populations to characterize the impact of prolonged training on the health of the athletes with sufficient sensitivity.

Chapter 2. Specific Aims

The overarching aim of this project was to explore the impact of psychological and training-induced stressors on the immune function of NCAA-level collegiate swimmers (Specific Aim #1). Data have been collected in athletes at rest to examine subjective and objective measures of stress throughout the competition season. It was hypothesized that higher resting serum cortisol concentrations would be associated with subjective measures of stress along with greater proportions of late-differentiated and dysfunctional senescent T-cells (Hypothesis #1).

Additionally, we examined the impact of acute exercise bouts on the immune response of collegiate swimmers throughout the season (Specific Aim #2). It was hypothesized that degree and composition of lymphocytosis induced by acute exercise would be affected by resting serum cortisol concentration and overall well-being of the athletes (Hypothesis #2).

Chapter 3. Methods

3.1. Participants & Study Timeline

Volunteers were recruited using word of mouth and presentations. Data collection occurred at 3 time points: off-season (June), early-season (October), and immediate post-season (April). At each time point, volunteers gave early-morning pre- and post-exercise blood samples, and completed questionnaires (Pittsburgh Sleep Quality Index, Daily Analysis of Life Demands for Athletes, Activation-Deactivation Adjective Checklist, and Wisconsin Upper Respiratory Symptoms Survey) prior to completing an in-water anaerobic capacity power test.

The volunteers were all healthy, college-aged members of a NCAA Division 1 swimming team who met inclusion criteria. Volunteers provided written informed consent. Only volunteers who were cleared by a Team Physician were eligible to participate. A regulation was in place so that if volunteers presented with any medical, psychiatric, or behavioral condition(s) that might interfere with protocol adherence, they would have been excluded from this study.

3.2. Exercise Protocol

Participants completed an in-water anaerobic capacity power test. The in-water anaerobic capacity power test began after a 15 minute warm-up period and test was comprised of a series of resisted 25-yard swims. Participants wore a harness that attached to a pulley system connected to a terminal 35-gallon bucket. After each successful 25-yard swim, the swimmer was allowed 3 minutes of active recovery (light swimming). Resistance was increased after each successful trial by adding weight to the bucket: 20 lbs added for men and 15 lbs added for women. Men

performed the first trial with 40 lbs in the bucket; women performed the first trial with 20 lbs in the bucket. Trials of increasing weight were completed every 3 minutes until the swimmer was unable to reach the other side of the pool. At this point, the swimmer was offered the option of attempting one additional trial with weight in the bucket equaling that during the previous failed attempt or stopping the test.

Heart rate, relative workload intensity, and blood lactate concentrations were taken and recorded after each trial, regardless of whether the athlete was successful or not. Blood samples were collected immediately before and immediately after the in-water exercise performance test.

3.3. Objective Measures of Stress

Serum cortisol was measured using enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, MN, USA). Samples and cortisol-enzyme conjugate were added to wells coated with anti-cortisol monoclonal antibodies (mAb). Serum cortisol competes with the cortisol-enzyme conjugate for binding sites on microwell plates, and wash buffer removes unbound protein. Substrate was added and the intensity of color of the sample was compared to that set by a standard curve. CMV serostatus was determined using ELISA (GenWay Biotech Inc, San Diego, CA, USA; Abcam, Cambridge, MA, USA).

3.4. Subjective Measures of Stress

Subjective measures of stress were determined by self-reported questionnaire responses. Participants completed questionnaires prior to being phlebotomized and exercise testing. Self-reported measures of sleep, overall well-being, activation-arousal, and upper respiratory tract infection were assessed at each visit. Females completed menstrual cycle questionnaires.

Sleep quality was assessed at each time point through the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al. 1989). This is a non-invasive, reliable, and validated measure (Mollayeva et al. 2016) that assesses dimensions of sleep quality, namely (a) overall sleep quality, (b) time to fall asleep, (c) sleep duration (d) daytime effects of sleep quality, and (e) sleep efficiency. The PSQI uses 19 items, consisting of open-ended questions (e.g., "When have you usually gone to bed?") as well as pointed response options (e.g., "During the last month, how often have you had trouble sleeping because you cannot get to sleep within 30 minutes?") of "Not during the last month," "Less than once a week," "Once or twice a week," or "Three or more times a week".

Participants self-assessed symptoms & sources of psychological well-being through the use of the Daily Analysis of Life Demands for Athletes (DALDA) questionnaire. This measure assessed the degree of general psychological well-being of the athlete, as well as sport-specific psychological well-being (Freitas et al. 2013; Rushall 1990). The DALDA questionnaire consists of nine items that address home-life, school/work, friends, and training while the assessment of sport-based psychological well-being addresses muscle pains, irritability, boredom, and tiredness. Athletes are asked to characterize each source and symptom as "worse than normal", "normal", or "better than normal".

Participants self-assessed activation and arousal at each time point by completing the Activation-Deactivation Adjective Checklist (AD-ACL). This twenty-item scale uses adjectives

to provide calculated dimensions of *energy arousal* (i.e. sleep-energy continuum) and *tension arousal* (i.e. placidity-tension continuum). Participants report the degree to which they feel each adjective as "Definitely feel," "Feel slightly," "Cannot decide," or "Definitely do not feel." Sample adjectives include *active* & *vigorous* (Energy), *sleepy* & *wakeful* (Tiredness), *jittery* & *clutched-up* (Tension), and *at-rest* & *quiet* (calmness) (Peters & Ziemainz 2014; Thayer 1986).

Upper respiratory tract infection symptoms were self-assessed using the Wisconsin Upper Respiratory Symptoms Survey (WURSS-21). The WURSS-21 is a condensed version of the original WURSS-44, and has been argued by the authors to be more responsive than the original (Barrett et al. 2005). This measure assesses the degree to which an athlete may experience upper respiratory complications (i.e. common cold-like symptoms). This measure, administered three times during the spring season, asks participants to report how they are feeling that day as well as over the previous 24 hours using 8-point Likert response options of "Not sick/Do not have this symptom/Not at all," (0), "Very mildly," (1), "Mildly," (3), "Moderately," (5), "Severely" (7). Participants reported how they feel, the degree of their symptoms, the degree to which their symptoms interfere with daily function, and how they feel compared to the previous day.

3.5. Cell Separation and Analysis

Venous blood was collected in EDTA-coated vacutainers, from which peripheral blood mononuclear cells (PBMCs) were isolated using gradient centrifugation. Blood samples were diluted in equal volume of Phosphate Buffer Saline (PBS) and layered on density-gradient media (Histopaque, Sigma Aldrich, MO, USA) before being centrifuged at 800 g for 30 minutes. Serum tubes were centrifuged and the resultant serum samples were frozen and kept at -80°C until all participants completed the study.

Immune cell phenotype was assessed using a BD Accuri C6 flow cytometer (Accuri, Ann Arbor, MI, USA) equipped with a blue laser emitting light at a fixed wavelength of 488 nm and a red laser emitting light at a fixed wavelength of 640 nm. Isolated PBMCs (1.0×10^6) were labeled with pre-diluted monoclonal antibodies (mAbs) in a 4-color direct immunofluorescence assay. Cells were incubated at room temperature in the dark for 30-minutes. The mAb combinations consisted of KLRG1, CD28 FITC, CD45RA FITC, CD27 PE, CD57 PE or CCR7 PE, CD4 PerCP, CD8 PerCP and CD3 APC. Specific combinations of mAbs allowed for the determination of various levels of T-cell differentiation. Following acquisition, FCS files were transferred to a third party software program (FCS Express v3.0, De Novo, Los Angeles, CA, USA) for analysis.

3.6. Statistical Analysis

All data were assessed for assumptions of normality using the Shapiro-Wilk test prior to subsequent formal statistical testing. While log transformation cannot always normalize data and, further, log-transformed data often loses relevance to the original data after standardized statistical testing (Feng et al. 2014), skewed data were normalized by logarithmic transformation. In instances when log transformation could not sufficiently normalize data, nonparametric analysis alternatives were utilized.

Paired samples t-tests and related samples Wilcoxon signed rank tests were performed to determine the main effect of time between two time points for resting and post-exercise peripheral blood T-cell proportion data. Paired samples t-tests and related-samples Wilcoxon signed rank tests were used to determine the effect of exercise on immune cell proportions. Spearman's rank-order correlation coefficients were used to identify linear correlations between

serum cortisol concentration and questionnaire responses. Linear mixed models were used to determine the interaction effects of covariates – CMV serostatus, cortisol, and time – on pre-exercise T-cell proportions, post-exercise T-cell proportions, and exercise-induced T-cell mobilization. Statistical significance was set at $p=0.1$ (i.e. $\alpha=0.1$). This significance level, the probability of rejecting the null hypothesis when it is true (Type I error), was chosen given that this is a preliminary examination and the real-life losses incurred by errors as a result of these findings are limited. All values given are as mean ± standard error (SE) unless otherwise stated (Altman & Bland 2005). All statistical analyses were performed using "Statistical Package for Social Sciences" (SPSS Inc., Chicago, IL, USA).

Chapter 4. Results

4.1. Anthropometric Measurements

All participants completed the exercise protocol described above. Anthropometric data of all participants is presented in Table 2. Differing numbers of athletes participated at each of the time points (April, *n*=23; June, *n*=10; and October, *n*=18).

Table 2. Anthropometric data of all participants.

Physical Characteristics	All participants (n=25)	Female (n=12)	Male (n=13)	CMV– (n=16)	CMV+ (n= 9)
Age (years)	19.7 ± 0.8	19.5 ± 0.9	19.9 ± 0.5	19.6 ± 0.6	19.9 ± 1.0
Body mass (kg)	73.4 ± 9.9	67.2 ± 9.3	79.6 ± 5.8	71.2 ± 9.5	77.8 ± 9.7
Height (cm)	177.9 ± 8.9	171.2 ± 7.3	184.1 ± 4.9	176.1 ± 9.3	181.2 ± 7.6
BMI (kg/m^2)	23.1 ± 1.7	22.9 ± 2.2	23.4 ± 1.1	22.9 ± 2.0	23.5 ± 1.0
CMV seropositivity	36%	17%	54%	–	–

Data presented as (mean ± SD).

4.2. Effect of Time on T-Cell Proportions at Rest

Results for the effect of time on resting T-cell proportions are presented in Table 3. The effect of time on early-differentiated CD4+, late-differentiated CD4+, early-differentiated CD8+, and late-

differentiated CD8+ T-cell proportions at rest (pre exercise) is presented in Figures 5, 6, 7, and 8, respectively.

Resting early-differentiated CD4+ T-cell proportions increased from 86.8 % ± 1.6 % in April to 91.0 % ± 0.8 % in October ($p=0.025$). Proportions of resting late-differentiated CD4+ T-cells decreased from 3.7 % ± 0.7 % in April to 1.4 % ± 0.3 % in October ($p=0.001$). Proportions of early-differentiated CD8+ T-cells decreased from April to June, increased from June to October (Figure 7), however, these changes were not statistically significant. Conversely, proportions of late-differentiated CD8+ T-cells increased from April to June and decreased from June to October. These values, too, were not statistically significant. Proportions of late-differentiated CD8+ T-cells decreased from 15.2 % ± 2.6 % in April to 8.6 % ± 2.4 % in October ($p=0.006$).

Table 3. Proportions of pre-exercise T-cell subsets – early-differentiated CD4+, late-differentiated CD4+, early-differentiated CD8+, and late-differentiated CD8+ T-cell proportions – at each time point.

T-cell subset	Cell phenotype	Time point comparison (1st vs 2nd)	1st time point pre-exercise cell frequency (%)	2nd time point pre-exercise cell frequency (%)	p-value
early- differentiated CD4+ T-cells	CD3+/CD4+/ CD57–/KLRG1–	April vs June (n = 10)	88.63 ± 1.4	88.39 ± 2.1	0.919
		June vs October (n = 10)	88.39 ± 2.1	90.50 ± 0.9	0.262
		October vs April (n = 17)	90.98 ± 0.8	86.78 ± 1.6	**0.025**
late-differentiated CD4+ T-cells	CD3+/CD4+/ CD57+/KLRG1+	April vs June (n = 10)	3.70 ± 0.8	4.56 ± 2.2	0.766
		June vs October (n = 10)	4.56 ± 2.2	1.72 ± 0.6	0.155
		October vs April (n = 17)	1.40 ± 0.3	3.72 ± 0.7	**0.001**
early- differentiated CD8+ T-cells	CD3+/CD8+/ CD57–/KLRG1–	April vs June (n = 10)	66.37 ± 4.9	61.02 ± 4.5	**0.070**
		June vs October (n = 10)	61.02 ± 4.5	65.02 ± 5.3	0.267
		October vs April (n = 17)	69.65 ± 4.0	66.97 ± 3.2	0.405
late-differentiated CD8+ T-cells	CD3+/CD8+/ CD57+/KLRG1+	April vs June (n = 10)	16.90 ± 4.1	18.53 ± 4.3	0.455
		June vs October (n = 10)	18.53 ± 4.4	11.13 ± 3.7	**0.059**
		October vs April (n = 17)	8.57 ± 2.4	15.16 ± 2.6	**0.006**

Values presented are means ± SEM. All p-values reported. Statistically significant differences between pre-exercise cell proportions between time points are indicated by boldface text.

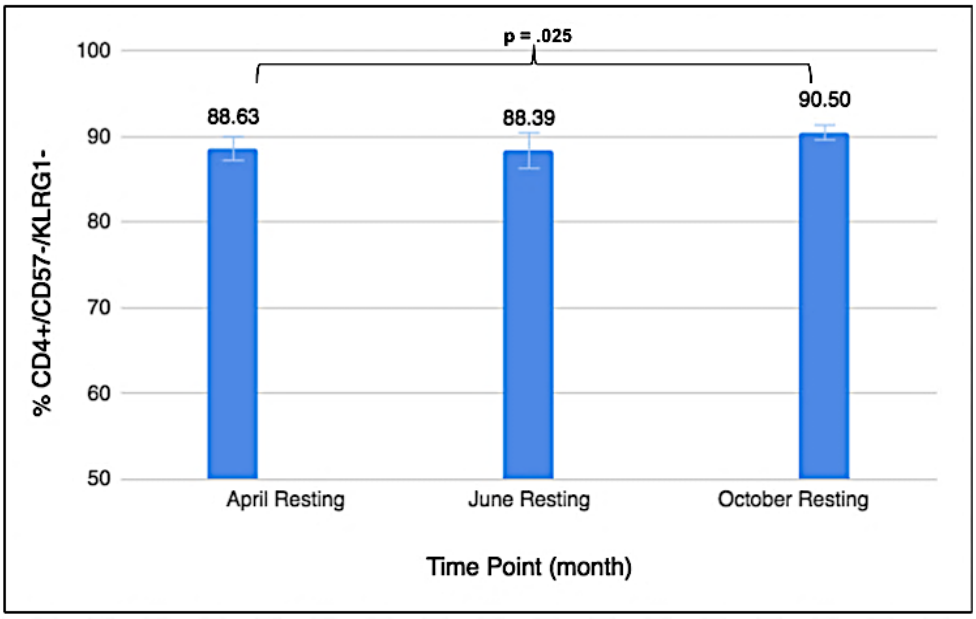

Figure 5. Effect of time on resting early-differentiated CD4+ T-cell proportions. Values presented are mean ± SE.

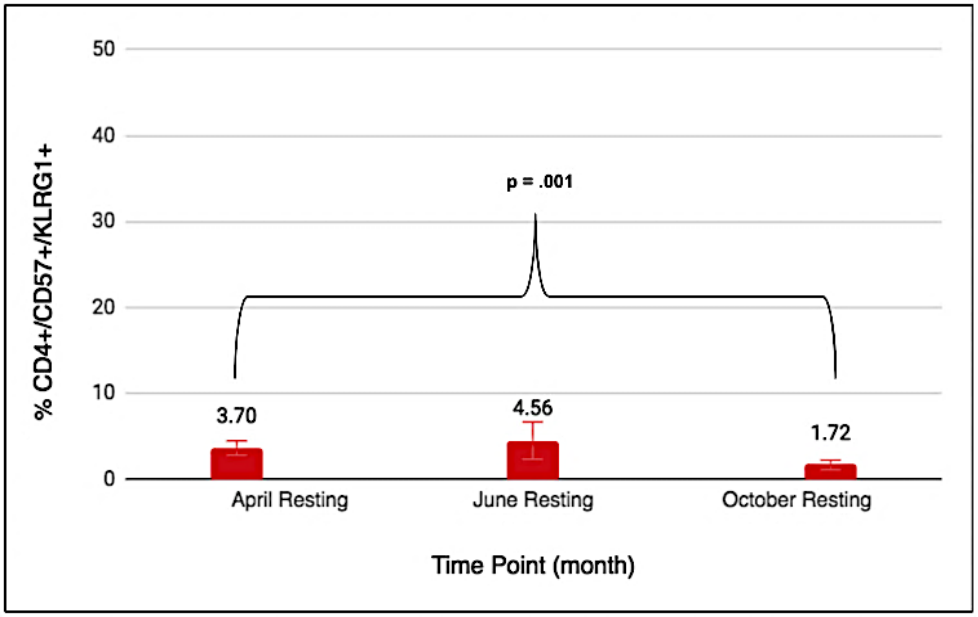

Figure 6. Effect of time on resting late-differentiated CD4+ T-cell proportions. Values presented are mean ± SE.

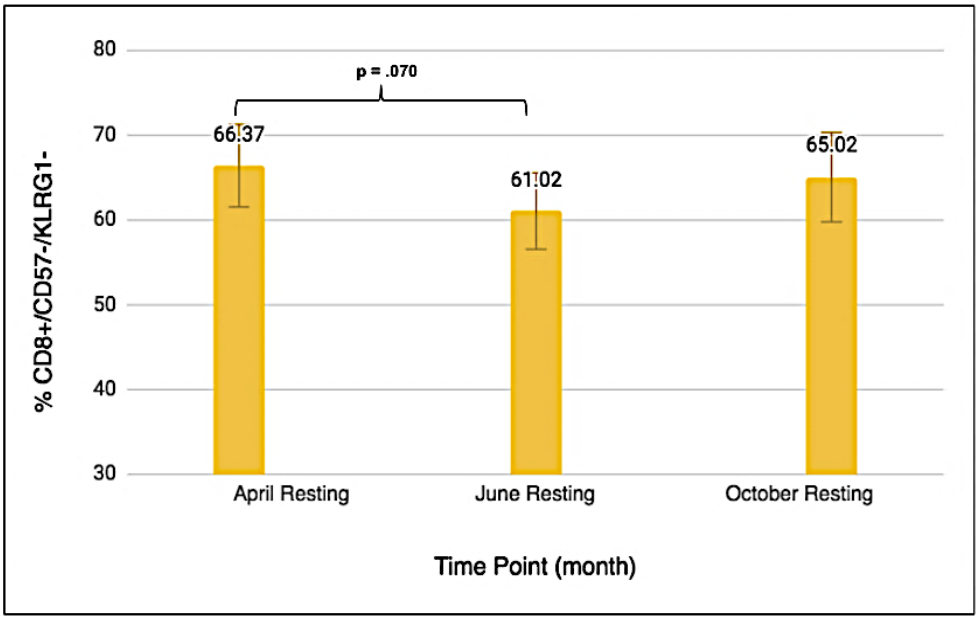

Figure 7. Effect of time on resting early-differentiated CD8+ T-cell proportions. Values presented are mean ± SE.

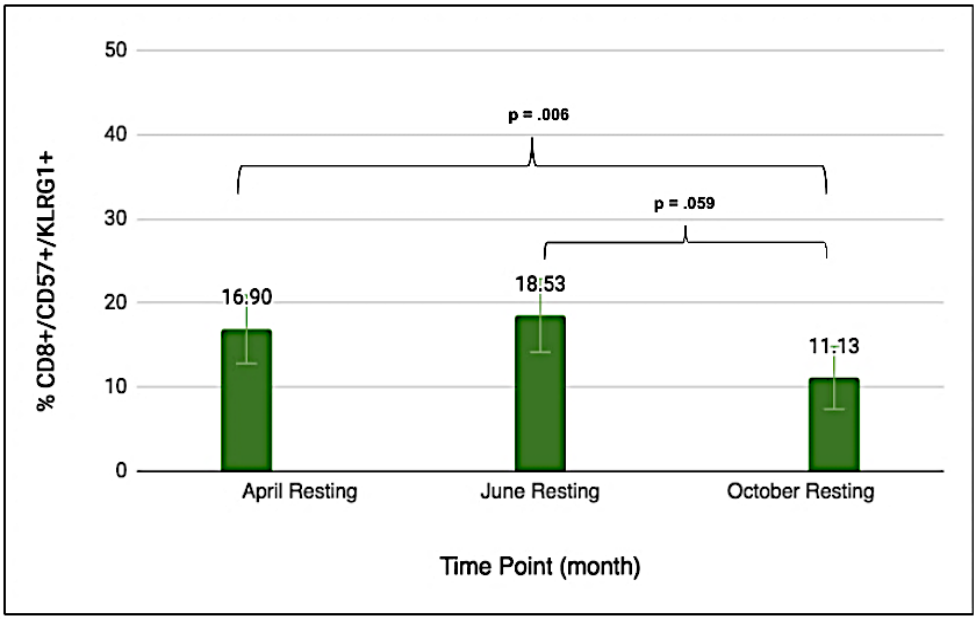

Figure 8. Effect of time on resting late-differentiated CD8+ T-cell proportions. Values presented are mean ± SE.

4.3. Effect of Time on T-Cell Proportions Post Exercise

Results for the effect of time on post-exercise T-cell proportions are presented in Table 4. The effect of time on early-differentiated CD4+, late-differentiated CD4+, early-differentiated CD8+, and late-differentiated CD8+ T-cell proportions post exercise is presented in Figures 9, 10, 11, and 12, respectively.

Proportions of post-exercise early-differentiated CD4+ T-cells increased slightly from April to June and June to October ($p=0.093$). Similarly, proportions of this cell type were marginally elevated from April to October ($p=0.079$), too (Figure 9). Late-differentiated CD4+ proportions decreased from 8.0 % ± 3.4 % in June to 3.7 % ± 2.2 % in October ($p=0.017$) and increased from 2.7 % ± 1.4 % in April to 6.8 % ± 1.6 % in October ($p<0.001$). Proportions of post-exercise early-differentiated CD8+ T-cells decreased from April (53.2 % ± 6.3 %) to June (8.0 % ± 3.4 %) ($p=0.006$) and increased from June (43.3 % ± 5.6 %) to October (52.1 % ± 6.4 %) ($p=0.034$). Finally, post-exercise late-differentiated CD8+ T-cells increased from 25.7 % ± 5.4 % in June to 17.7 % ± 5.1 % in October ($p=0.043$). Proportions of this cell type were higher in October (22.6 % ± 3.7 %) compared to those in April (15.7 % ± 3.8 %) ($p=0.008$).

Table 4. Proportions of post-exercise T-cell subsets – early-differentiated CD4+, late-differentiated CD4+, early-differentiated CD8+, and late-differentiated CD8+ T-cell proportions – at each time point.

T-cell subset	Cell phenotype	Time point comparison (1st vs 2nd)	1st time point post-exercise cell frequency (%)	2nd time point post-exercise cell frequency (%)	p-value
early- differentiated CD4+ T-cells	CD3+/CD4+/ CD57–/KLRG1–	April vs June (n = 10)	83.20 ± 2.8	84.55 ± 3.3	0.959
		June vs October (n = 10)	84.55 ± 3.3	87.68 ± 2.6	**0.093**
		October vs April (n = 16)	88.17 ± 1.6	83.44 ± 2.0	**0.079**
late-differentiated CD4+ T-cells	CD3+/CD4+/ CD57+/KLRG1+	April vs June (n = 10)	7.98 ± 2.3	8.03 ± 3.4	0.878
		June vs October (n = 10)	8.03 ± 3.4	3.65 ± 2.2	**0.017**
		October vs April (n = 16)	2.65 ± 1.4	6.81 ± 1.6	**<0.001**
early- differentiated CD8+ T-cells	CD3+/CD8+/ CD57–/KLRG1–	April vs June (n = 10)	53.21 ± 6.3	43.29 ± 5.6	**0.006**
		June vs October (n = 10)	43.29 ± 5.6	52.06 ± 6.4	**0.034**
		October vs April (n = 16)	54.03 ± 4.8	51.61 ± 4.4	0.386
late-differentiated CD8+ T-cells	CD3+/CD8+/ CD57+/KLRG1+	April vs June (n = 10)	24.35 ± 5.1	25.68 ± 5.4	0.588
		June vs October (n = 10)	25.68 ± 5.4	17.70 ± 5.1	**0.043**
		October vs April (n = 16)	15.70 ± 3.8	22.58 ± 3.7	**0.008**

Values presented are means ± SEM. All p-values reported. Statistically significant differences between post-exercise cell proportions between time points are indicated by boldface text.

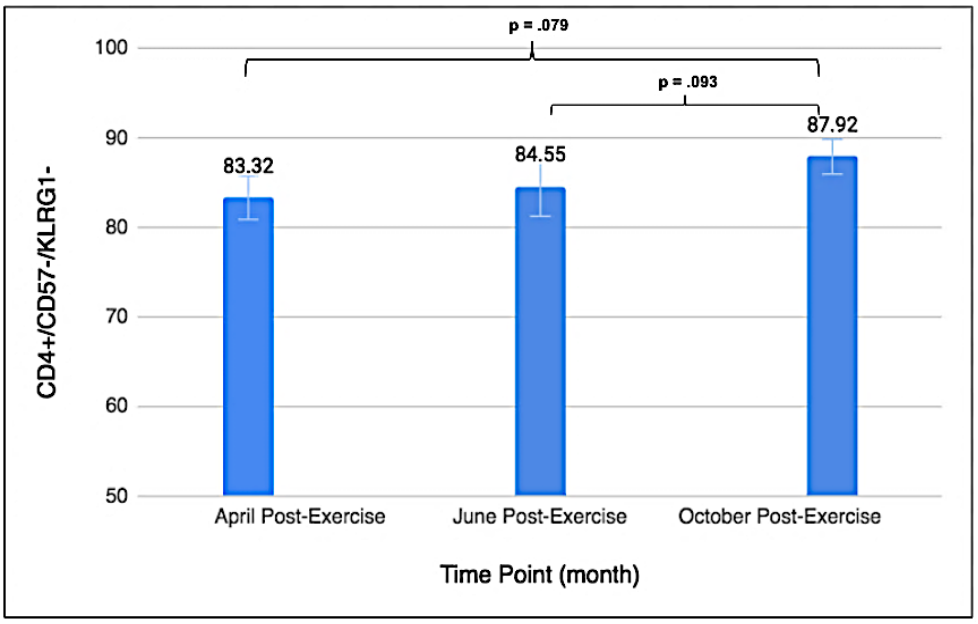

Figure 9. Effect of time on post-exercise early-differentiated CD4+ T-cell proportions. Values presented are mean ± SE.

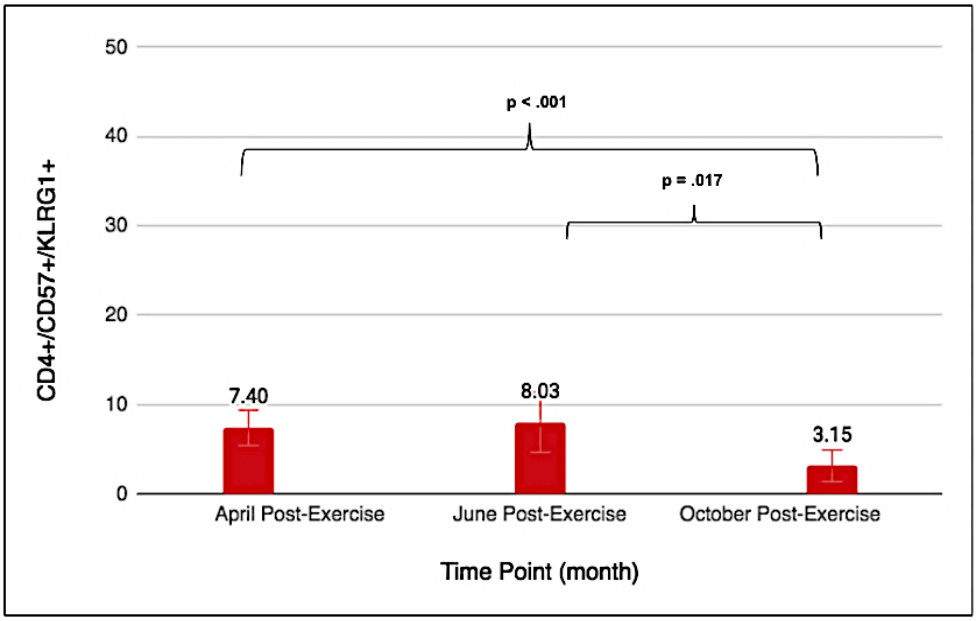

Figure 10. Effect of time on post-exercise late-differentiated CD4+ T-cell proportions. Values presented are mean ± SE.

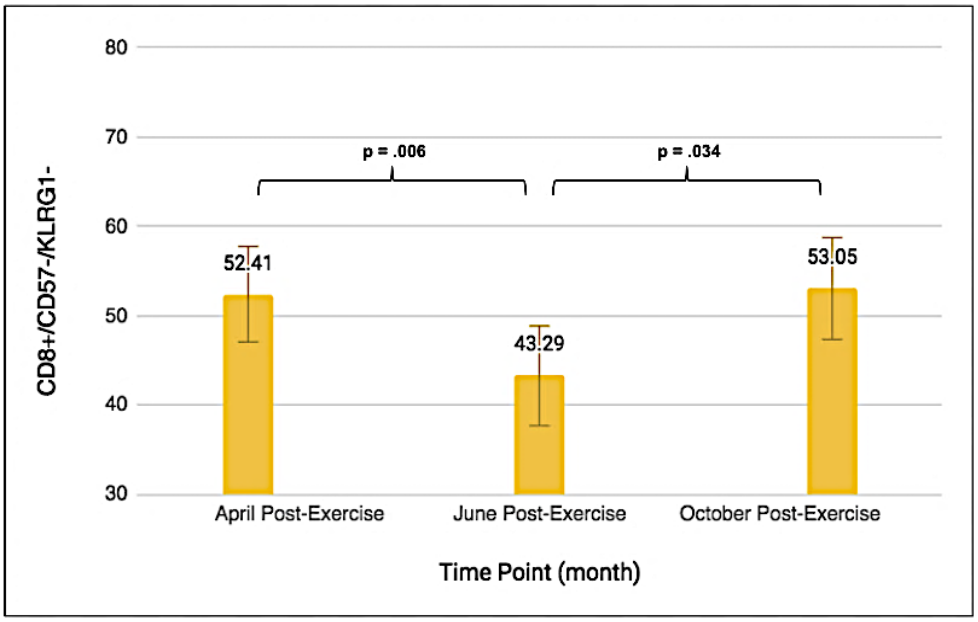

Figure 11. Effect of time on post-exercise early-differentiated CD8+ T-cell proportions. Values presented are mean ± SE.

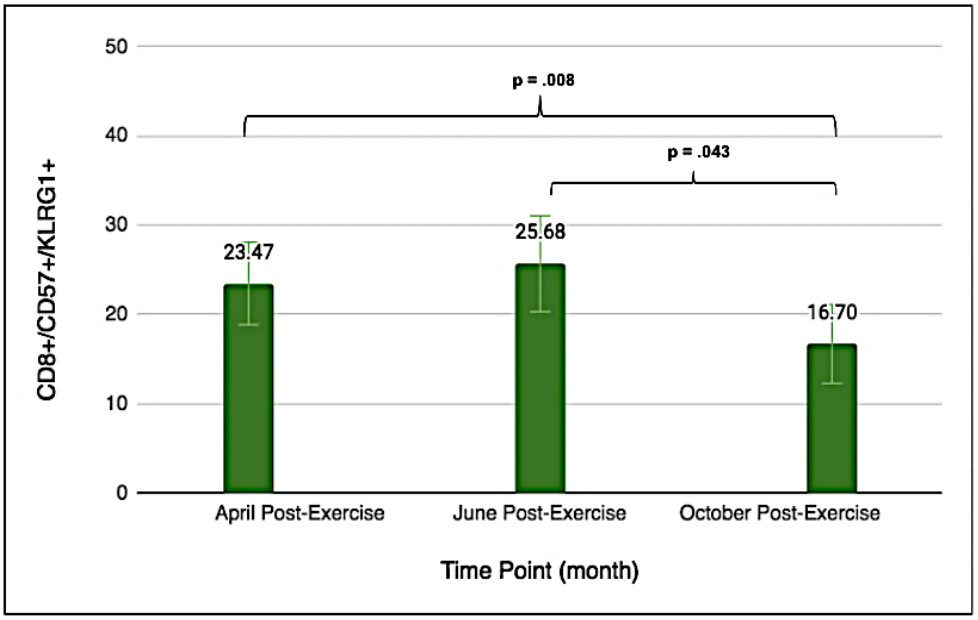

Figure 12. Effect of time on post-exercise late-differentiated CD8+ T-cell proportions. Values presented are mean ± SE.

4.4. Effect of Exercise on T-Cell Proportions

Results for the effect of exercise (pre- vs. post-exercise) at each time point are presented in Table 5. Pre- vs. post-exercise mean values, Δ (post-exercise proportion minus pre-exercise proportion), and % change (from pre-exercise to post-exercise) are also presented in Table 5. Figures 13, 14, 15, and 16 correspond to early-differentiated CD4+, late-differentiated CD4+, early-differentiated CD8+, and late-differentiated CD8+ T-cell proportions, respectively.

A statistically-significant reduction in early-differentiated CD4+ T-cells was found for the April ($p=0.001$) June ($p=0.017$), and October ($p=0.071$) time points (Figure 13) as a result of exercise. For late-differentiated CD4+ T-cells, exercise resulted in a statistically-significant increase for April ($p=0.001$) and June ($p=0.005$). Exercise resulted in a statistically-significant decrease in early-differentiated CD8+ T-cell proportions at all three time points ($p<0.001$, Figure 15). Finally, exercise resulted an increase in late-differentiated CD8+ T-cell proportions at the April ($p<0.001$), June ($p=0.006$), and October ($p<0.001$) time points (Figure 16).

Table 5. Proportions of T-cell subsets – early-differentiated CD4+, late-differentiated CD4+, early-differentiated CD8+, and late-differentiated CD8+ T-cell proportions – in response to exercise.

T-cell subset	Cell phenotype	Time point	Pre-exercise cell frequency (%)	Post-exercise cell frequency (%)	Δ post% - pre%	% change	p-value
early-differentiated CD4+ T-cells	CD3+/CD4+/ CD57–/KLRG1–	April	86.31 ± 1.7	82.12 ± 2.5	-4.67 ± 1.2	-5.72 ± 1.6	**<0 .001**
		June	88.39 ± 2.1	84.55 ± 3.3	-3.84 ± 1.6	-4.6 ± 2.0	**0.017**
		October	90.98 ± 0.7	88.57 ± 1.5	-2.41 ± 1.2	-2.69 ± 1.3	**0.070**
late-differentiated CD4+ T-cells	CD3+/CD4+/ CD57+/KLRG1+	April	4.25 ± 0.8	8.10 ± 1.9	4.09 ± 1.1	111.77 ± 32.5	**0.001**
		June	4.56 ± 2.2	8.03 ± 3.4	3.47 ± 1.7	109.42 ± 25.0	**0.005**
		October	1.34 ± 0.3	2.46 ± 1.2	1.12 ± 0.9	57.29 ± 26.3	0.221
early-differentiated CD8+ T-cells	CD3+/CD8+/ CD57–/KLRG1–	April	66.67 ± 2.7	49.82 ± 3.6	-15.34 ± 1.6	-25.35 ± 3.2	**<0.001**
		June	61.02 ± 4.5	43.29 ± 5.6	-17.73 ± 2.2	-31.30 ± 4.5	**< 0.001**
		October	69.63 ± 3.7	54.81 ± 4.5	-14.82 ± 2.1	-22.92 ± 3.6	**< 0.001**
late-differentiated CD8+ T-cells	CD3+/CD8+/ CD57+/KLRG1+	April	16.49 ± 2.2	25.54 ± 3.4	8.71 ± 1.7	54.75 ± 7.9	**< 0.001**
		June	18.53 ± 4.3	25.68 ± 5.4	7.15 ± 2.0	44.51 ± 15.1	**<0.001**
		October	8.35 ± 2.3	14.92 ± 3.4	6.57 ± 1.8	272.8 ± 135.5	**< 0.001**

Values are presented as mean ± SE. All p-values reported. Statistically significant differences between pre- and post-exercise cell proportion averages are indicated by boldface text.

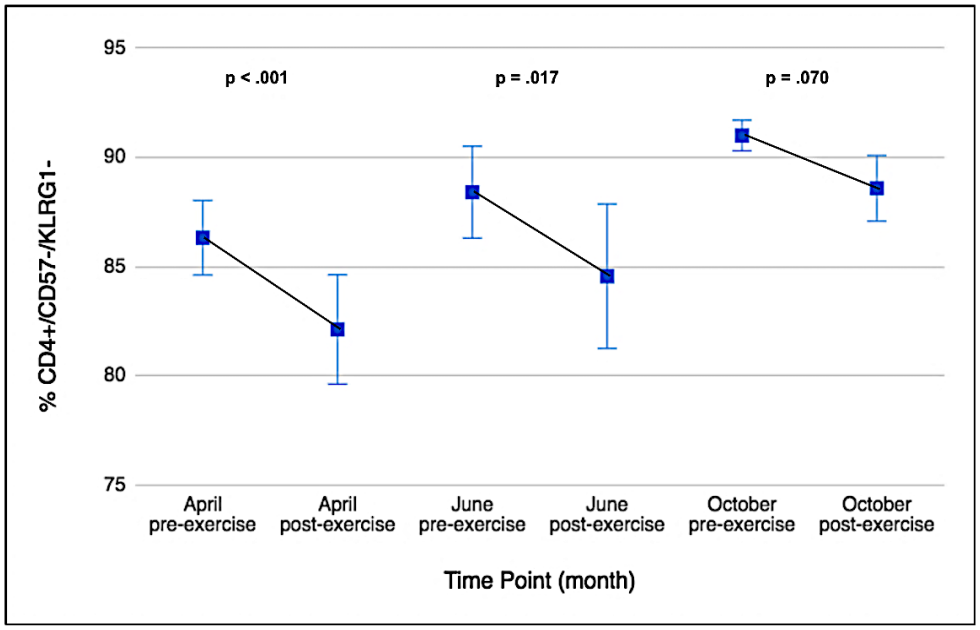

Figure 13. The effect of exercise on early-differentiated CD4+ T-cell proportions. Values presented are mean ± SE. Statistically significant differences between resting (pre-exercise) and post-exercise values are indicated by corresponding p-values.

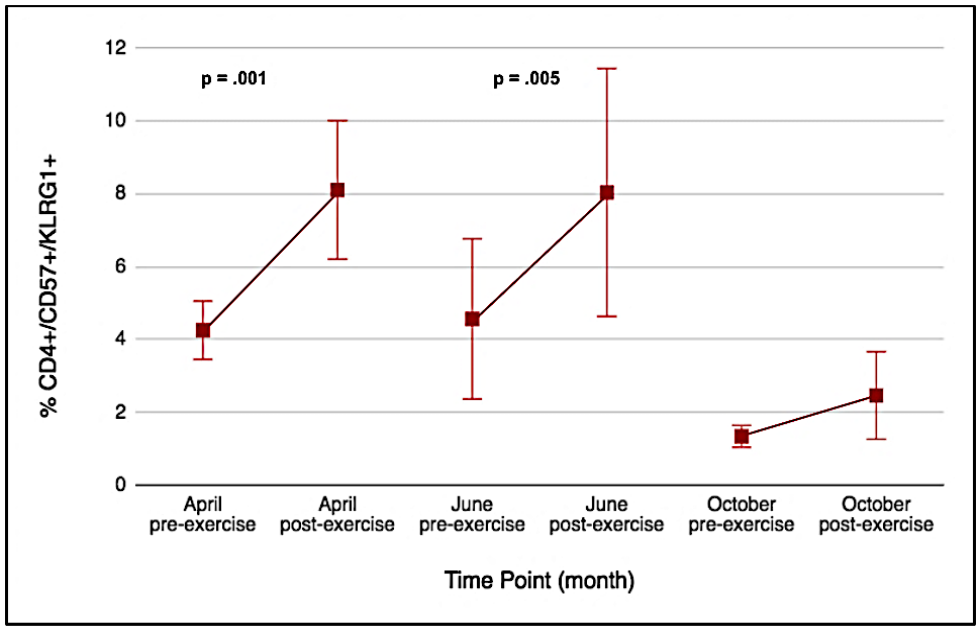

Figure 14. The effect of exercise on late-differentiated CD4+ T-cell proportions. Values presented are mean ± SE. Statistically significant differences between resting (pre-exercise) and post-exercise values are indicated by corresponding p-values.

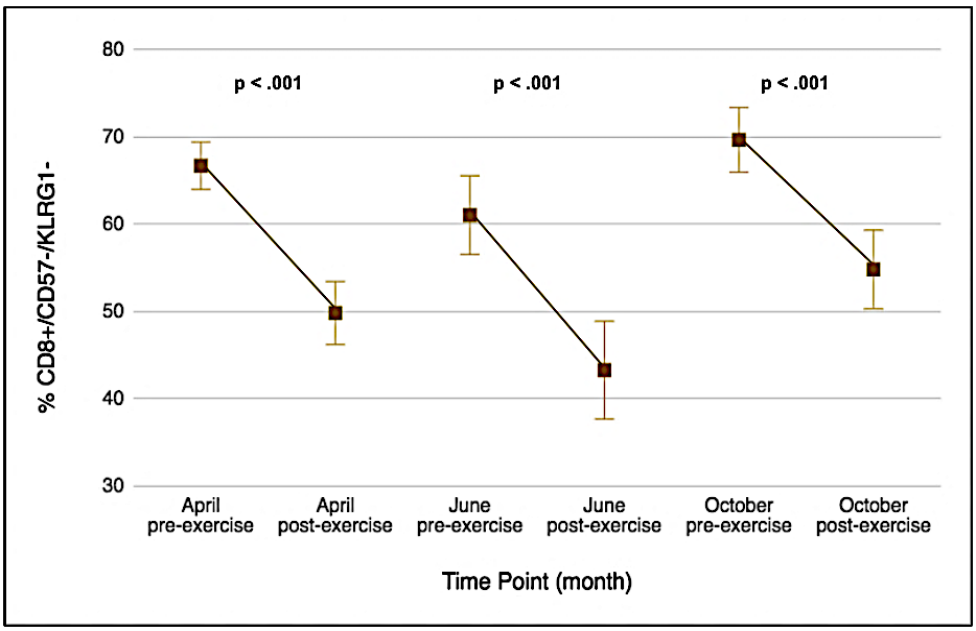

Figure 15. The effect of exercise on early-differentiated CD8+ T-cell proportions. Values presented are mean ± SE. Statistically significant differences between resting (pre-exercise) and post-exercise values are indicated by corresponding p-values.

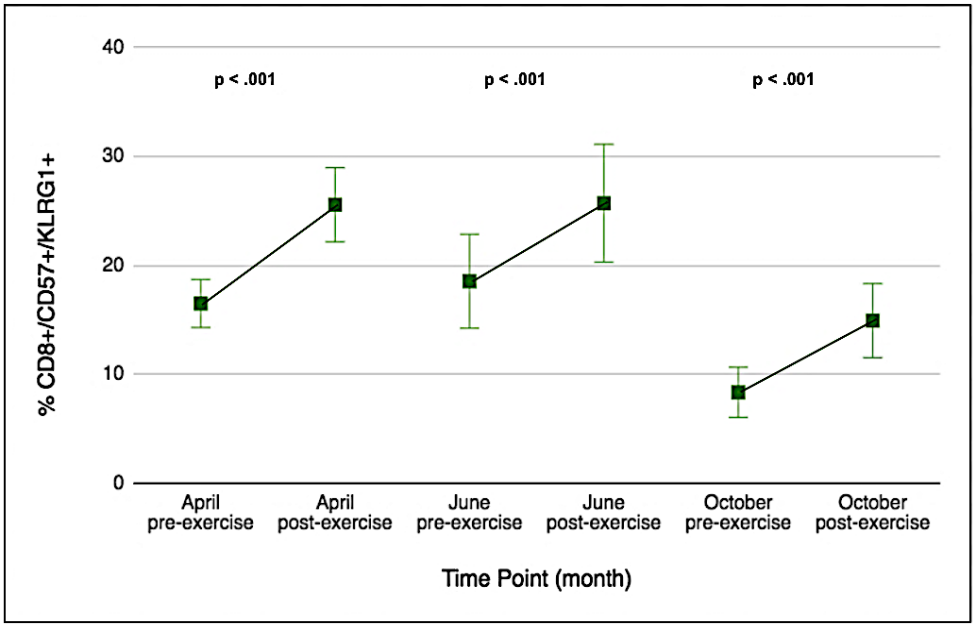

Figure 16. The effect of exercise on late-differentiated CD8+ T-cell proportions. Values presented are mean ± SE. Statistically significant differences between resting (pre-exercise) and post-exercise values are indicated by corresponding p-values.

4.5. Correlations Between Measures of Stress and T-Cell Subsets

Tables 6, 7, and 8 present the Spearman's rank-order correlation coefficients and p-values determined for correlations between objective or subjective measures of stress and proportions and % change of T-cell subsets at the April, June, and October time points, respectively. Statistically significant ($p \leq 0.05$) Spearman's rank-order correlation coefficients are indicated by boldface text (Tables 6-8).

At the April time point (Table 6), resting serum cortisol concentration correlated positively with resting early-differentiated CD8+ T-cells and % change of late-differentiated CD8+ T-cells. PSQI questionnaire data exhibited a positive correlation with % change of early-differentiated CD4+ T-cells and an equal but negative correlation with % change of late-differentiated CD4+ T-cells. Finally, DALDA questionnaire responses correlated positively with resting late-differentiated CD4+ and CD8+ T-cells.

Table 6. Spearman's rank-order correlation coefficients for subjective and objective measures of stress and resting and exercise-induced immune cell distributions for the April time point.

Measure of Stress	Resting early-diff. CD4+	Resting late-diff. CD4+	Resting early-diff. CD8+	Resting late-diff. CD8+	% change early-diff. CD4+	% change late-diff. CD4+	% change early-diff. CD8+	% change late-diff. CD8+
Serum Cortisol Concentration	r=0.329 p=0.106	r=-0.230 p=0.196	**r=0.431** p=0.048	r=-0.300 p=0.129	r=-0.304 p=0.136	r=0.434 p=0.053	r=0.232 p=0.203	**r=0.479** p=0.036
PSQI	r=-0.207 p=0.172	r=0.221 p=0.155	r=0.146 p=0.253	r=0.069 p=0.377	**r=-0.496** p=0.010	**r=0.495** p=0.010	r=-0.180 p=0.212	r=0.213 p=0.171
WURSS	r=-0.021 p=0.463	r=0.100 p=0.325	r=-0.183 p=0.201	r=0.173 p=0.215	r=-0.083 p=0.356	r=0.147 p=0.257	r=0.049 p=0.414	r=0.029 p=0.449
Tired	r=-0.170 p=0.219	r=0.072 p=0.372	r=-0.311 p=0.075	r=0.243 p=0.132	r=-0.203 p=0.182	r=0.100 p=0.329	r=-0.212 p=0.172	r=0.093 p=0.340
DALDA	r=-0.334 p=0.060	**r=0.420** p=0.023	r=-0.285 p=0.094	**r=0.391** p=0.033	r=-0.302 p=0.086	r=0.200 p=0.186	r=-0.188 p=0.201	r=-0.077 p=0.367

Statistically significant ($p \leq 0.05$) Spearman's rank-order correlation coefficients are indicated by boldface text.

At the June time point (Table 7), resting serum cortisol concentration correlated positively with proportions resting early-differentiated CD4+ T-cells and negatively with late-differentiated CD4+ T-cells and % change of late-differentiated CD8+ T-cells. WURSS questionnaire responses showed a negative correlation with resting and % change of early-differentiated CD8+ T-cells. Finally, DALDA responses correlated negatively with resting proportions and % change of late-differentiated CD8+ T-cells.

Table 7. Spearman's rank-order correlation coefficients for subjective and objective measures of stress and resting and exercise-induced immune cell distributions for the June time point.

Measure of Stress	Resting early-diff. CD4+	Resting late-diff. CD4+	Resting early-diff. CD8+	Resting late-diff. CD8+	% change early-diff. CD4+	% change late-diff. CD4+	% change early-diff. CD8+	% change late-diff. CD8+
Serum Cortisol Concentration	**r=0.685** p=0.014	**r=-0.745** p=0.007	r=-0.152 p=0.338	r=0.006 p=0.493	r=0.200 p=0.290	r=0.261 p=0.234	r=0.042 p=0.454	**r=-0.564** p=0.045
PSQI	r=-0.377 p=0.142	r=0.280 p=0.217	r=-0.140 p=0.350	r=0.235 p=0.257	r=-0.103 p=0.388	r=-0.286 p=0.212	r=-0.243 p=0.249	r=-0.134 p=0.356
WURSS	r=-0.243 p=0.249	r=0.061 p=0.434	**r=-0.565** p=0.044	r=0.280 p=0.216	r=-0.304 p=0.197	r=0.413 p=0.118	**r=-0.644** p=0.022	r=-0.182 p=0.307
Tired	r=-0.142 p=0.384	r=0.271 p=0.225	r=-0.271 p=0.225	r=-0.519 p=0.062	r=-0.455 p=0.093	r=0.055 p=0.440	r=-0.332 p=0.174	r=-0.135 p=0.355
DALDA	r=0.326 p=0.179	r=-0.433 p=0.106	r=0.151 p=0.339	**r=-0.563** p=0.045	r=0.470 p=0.085	r=-0.013 p=0.486	r=0.263 p=0.231	**r=-0.634** p=0.025

Statistically significant ($p \leq 0.05$) Spearman's rank-order correlation coefficients are indicated by boldface text.

At the October time point (Table 8), serum cortisol concentration correlated negatively with proportions of resting early-differentiated CD4+ T-cells and positively with % change of early-differentiated CD4+ T-cells. WURSS questionnaire responses correlated positively with resting proportions of late-differentiated CD4+ and CD8+ T-cell proportions and correlated negatively with proportions of resting early-differentiated CD8+ T-cells. Finally, questionnaire

responses indicating participant tiredness correlated negatively with % change of late-differentiated CD8+ T-cells.

Table 8. Spearman's rank-order correlation for subjective and objective measures of stress and resting and exercise-induced immune cell distributions for the October time point.

Measure of Stress	Resting early-diff. CD4+	Resting late-diff. CD4+	Resting early-diff. CD8+	Resting late-diff. CD8+	% change early-diff. CD4+	% change late-diff. CD4+	% change early-diff. CD8+	% change late-diff. CD8+
Serum Cortisol Concentration	**r=-0.486** p=0.028	r=-0.103 p=0.351	r=-0.297 p=0.132	r=0.259 p=0.166	**r=0.545** p=0.015	r=-0.142 p=0.300	r=0.288 p=0.139	r=-0.403 p=0.061
PSQI	r=-0.134 p=0.297	r=0.107 p=0.337	r=0.255 p=0.154	r=-0.224 p=0.186	r=-0.334 p=0.088	r=-0.140 p=0.289	r=0.007 p=0.488	r=0.112 p=0.329
WURSS	r=0.031 p=0.452	**r=0.422** p=0.041	**r=-0.431** p=0.037	**r=0.404** p=0.048	r=-0.130 p=0.304	r=-0.236 p=0.173	r=-0.224 p=0.186	r=-0.194 p=0.221
Tired	r=-0.177 p=0.241	r=0.341 p=0.083	r=-0.185 p=0.231	r=0.227 p=0.182	r=-0.026 p=0.459	r=0.011 p=0.482	r=0.191 p=0.224	**r=-0.494** p=0.019
DALDA	r=0.236 p=0.173	r=0.181 p=0.236	r=-0.137 p=0.294	r=0.183 p=0.234	r=0.320 p=0.097	r=-0.218 p=0.192	r=-0.121 p=0.316	r=-0.246 p=0.163

Statistically significant ($p \leq 0.05$) Spearman's rank-order correlation coefficients are indicated by boldface text.

4.6. Effect of CMV on T-Cell Proportions at Rest and with Exercise

Results from statistical analyses assessing a main effect of Time and interaction effects of CMV, Time * CMV, and Time * CMV * Cortisol are presented in Table 9; boldface text indicates statistical significance ($p \leq 0.1$). Graphical data corresponding to p-values for CMV as a moderator, presented in Table 9, column 5, is shown in Figures 17-24. CMV seronegative (CMV–) and CMV seropositive (CMV+) grouping is indicated in Figures 17 ,18, 19, and 20 which correspond to resting early-differentiated CD4+, late-differentiated CD4+, early-differentiated CD8+, and late-differentiated CD8+ T-cell proportions, respectively. Figures 21,

22, 23, and 24 correspond to post-exercise early-differentiated CD4+, late-differentiated CD4+, early-differentiated CD8+, and late-differentiated CD8+ T-cell proportions, respectively.

A statistically-significant main effect of Time was detected for all T-cell subsets with exception of resting and post-exercise proportions of early-differentiated CD4+ T-cells (Table 9). Additionally, a significant main effect for Time was determined for mobilization of all T-cell subsets except late-differentiated CD4+ T-cells.

An interaction effect of CMV was found for resting proportions of early- ($p=0.007$) and late-differentiated ($p<0.001$) CD4+ and early- ($p=0.045$) and late-differentiated ($p=0.038$) CD8+ T-cells. Additionally, a CMV interaction effect was determined for post-exercise proportions of early- ($p<0.001$) and late-differentiated ($p<0.001$) CD4+ and late-differentiated ($p=0.033$) CD8+ T-cells. Finally, a significant interaction effect of CMV was determined for mobilization of early-differentiated CD4+ T-cells ($p<0.001$) with exercise.

A Time * CMV interaction was detected for the following T-cell subsets: post-exercise proportions of early-differentiated CD4+ ($p=0.017$) and late-differentiated CD8+ ($p=0.020$) T-cells, and mobilization of early-differentiated CD4+ ($p=0.074$) T-cells. A statistically-significant interaction of Time * CMV * Cortisol was found for all T-cell subsets except for resting early- and late-differentiated CD8+ T-cells and mobilization of late-differentiated CD8+ T-cells.

Table 9. Effect of time, CMV, Time * CMV interaction, and Time * CMV * Cortisol interaction on pre-exercise T-cell proportions, post-exercise T-cell proportions, and exercise-induced T-cell mobilization.

Relation to Exercise	T-cell subset (dependent variable)	Cell phenotype	p-value for parameter= Time	p-value for parameter= CMV+	p-value for parameter=Time * CMV+	p-value for parameter= Time * CMV+ * Cortisol
pre-exercise	early-differentiated CD4+ T-cells	CD3+/CD4+/ CD57–/KLRG1–	0.361	**0.007**	0.180	**0.071**
	late-differentiated CD4+ T-cells	CD3+/CD4+/ CD57+/KLRG1+	**0.040**	**<0.001**	0.148	**0.023**
	early-differentiated CD8+ T-cells	CD3+/CD8+/ CD57–/KLRG1–	**0.090**	**0.045**	0.903	0.275
	late-differentiated CD8+ T-cells	CD3+/CD8+/ CD57+/KLRG1+	**0.022**	**0.038**	0.445	0.521
post-exercise	early-differentiated CD4+ T-cells	CD3+/CD4+/ CD57–/KLRG1–	0.393	**<0.001**	**0.017**	**0.001**
	late-differentiated CD4+ T-cells	CD3+/CD4+/ CD57+/KLRG1+	**0.018**	**<0.001**	0.359	**0.001**
	early-differentiated CD8+ T-cells	CD3+/CD8+/ CD57–/KLRG1–	**<0.001**	0.139	0.989	**0.063**
	late-differentiated CD8+ T-cells	CD3+/CD8+/ CD57+/KLRG1+	**0.003**	**0.033**	**0.020**	**0.001**
% change ((post-pre)/pre*100 as % change)	early-differentiated CD4+ T-cells	CD3+/CD4+/ CD57–/KLRG1–	**0.003**	**< 0.001**	**0.074**	**<0.001**
	late-differentiated CD4+ T-cells	CD3+/CD4+/ CD57+/KLRG1+	0.568	0.830	0.723	**0.078**
	early-differentiated CD8+ T-cells	CD3+/CD8+/ CD57–/KLRG1–	**0.004**	0.306	0.884	**0.018**
	late-differentiated CD8+ T-cells	CD3+/CD8+/ CD57+/KLRG1+	**0.006**	0.158	0.141	0.129

Statistically significant effects ($p \leq 0.1$) indicated by boldface text.

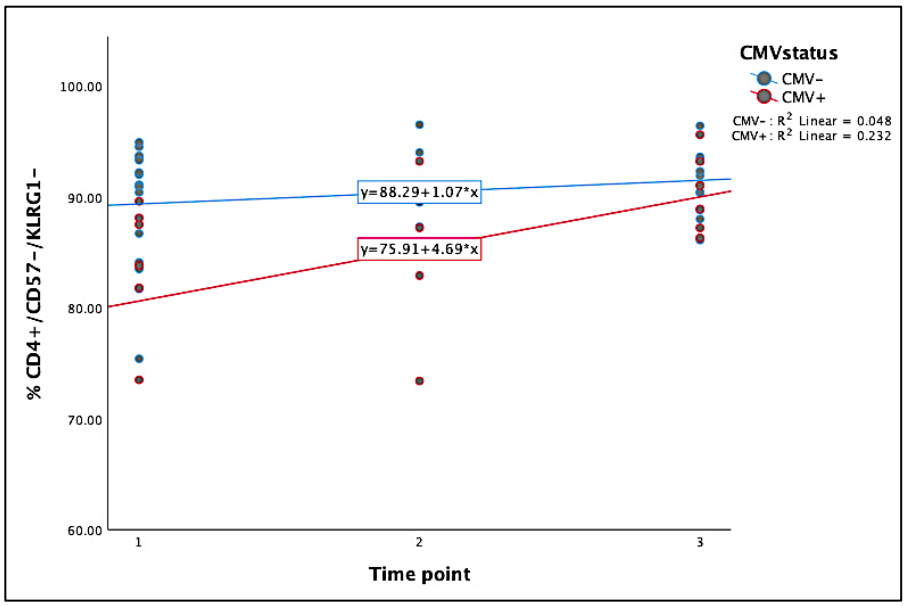

Figure 17. Proportions (%) of resting early-differentiated CD4+ (CD4+/CD57-/KLRG1-) T-cells with time (1=April, 2=June, 3=October) as main effect and CMV as covariate (p=0.007). Data corresponds to p-values presented in Table 9, column 5.

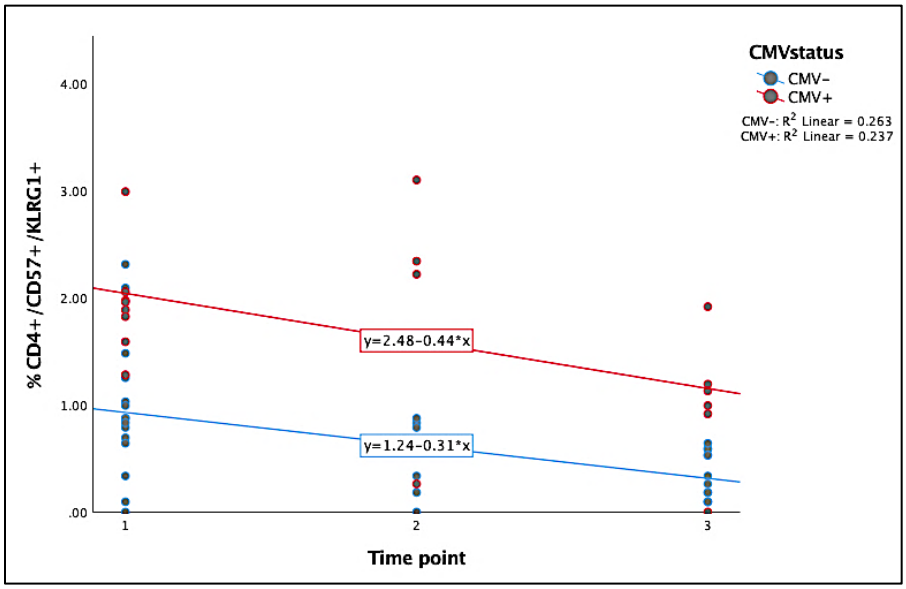

Figure 18. Proportions (%) of resting late-differentiated CD4+ (CD4+/CD57+/KLRG1+) T-cells with time (1=April, 2=June, 3=October) as main effect and CMV as covariate (p<0.001). Data corresponds to p-values presented in Table 9, column 5. (Note: Data were log-transformed prior to analysis.)

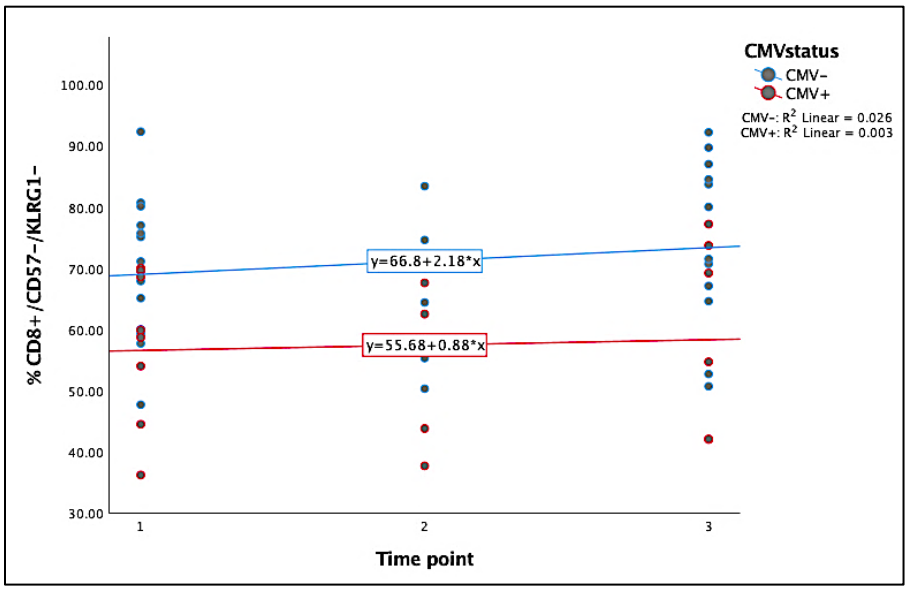

Figure 19. Proportions (%) of resting early-differentiated CD8+ (CD8+/CD57-/KLRG1-) T-cells with time (1=April, 2=June, 3=October) as main effect and CMV as covariate (p=0.045). Data corresponds to p-values presented in Table 9, column 5.

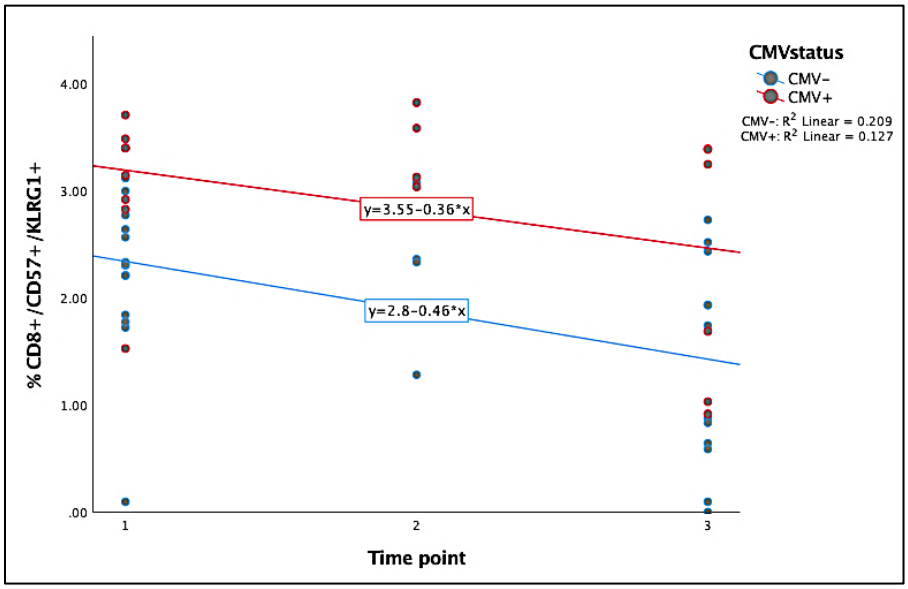

Figure 20. Proportions (%) of resting late-differentiated CD8+ (CD8+/CD57+/KLRG1+) T-cells with time (1=April, 2=June, 3=October) as main effect and CMV as covariate (p=0.038). Data corresponds to p-values presented in Table 9, column 5. (Note: Data were log-transformed prior to analysis.)

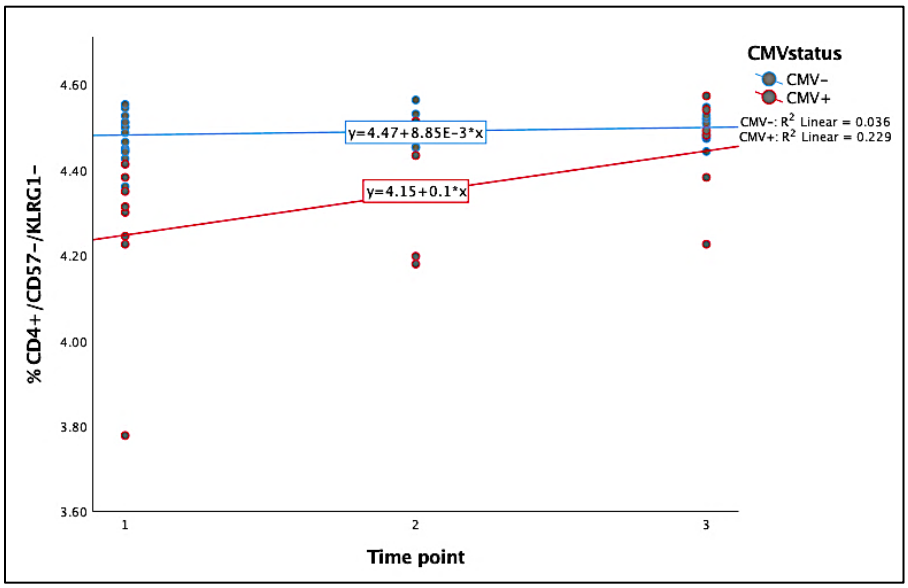

Figure 21. Proportions (%) of post-exercise early-differentiated CD4+ (CD4+/CD57-/KLRG1-) T-cells with time (1=April, 2=June, 3=October) as main effect and CMV as covariate ($p<0.001$). Data corresponds to p-values presented in Table 9, column 5. (Note: Data were log-transformed prior to analysis.)

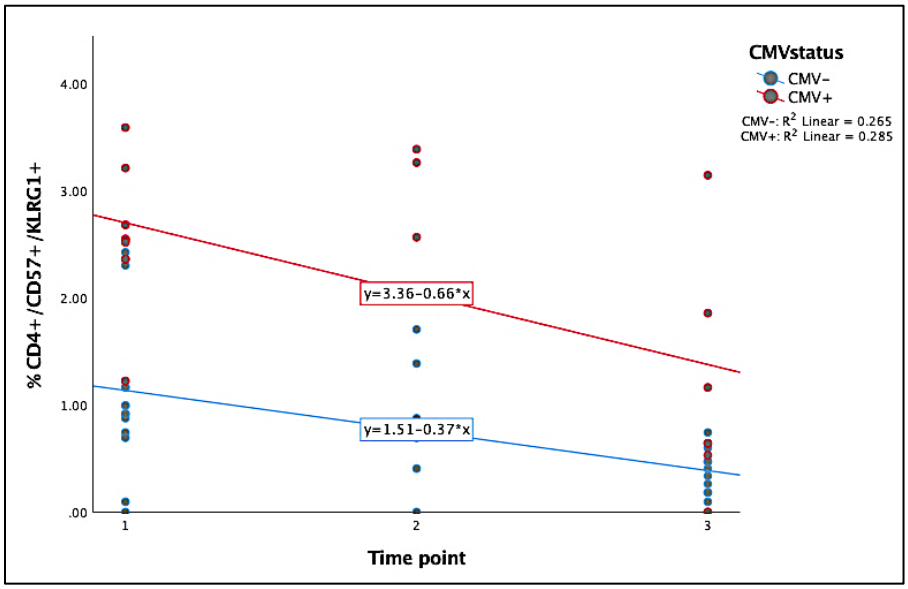

Figure 22. Proportions (%) of post-exercise late-differentiated CD4+ (CD4+/CD57+/KLRG1+) T-cells with time (1=April, 2=June, 3=October) as main effect and CMV as covariate ($p<0.001$). Data corresponds to p-values presented in Table 9, column 5. (Note: Data were log-transformed prior to analysis.)

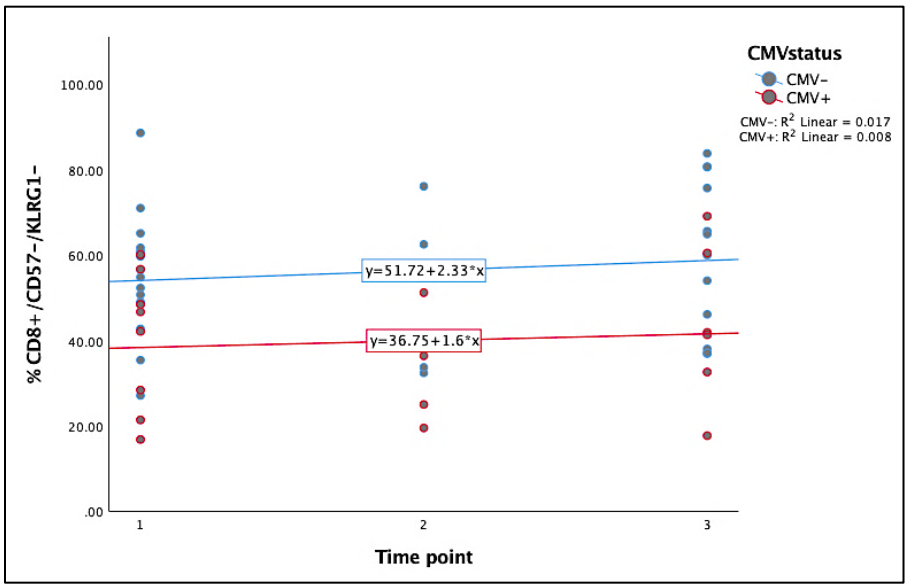

Figure 23. Proportions (%) of post-exercise early-differentiated CD8+ (CD8+/CD57-/KLRG1-) T-cells with time (1=April, 2=June, 3=October) as main effect and CMV as covariate. Data corresponds to p-values presented in Table 9, column 5.

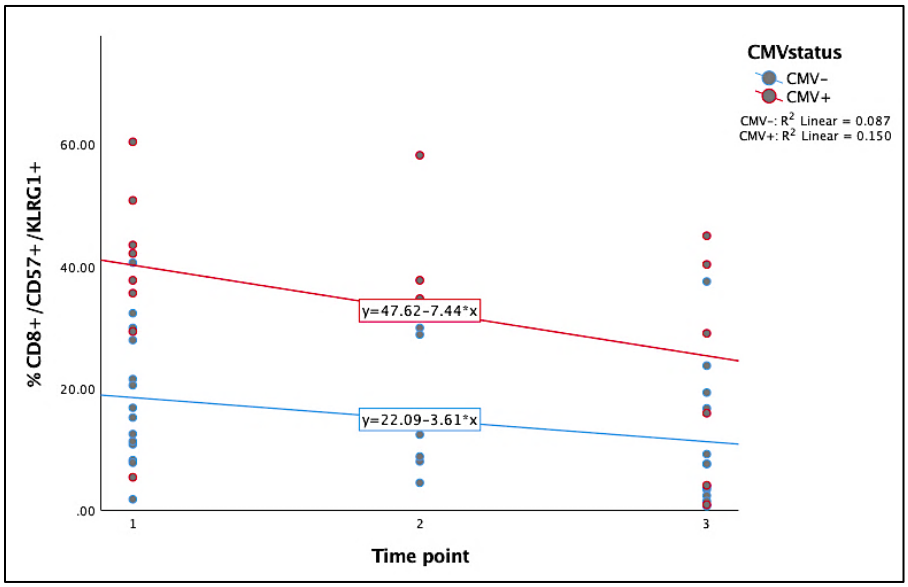

Figure 24. Proportions (%) of post-exercise late-differentiated CD8+ (CD8+/CD57+/KLRG1+) T-cells with time (1=April, 2=June, 3=October) as main effect and CMV as covariate (p=0.033). Data corresponds to p-values presented in Table 9, column 5.

Chapter 5. Discussion

The first aim of this project was to explore the impact of psychological and training-induced stressors on the immune function of NCAA-level collegiate swimmers. Data was collected in athletes at rest to examine subjective and objective measures of stress throughout the competition season. Previous studies demonstrated chronic job stress was associated with a reduced CD4+:CD8+ T-cell ratio and higher percentage of senescent T-cells (Bosch et al. 2009), so it was hypothesized that higher resting serum cortisol concentrations would be associated with subjective measures of stress along with greater proportions of late-differentiated and dysfunctional senescent T-cells.

The second aim of this project was to examine the impact of acute exercise bouts on the immune response in collegiate swimmers throughout the competition season. It was hypothesized that degree and composition of lymphocytosis induced by acute exercise would be affected by resting serum cortisol concentration and overall athlete well-being.

Finally, CMV infection status was determined. ~58% of Americans are infected by CMV (Dollard et al. 2011; Staras et al. 2006), however, nearly all studies that have examined the immune effects of CMV have done so in older adult populations. This study was a unique opportunity to examine CMV in collegiate athletes. Further, stress has been shown to increase CMV viral titers in infected individuals via latent viral reactivation (Rector et al. 2014). Just as herpes simplex virus reactivation has been shown to result from psychological stress (Padgett et al. 1998), stress-triggered viral reactivation has been demonstrated in CMV seropositive individuals, too. Rector et al. (2014) found mental health variables (anxiety, depression, sleep problems, etc.) increased CMV-IgG titers in a dose-responsive manner. Therefore, this present

study examined the impact of chronic stress concomitant with CMV infection status on resting lymphocyte distribution and exercise-induced immune responses.

5.1. Aim 1.A: Impact of Subjective & Objective Measures of Stress

The first specific aim of this experiment was to determine the impact of psychological and training-induced stressors on the immune function in this population of NCAA-level collegiate swimmers. Questionnaire responses correlated with immune cell parameters, however, these correlations were inconsistent across time points (Tables 6-9).

Cortisol, too, did correlate with resting proportions of some T-cell subsets and % change from pre to post exercise albeit inconsistently across time points (Tables 6-9). Similarly, neither serum cortisol concentration nor the Wisconsin Upper Respiratory Symptoms Survey, WURSS, correlated to any immune cell parameter across all time points. Barrett et al. (2005) examined a condensed version of the original WURSS-44 assessment referred to as WURSS-21. The authors claim the construct validity of WURSS-21 validated by its high responsiveness, reliability, and importance to patients. The present study, however, found no evidence to support the construct validity of WURSS-21. Correlations between WURSS-21 responses did not correlate well, frequently, or regularly between each time point. Barrett et al. (2005) report high Pearson's correlation coefficients between responses from WURSS-21 and responses from other, well-established surveys designed to assess URTI illness severity. These findings suggest that WURSS-21 may not have strong construct validity. Therefore, questionnaires may be an ineffective tool for coaches and training staff to monitor the well-being and response to stressors in athletes.

Additionally, these findings suggest that resting serum cortisol concentration alone may not be a sufficient indicator of T-cell mobilization with exercise. This study did not present cortisol changes that occurred as a result of exercise. However, these findings suggest that regardless of baseline levels of stress, increases in catecholamines and cortisol due to exercise produce an immune response as expected. This distinction between resting serum cortisol concentration and the exercise-induced *change in magnitude* of cortisol may be important to consider for future studies.

This idea aligns with findings and conclusions from other studies (Schwab et al. 2005; Dhabhar et al. 2012). In addition, Anane et al. (2009) examined specific lymphocyte mobilization in response to three different stress tasks including a psychological public speaking stress test, acute exercise, and pharmacological infusion of a β-agonist (isoproterenol). γδ T-cells, CD8+ T-cells, and NK cells were mobilized in a dose-dependent manner during all three tasks. This mobilization of T-lymphocytes results from stimulation by catecholamines and cortisol (McCarthy & Dale 1988). Future studies should aim to examine the impact of the magnitude of the cortisol in the context of a given stress response. That is, as a result of acute exercise, how does the % change of cortisol affect the ingress of immune cells into peripheral circulation? Future studies should attempt to elucidate the relationship between serum cortisol concentration at rest and the magnitude of the cortisol response to an acute stressor and the resulting immune response.

5.2. Aim 1.B: Changes in T-Cell Subsets Over Time

Proportions of resting T-cell subsets did differ over time (Table 3 & Table 9). This finding is consistent with the idea that the immune cell repertoire changes over time (Brown et al. 2014;

Minuzzi et al. 2018; Teixeira et al. 2014). Apropos of this current study, differences in proportions of all T-cell subsets varied between the April and October time point (Table 3).

This finding can be explained by different training volume and periodization at the time of data collection. In April, data collection occurred just after the end of competition season, while the October data collection occurred during high or increasing training volume. Other studies have demonstrated the effect of training status on mobilization of early-differentiated and late-differentiated T-cells. Brown et al. (2014) found that training status was associated with increased mobilization of senescent T-cells. Here, they found acute exercise to mobilize senescent CD4+ & CD8+ T-cells in trained adults (n=16) to a greater extent than in untrained adults (n=16). Similarly, Minuzzi et al. (2018) found trained masters athletes (53.5 ± 8.9 yrs) had lower proportions of senescent CD4+ and senescent CD8+ T-cells compared to untrained controls (53.7 ± 6.0 yrs). Together, the findings by Brown et al. (2014) and (Minuzzi et al. 2018) suggest training status modulates T-cell proportions. Findings from the present study support these findings and, further, suggest that composition of the T-cell repertoire in trained collegiate athletes may be sensitive enough to reflect even slight changes in training status.

5.3. Aim 2: Impact of Exercise on T-Cell Proportions over Time

In this study, acute exercise mobilized all early- and late-differentiated T-cell subsets ($p<0.10$) at each time point with exception of late-differentiated CD4+ T-cells at the October time point ($p=0.221$, Table 5). Acute aerobic exercise preferentially mobilized late-differentiated CD4+ and CD8+ T-cells compared to early-differentiated CD4+ and CD8+ T-cells (Figures 13-16). This finding is consistent with previous studies (Simpson et al. 2007; Campbell et al. 2009). Two explanations for this preferential mobilization of late-differentiated T-cells have been proposed.

First, mobilization of late-differentiated T-cells may allow "younger" naïve T-cells to occupy that space previously occupied by late-differentiated T-cells, possibly of a senescent phenotype. After acute exercise, when senescent T-cells are mobilized into peripheral tissues from systemic circulation, they are be exposed to greater amounts of catecholamines, cortisol, and reactive oxygen species which may facilitate their clearance (Campbell et al. 2009; Krüger et al. 2009). Second, late-differentiated T-cells are highly-cytotoxic with effector memory function (Hamann et al. 1997).

Alternatively, preferential mobilization of these cells may occur for the purpose of protection against pathogens that the body may become exposed to during exercise, such as a predator bite, injury, etc. (Turner and Brum 2017; Franceschi et al. 2000; Simpson et al. 2007). These preferential mobilization of late-differentiated T-cells may represent changes beneficial for maintenance of the immune cell repertoire and proactive measures against injury or exposure to novel pathogens.

5.4. Aim 3: Impact of CMV on Immune Cell Proportions

A moderating effect was determined for CMV serostatus within this population of collegiate swimmers. CMV was determined to have a moderating effect on proportions of all resting T-cell subsets and all post-exercise T-cell subsets with exception of early-differentiated CD8+ T-cells (Table 9). Specifically, in this study, CMV+ participants exhibited lower proportions of resting early-differentiated CD4+ and CD8+ T-cells and higher proportions of resting late-differentiated CD4+ and CD8+ T-cells compared to those in CMV– participants (Figures 17-20). This finding suggests CMV infection modulates the immune cell repertoire and within otherwise healthy individuals. These findings are consistent with other studies, however such studies examined

only elderly individuals early all studies demonstrated this in individuals (Pourgheysari et al. 2007; La Rosa and Diamond 2012; Pawelec 2012; Khan et al. 2002). The findings from this present study are novel in that they demonstrate the impact of CMV on the immune cell repertoire in a population young, immunocompetent individuals.

These findings are consistent with those determined by Brodin et al. (2015) who examined monozygous twin pairs with concordant or discordant for CMV infection status. Brodin et al. found CMV discordant monozygous twins exhibited diminished correlations of immune cell frequencies. This finding, Brodin et al. note, was true for effector CD8+ T-cells and γδ T-cells. Other studies, too, support to theory that CMV infection results in the accumulation of late-differentiated T-cells, including those of the senescent phenotype, with age (Khan et al. 2002; Koch et al. 2007). This accumulation of late-differentiated T-cells, especially those of the senescent phenotype, with increasing age is supported throughout the pool of literature, however, few studies have examined the effects of CMV infection in young, immunocompetent populations.

Turner et al. (2010) examined the effects of CMV infection on the exercise-induced mobilization of CD8+ T-cells in a population of older young adults (mean age 35, SD ± 14 yrs). Here, Turner and colleagues found magnitude of mobilization of CD8+ T-cells to be twice as large in CMV+ participants compared to CMV– participants. However, this study did not find a difference in CD4+ T-cell mobilization between CMV+ and CMV– participants. While Turner and colleagues (2010) demonstrated the effects of CMV infection on exercise-induced mobilization of CD8+ T-cells, the mean age of 35 ± 14 years of the participants is greater than that of the present study.

The time elapsed since initial CMV infection, degree of CMV reactivation, and chronological age may contribute to changes in immune cell repertoire. Two interactions were

determined, which also may contribute to changes of T-cell subsets seen over time in this population of collegiate swimmers.

A Time * CMV interaction was determined for 3 of 16 of the immune cell parameters examined in this study (Table 9). This finding suggests CMV infection may modulate changes in immune cell distribution to a greater extent compared to CMV seronegative individuals.

Finally, a Time * CMV * Cortisol interaction was determined for 9 of the 16 immune cell parameters examined in this study (Table 9). Interestingly, no consistent interaction was determined for Cortisol alone. These findings may suggest cortisol may have a stronger modulating effect on immune cell parameters in CMV seropositive individuals. This finding is in some ways similar to those of a previous study (Mehta et al. 2000). In this study, Mehta and colleagues (2000) examined CMV titers in urine samples of astronauts before (baseline), 10 days prior to, and 3 days after spaceflight. CMV antibody titers increased from baseline to 10 days prior to spaceflight ($p<0.001$) and again from levels 10 days prior to after spaceflight ($p<0.001$). While Mehta et al. (2000) did not examine lymphocyte proportions in their study, these findings are valuable to the current study, because they demonstrate the ability of CMV antibody titers to change over time in response to stressors.

Chapter 6. Conclusions

The objective of Aim 1.A was to explore the impact of psychological and training-induced stressors on the immune function of NCAA-level collegiate swimmers. Serum cortisol concentration correlated with various questionnaire responses rarely and irregularly. Questionnaires may be an ineffective too for coaches and training staff to monitor the well-being of athletes.

Additionally, this study examined resting proportions of immune cells over 7 months of training and competition in a population of collegiate swimmers (Aim 1.B). Resting proportions of several T-cell subtypes varied significantly over time. Differences in resting proportions of 3 of 4 T-cell subtypes examined in this study were found between April and October time points. These differences between time points may represent a change, and possibly an improvement, in training status. This claim is supported by the decrease in proportions of resting late-differentiated CD4+ and CD8+ T-cells from the April time point to the October time point. Similarly, Minuzzi et al. (2018) found trained masters athletes had lower proportions of senescent CD4+ and senescent CD8+ T-cells when compared to untrained counterparts. Similarities between this finding from the present study and those from previous studies support the claim that exercise training can favorably impact the immune cell repertoire.

The objective of Aim 2 was to examine the effect of acute exercise on immune responses in collegiate swimmers throughout the competition season. Within this population of collegiate swimmers, acute aerobic exercise mobilized immune cells in a manner similar to that which has been well documented in the current pool of literature (Campbell et al. 2009; Turner et al. 2010; Simpson et al. 2007). In this study, late-differentiated CD4+ and CD8+ T-cells were mobilized preferentially into peripheral circulation. Conversely, acute aerobic exercise resulted in reduction

of proportions of early-differentiated CD4+ and CD8+ T-cells. As discussed in previous sections, this preferential mobilization of late-differentiated T-cells, including those of the senescent phenotype, may occur as a preemptive protective action and/or to make room for naïve T-cells within the immunological space (Turner and Brum 2017; Franceschi et al. 2000; Simpson et al. 2007). Thus, these findings from the present study support the idea that regular exercise is beneficial for maintenance of immune profile homeostasis.

Finally, Aim 3 sought to examine the impact of CMV infection on proportions of resting early- and late-differentiated T-cells and its effect on exercise-induced immune cell changes in peripheral blood. Because chronic stressors contribute to suppression of immune function in healthy individuals (Segerstrom and Miller 2004), those infected with CMV should be aware that their immunity may become compromised as a result.

This study added to the current pool of literature regarding CMV and its effects on proportions of immune cells. In this study, CMV was found to moderate changes of the immune cell repertoire in a population of young, immunocompetent swimmers. Here, CMV infection contributed to greater reduction in proportions of early-differentiated CD4+ and CD8+ T-cells and increase in proportions of late-differentiated CD4+ and CD8+ T-cells compared to CMV seronegative individuals. These findings could be interpreted as CMV infection elicit changes on the immune cell repertoire by causing an increase in proportions of late-differentiated T-cells, some of which may possess the senescent phenotype and its accompanying dysfunctions. Additionally, CMV infection causes a reduction in the proportion of early-differentiated T-cells, possibly blunting such an individual's ability to defend against novel pathogens. While the findings of this study do not suggest clinically-relevant immunocompromise in CMV-infected individuals within this population of collegiate swimmers, an abundance of evidence in the literature pool suggests a strong link between CMV infection and its immunosuppressive effects

that can lead to poor health outcomes (Varani and Landini 2011). This novel finding suggests CMV may begin exerting changes on immune cell parameters before immunosuppression occurs with aging.

These findings highlight CMV-induced immune cell repertoire changes in a young, immunocompetent population many years before CMV-associated immunosuppression seen with increasing age. CMV reactivation has been found to be associated with sepsis and other inflammatory states (von Müller and Mertens 2008; Aiello et al. 2008) and can occur in periods of immunosuppression and immune activation, such as chronic stress or inflammation (Cook and Trgovcich 2011). Therefore, young CMV-infected individuals, especially collegiate athletes, may become vulnerable to viral reactivation following periods of chronic psychological stress. For collegiate athletes, an episode of latent CMV reactivation may impede their ability to perform activities they deem important to their daily life (i.e. training, academics, etc.). Therefore, athletes, in particular, might consider consulting their physician to determine their CMV infection status. Knowledge of their CMV infection status could allow athletes to make lifestyle and training modifications that will prevent illness. Broadly, this study highlights the importance for athletes to develop healthy coping strategies to manage psychological stress that will optimize well-being, immune health, and athletic performance.

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Appendix. Supplemental Data

Individual participant pre- and post-exercise cell proportions are presented in Figures A.1–A.3, A.4–A.6, A.7–A.9, and A.10–A.12 which correspond to early-differentiated CD4+, late-differentiated CD4+, early-differentiated CD8+, and late-differentiated CD8+ T-cell proportions, respectively. These data are provided for reference. These graphs are meant to serve as a reference for comparison between individual participants.

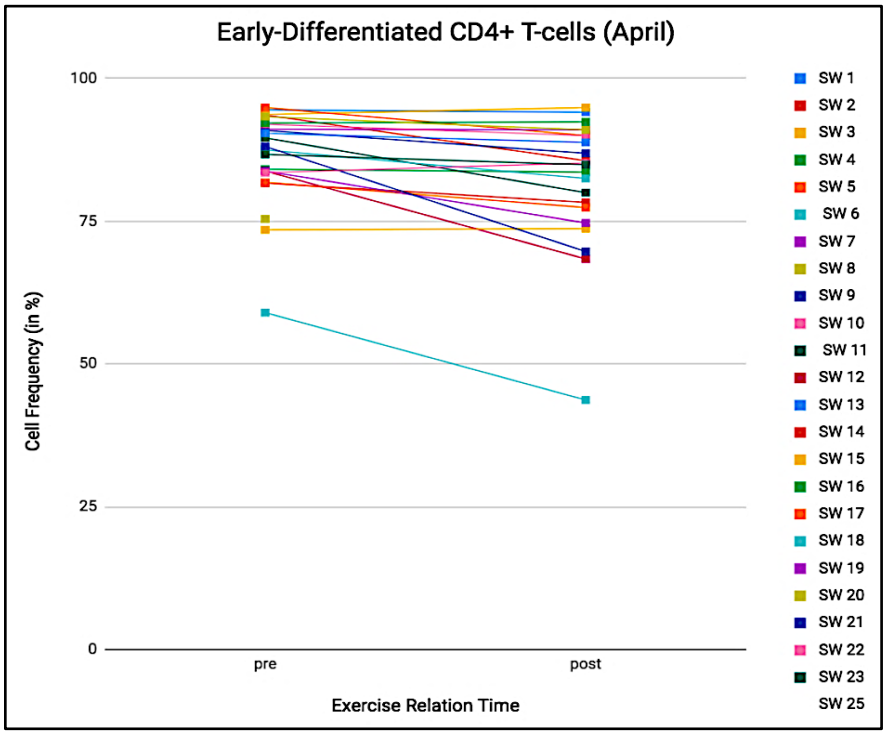

Figure A.1. Individual participant pre- and post-exercise early-differentiated CD4+ T-cell proportions for the April time point.

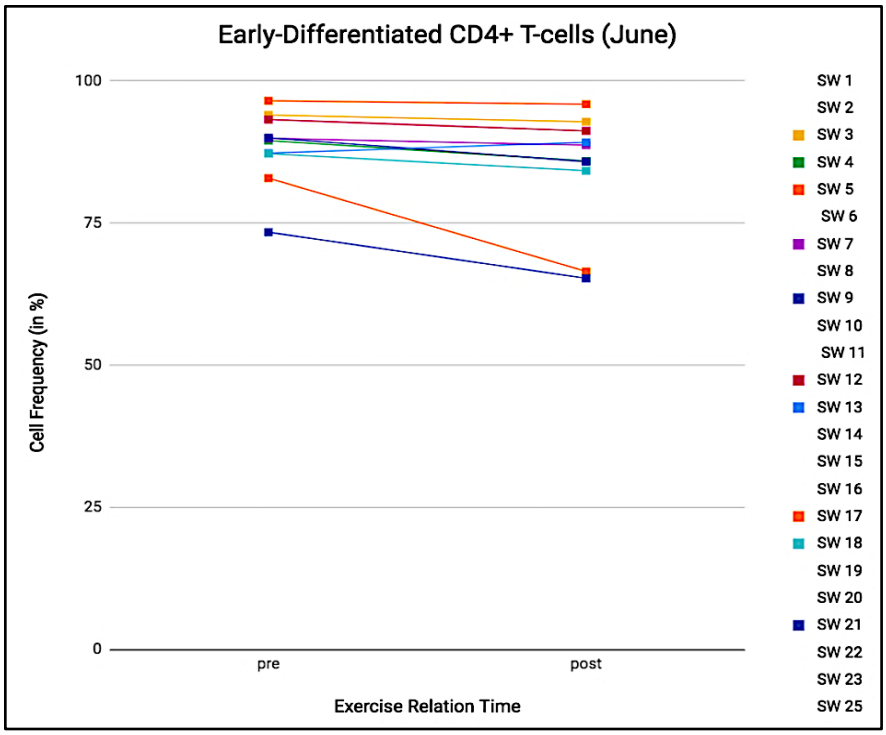

Figure A.2. Individual participant pre- and post-exercise early-differentiated CD4+ T-cell proportions for the June time point.

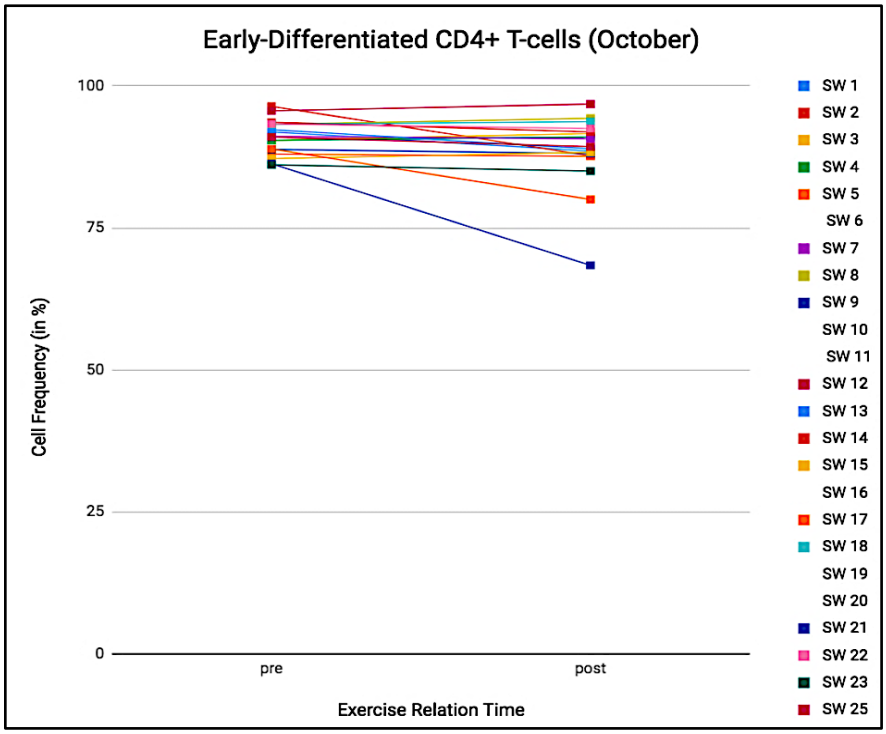

Figure A.3. Individual participant pre- and post-exercise early-differentiated CD4+ T-cell proportions for the October time point.

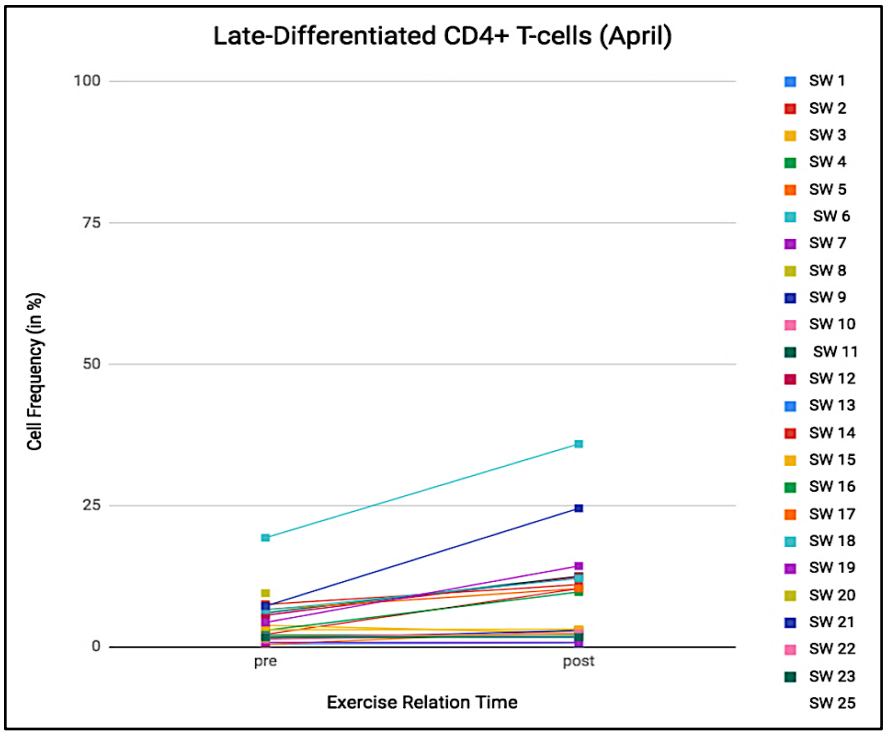

Figure A.4. Individual participant pre- and post-exercise late-differentiated CD4+ T-cell proportions for the April time point.

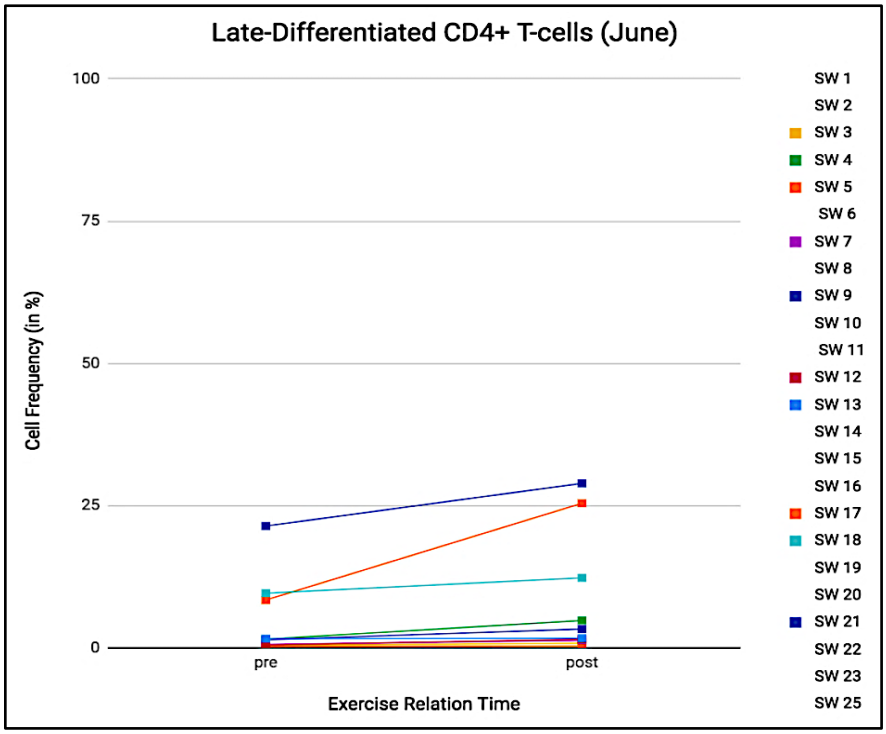

Figure A.5. Individual participant pre- and post-exercise late-differentiated CD4+ T-cell proportions for the June time point.

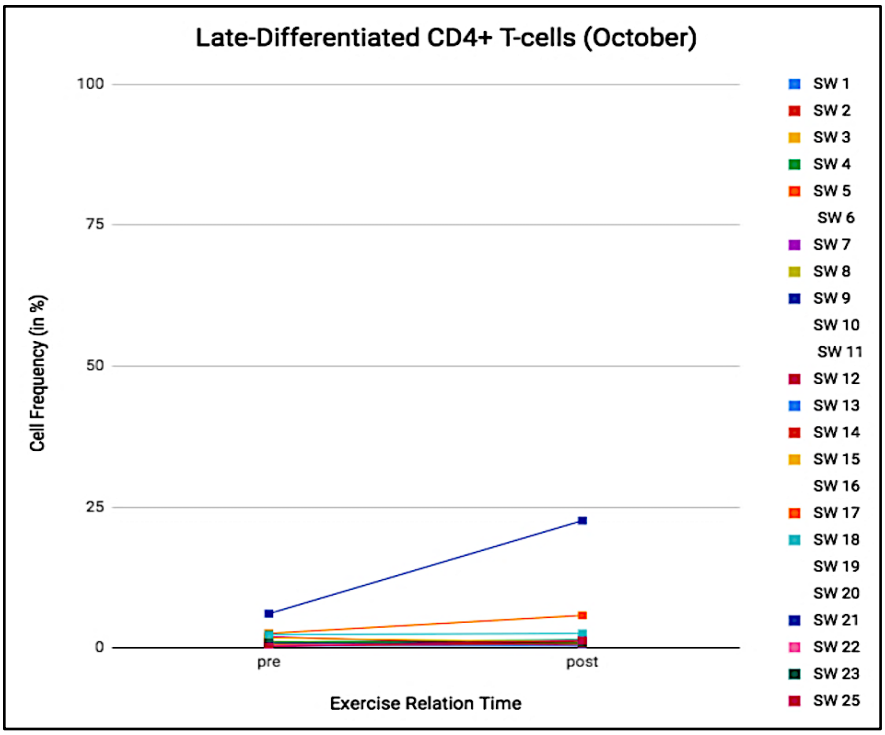

Figure A.6. Individual participant pre- and post-exercise late-differentiated CD4+ T-cell proportions for the October time point.

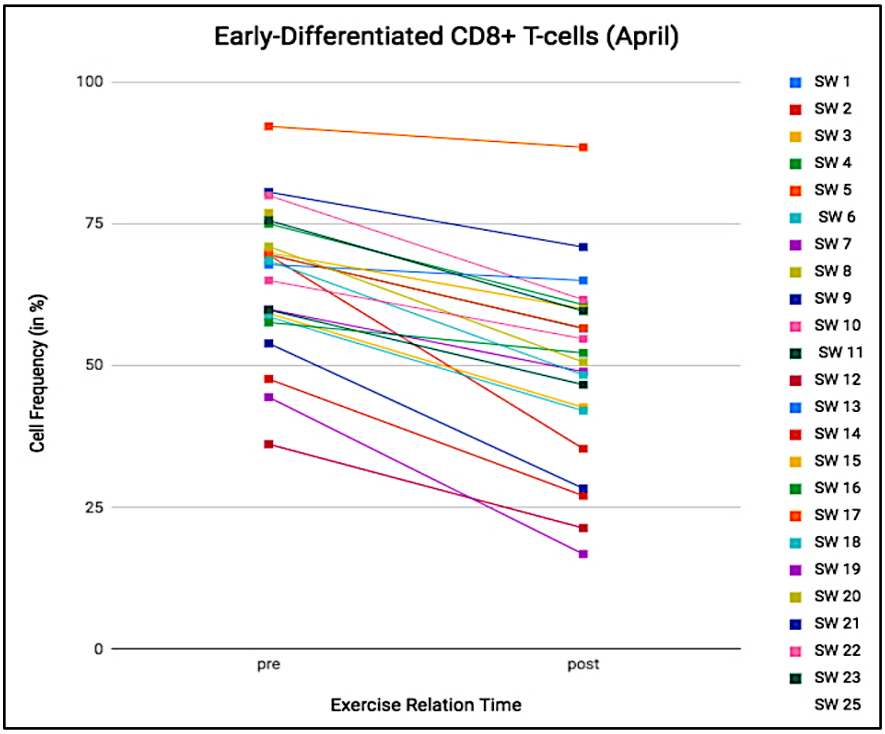

Figure A.7. Individual participant pre- and post-exercise early-differentiated CD8+ T-cell proportions for the April time point.

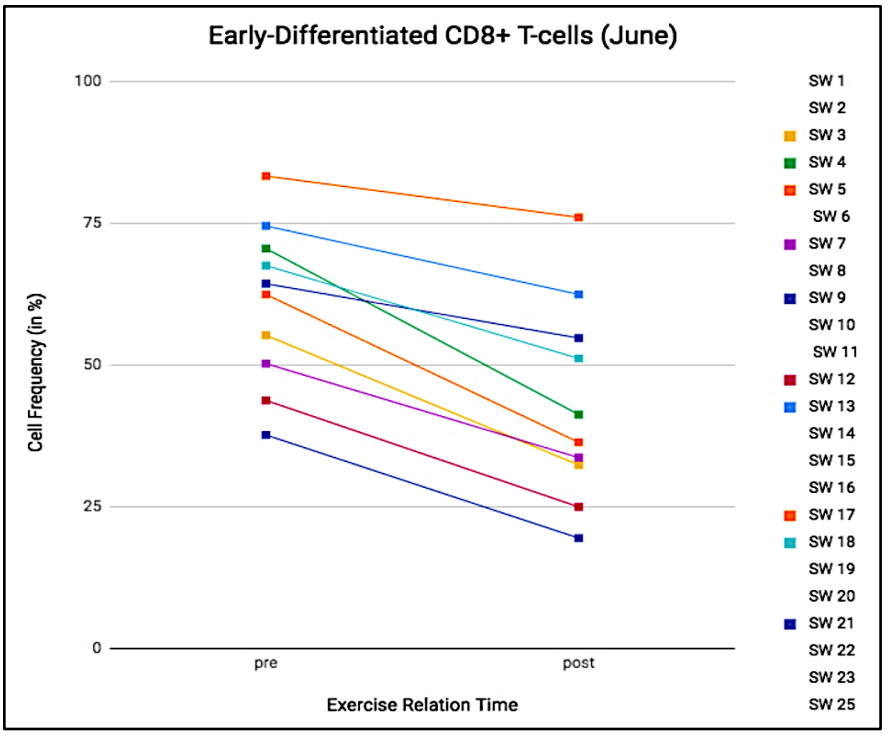

Figure A.8. Individual participant pre- and post-exercise early-differentiated CD8+ T-cell proportions for the June time point.

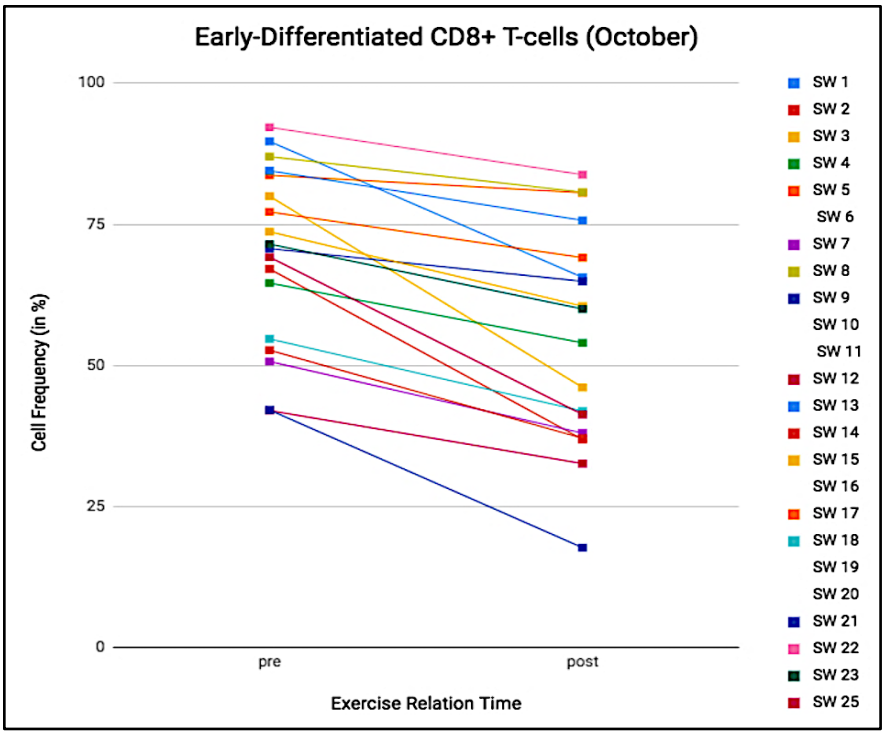

Figure A.9. Individual participant pre- and post-exercise early-differentiated CD8+ T-cell proportions for the October time point.

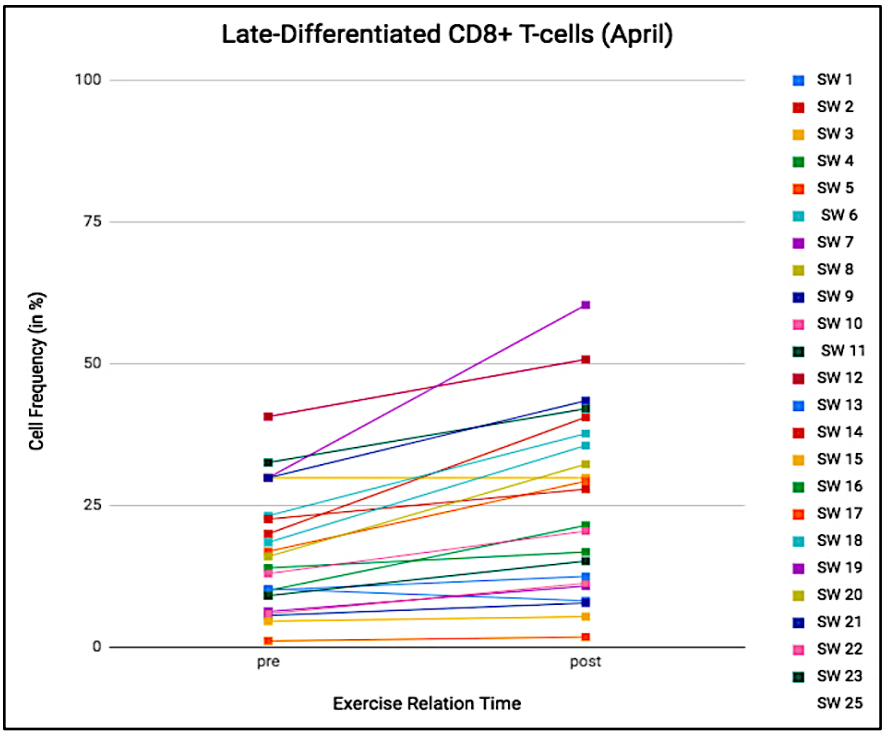

Figure A.10. Individual participant pre- and post-exercise late-differentiated CD8+ T-cell proportions for the April time point.

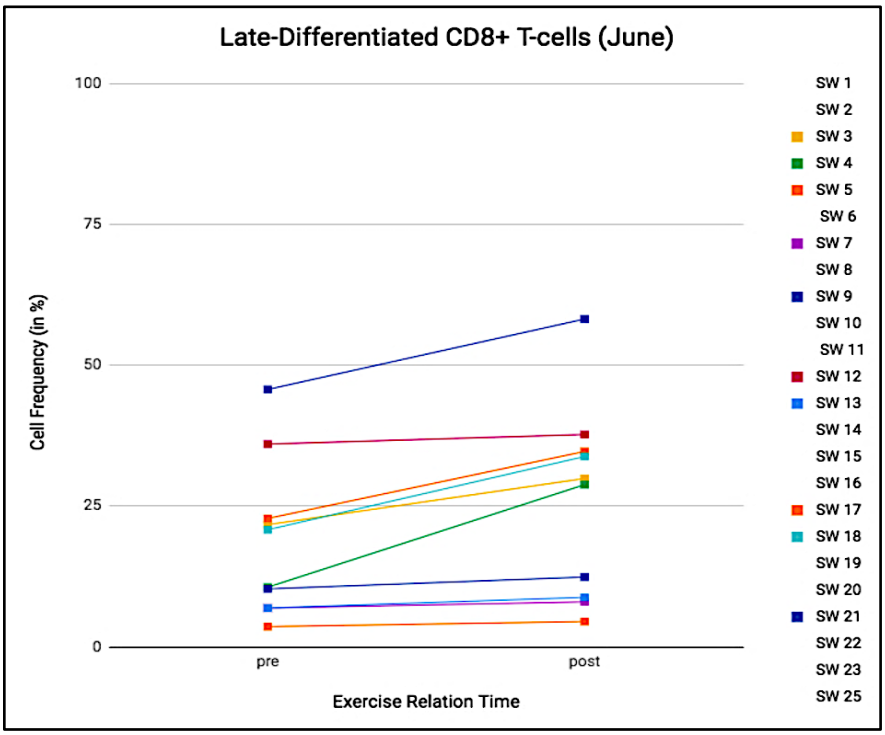

Figure A.11. Individual participant pre- and post-exercise late-differentiated CD8+ T-cell proportions for the June time point.

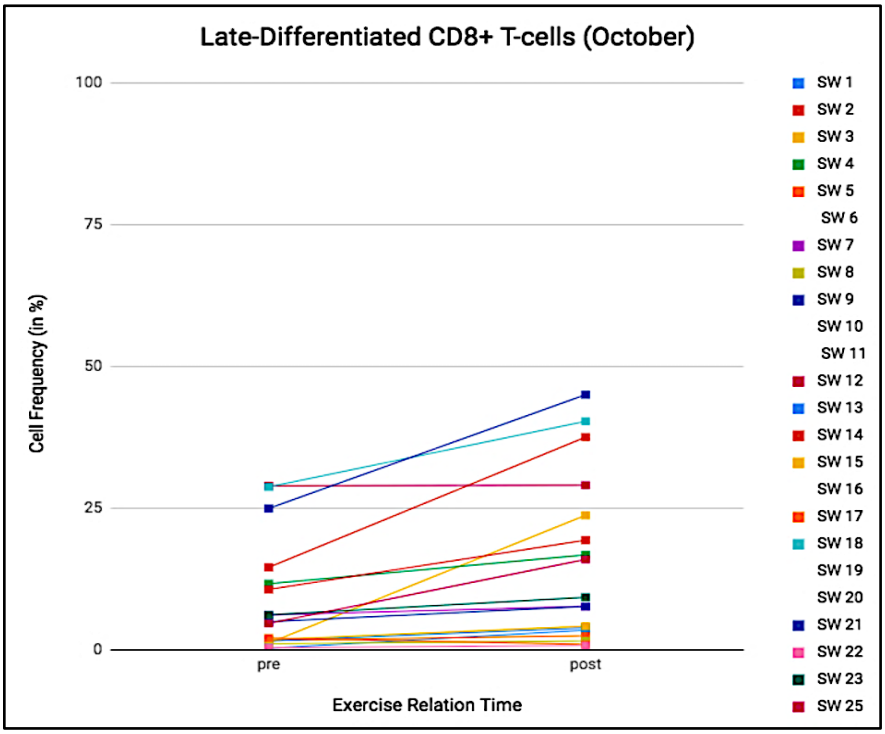

Figure A.12. Individual participant pre- and post-exercise late-differentiated CD8+ T-cell proportions for the October time point.

Vita

Connor Alexander Kuremsky was born in Pittsburgh, Pennsylvania. He studied Human Biology at Stanford University, where he was also a member of the Varsity Diving Team. After earning his bachelor's degree in 2016, he trained for and competed in the 2016 USA Diving Olympic Team Trials. He then began graduate school at Louisiana State University; he studied Exercise Physiology and coached the Varsity Diving Team. His interest in sports medicine and exercise science developed as he began research in LSU's Exercise Biochemistry Lab. After earning his master's degree, he plans to enter medical school.