EFFECTS OF PHYSICAL ACTIVITY STATUS AND EXERCISE MODALITY ON THE T-CELL AND MONOCYTE RESPONSE TO ACUTE EXERCISE IN OLDER ADULTS

by
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DEDICATION

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ABSTRACT

Older adults are at increased risk for many inflammation-associated chronic diseases, especially when aging is combined with physical inactivity. Maintaining an active lifestyle significantly reduces the risk of chronic disease in older adulthood, which may be due, in part, to the ability of regular exercise to maintain optimal immune function and minimize chronic inflammation. The specific immune system and inflammatory effects of cardiorespiratory (CRE) compared to resistance (RE) exercise in older adults are not fully understood. The purpose of this study was to examine the effects of exercise modality (cardiorespiratory vs. resistance training), and training status (physically active vs. inactive) on the immune system and inflammatory response to acute exercise of physically active older adults (OPA) and physically inactive older adults (OPI). Twenty-four healthy older adults (OPA n=12; OPI n=12) completed one bout of CRE and one bout of RE in a randomized order, both at a moderate intensity, and separated by at least 7 days. Blood samples were collected pre-exercise, post-exercise, and 1h post-exercise (recovery) time points and analyzed for CD4+ and CD8+ T-cells and T-cell subsets expressing surface markers CD45RA, CD62L, CD57, CD161, and CD196, and monocytes and monocyte subsets expressing surface markers CD11b, CCR5, CX3CR1, and CCR2. Monocyte function was assessed *in vitro* by LPS-stimulated cytokine (IL-6 and TNF) secretion and a glucocorticoid resistance assay. OPI had higher numbers of circulating CD57+ EMRA CD4+ T-cells (mean ± SE; OPA, 1 ± 2 cells/μL; OPI, 6 ± 2 cells/μL) and higher CCR2+ non-classical monocytes MFI (mean ± SE arbitrary units; OPA, 8 ± 4; OPI, 21 ± 4) than OPA at rest. Furthermore, RE mobilized more T-

cell and monocyte subsets than CRE in both OPA and OPI groups, and CRE resulted in increased LPS-stimulated TNF production and decreased Th17 cell mobilization post-exercise, while RE caused decreased LPS-stimulated TNF production post-exercise and increased Th17 cell mobilization. Taken together, there were not major differences between the OPA and OPI response to acute exercise. However, differences in the immune system and inflammatory response to CRE compared to RE may exist and these differences may provide important insight to informing future exercise prescriptions for older adults to minimize inflammation and promote optimal immune function.

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CHAPTER ONE

Introduction

Older adults are at increased risk of many inflammation-associated chronic diseases [1]. These risks are often increased when aging is combined with physical inactivity [2]. It is widely accepted that maintaining an active lifestyle significantly reduces the risk of chronic disease in older adulthood, and cross-sectional data supports that remaining physically active into older adulthood can offset many of the age-related immune and inflammatory changes often observed in the elderly [3]. Many researchers have proposed that this is due to the anti-inflammatory effects of exercise training [4]. Nevertheless, the mechanisms through which exercise is anti-inflammatory still remain unclear.

The cellular immune changes that occur with physical inactivity are likely responsible for increased systemic inflammation, which can be prevented or reversed with the implementation of habitual exercise [5]. Immunosenescence, defined as the age-related decline in immune function, is characterized by detriments in both the innate and adaptive arms of the immune system [6]. For example, immunosenescence is often described by a reduced T-cell repertoire, characterized by an accumulation of highly-differentiated, viral-specific T-cells and a reduction in antigen inexperienced naïve T-cells [5]. It is also associated with changes in monocyte subpopulations, function, and surface marker expression, reduced NK-cell cytotoxicity, and impairments in neutrophil functioning [5].

Exercise training can prevent and reverse a variety of these biomarkers often associated with immunosenescence and chronic disease. For example, Speilmann et al (2011) reported that fitness level was a better predictor of circulating senescent T-cells than age [3]. In other words, older adults with high cardiorespiratory fitness (VO_{2max}) did not display elevated proportions of highly-differentiated T-cells like their unfit counterparts. Similar findings were shown longitudinally by Shimizu et al (2011), who reported that 12 weeks of exercise training resulted in an increased number of circulating CD28+ T-cells, which is a marker of low-differentiation status [7]. Furthermore, additional research in older adults showed that inactive older adults had higher proportions of non-classical, pro-inflammatory monocytes than their active counterparts, but that 12 weeks of exercise training was able to normalize the proportions of these cells [8, 9]. Timmerman et al (2008) also reported that exercise training was able to reduce the amount of pro-inflammatory cytokine, TNF production in response to antigenic stimulation of the monocytes using lipopolysaccharide (LPS) [8]. Together, these findings are significant because they demonstrate potential mechanisms through which exercise training may reduce systemic inflammation and improve immunity.

Another potential mechanism in which exercise may improve immunity is via its ability to improve the physiological response to stress. Indeed, aging and chronic stress are associated with hypothalamic-pituitary-adrenal (HPA)-axis dysregulation and increased resistance of glucocorticoid receptors, which impair the anti-inflammatory effects of glucocorticoids (i.e. cortisol) and can potentially result in elevated chronic systemic inflammation [10-12]. Physical exercise, which is often

described as an acute stressor that affects the HPA-axis, may be able to “re-sensitize” the glucocorticoid receptors on immune cells such that less hormone is necessary to exert its physiological anti-inflammatory effects [13]. However, this has not yet been demonstrated experimentally. Nevertheless, exercise trained subjects secrete less cortisol than untrained subjects in response to an acute psychosocial stressor [14], and physical activity level is able to buffer the negative relationship between chronic stress and telomere length—which is a sign of immunological aging [15]. Thus, it appears that the effects of exercise on the body’s ability to cope with stress may serve as an important mechanism through which exercise training may improve immunity and reduce chronic inflammation.

While these findings are promising, much remains to be determined about the ways in which exercise may be used to improve immunity. For example, much of the exercise immunology research to date has been conducted using cardiorespiratory fitness-based exercise, with much less attention devoted to resistance training. Thus, the differences between the immune response to aerobic compared to resistance training remains largely unknown [16]. Additionally, more research is needed in order to determine the role of exercise training status on immune characteristics and the acute exercise response, particularly among older adults. Indeed, while the body of literature examining exercise immunology in older adults is growing, many unanswered questions still remain. To date, no study has compared the effects of exercise modality and training status in older adults within a single study design.

Statement of the Problem

The primary purpose of this study was to examine the effects of exercise modality (cardiorespiratory (CRE) vs. resistance (RE) exercise), and physical activity level (physically active vs. physically inactive) on the immune and inflammatory response to acute exercise in older adults (older physical active (OPA) and older physically inactive (OPI)). Each participant completed one bout of moderate-intensity cardiorespiratory exercise (CRE) and on a separate occasion, one bout of resistance exercise (RE), and their immune responses to the acute exercise sessions were measured.

Aims and Hypotheses

Aim 1 (A1): To compare resting immune characteristics between OPI and OPA.

Hypothesis 1 (H1): OPI participants will have higher proportions of highly-differentiated T-cells and non-classical monocytes than OPA. They will also secrete higher levels of TNF and IL-6 in response to LPS stimulation, and dexamethasone will be less effective at suppressing the inflammatory response when compared to OPA participants.

Aim 2 (A2): To examine the independent effects of acute cardiorespiratory (CRE) and resistance (RE) exercise on the immune response while accounting for physical activity level.

Hypothesis 2a (H2a): Both CRE and RE will independently elicit a leukocytosis along with a significant mobilization and subsequent egress of immune cells with increased effector functions and inflammatory properties,

including highly-differentiated T-cells and non-classical monocytes. However, the magnitude of the response will be greater following CRE compared to RE at both the post-exercise and recovery time points.

Hypothesis 2b (H2b): Although the relative proportions of mobilized immune cells will remain similar among the groups, we hypothesize that OPI will exhibit higher absolute mobilization of lymphocytes and monocytes in response to both CRE and RE (post-exercise), and also a higher absolute egress of these cells at the recovery time point.

Aim 3 (A3): To examine the effects of acute CRE and RE on the inflammatory response (TNF and IL-6 secretion) *in vitro* while accounting for physical activity level

Hypothesis 3a (H3a): Post-exercise and recovery blood will produce more pro-inflammatory cytokines when stimulated with LPS compared to resting blood, and dexamethasone will be less effective at suppressing the inflammatory responses at both the post-exercise and recovery time points when compared to resting conditions. We hypothesize that the magnitude of these responses will also be greater following CRE compared to RE.

Hypothesis 3b (H3b): While the relative inflammatory response to LPS and dexamethasone will remain similar among the groups, we hypothesize that OPI will exhibit larger increases in TNF and IL-6 at all 3 time points (pre-exercise, post-exercise, and recovery) compared to OPA.

Limitations

Limitations associated with this study include:

- Participants may not have accurately reported personal information (i.e. medications, whether they are fasted, physical activity status, etc.)
- During the muscular strength assessment, participants may not have accurately reported their effort, leading to an underestimation of their 1 RM
- Not testing or controlling for latent CMV infection
- OPA group consisted of 6 males and 6 females, while OPI group consisted of 1 male and 11 females
- Not measuring monocyte TLR4 expression

Assumptions

Assumptions associated with the study include:

- Participants were honest about their medical history and exercise training history
- Participants did not significantly change their diet or physical activity level between study visits
- That a true 8 RM was obtained during visit 2

Significance of the Problem

Immunosenescence is common among aging individuals, and it is associated with the development and perpetuation of many pro-inflammatory conditions and diseases [5]. Physical inactivity is also often accompanied by increased risk for inflammation-associated chronic disease [2, 5]. The negative cellular and inflammatory changes that have traditionally been associated with aging may be largely attributed to increases in physical inactivity [2]. Habitual exercise in older adults may be able to prevent or reverse many of the cellular and inflammatory

changes that often characterize immunosenescence [3, 8, 17]. However, there is not currently a specific exercise prescription for older adults. Specifically, the independent effects of resistance exercise compared to cardiorespiratory exercise on the immune and inflammatory response of older adults have not been examined. Delineating the independent contributions of resistance compared to cardiorespiratory exercise can help researchers identify potential mechanisms through which exercise may enhance immunity and reduce systemic inflammation, ultimately contributing to improved exercise prescription for older adults in a way that will maximize health and longevity.

CHAPTER TWO

Literature Review

Aging is associated with a deterioration in many physiological processes and an increased risk of many chronic diseases [2, 18]. While modern medical advances have contributed to increased life expectancy by reducing mortality, they have not yet been successful in preventing or reversing the detrimental effects of aging on overall health [18]. Thus, an increasing number of older adults are living with chronic diseases and conditions that have a profoundly negative impact on their quality of life. Many of these age-associated chronic diseases are characterized by immunological changes, known as immunosenescence, and increases in chronic systemic inflammation, which are thought to individually and additively perpetuate disease advancement. A growing body of research supports the notion that exercise training exerts systemic, anti-inflammatory effects and that regular exercise can offset the cellular immune changes that are frequently observed in aged, physically inactive populations [5], as well as improve the body's ability to combat disease and other stressors [14].

This review of the literature is divided into six sections. Section 1 will discuss the effects of aging on the immune system; section 2 will review the effects of stress on immunity; section 3 will introduce the effects of exercise on immunity; section 4 will discuss the anti-aging and anti-stress effects of exercise; section 5 will provide an overview of the current gaps in the literature; and section 6 will summarize the implications of exercise on aging, immunity, and stress and the significance of the present study.

Effects of Aging on Immunity

Currently, cardiovascular disease, hypertension, cancer, osteoarthritis, type 2 diabetes, and osteoporosis serve as some of the most common age-associated chronic diseases [1]. Immune dysregulation (i.e. senescence), and chronic inflammation are major contributors to the development and perpetuation of these diseases, serving as a common theme underlying age-associated chronic disease [2]. Indeed, immunosenescence, a term that accounts for a variety of unfavorable immune and inflammatory changes that occur with age, is largely implicated in the development of age-associated chronic disease and is responsible for increased susceptibility to infection as well as reduced vaccine efficacy in the elderly [5]. Recently, a new term, “inflamm-inactivity” was coined to describe the increase in chronic systemic inflammation that is often associated with age and perpetuated by physical inactivity [2]

Aging and Adaptive Immunity

T-cells are major components of the adaptive immune system and appear to be largely influence by age [5]. In response to a novel antigen, recognition occurs by naïve CD4+T-helper cells and CD8+T-cytotoxic cells, which rapidly differentiate and replicate in order to mount an immune response and eradicate the virus [19]. Once the body has cleared the active virus, viral-specific memory cells remain so that if encountered with the same virus in the future, a streamlined immune response can occur to clear the virus more efficiently [19]. These memory cells are no longer naïve, as they have encountered a virus and have undergone clonal replication, and thus, they exhibit markers of increased differentiation status [20, 21]. Nevertheless,

retaining a diverse naïve T-cell pool is critical to maintaining optimal health, as naïve T-cells are necessary to combat exposure to any novel antigens and a reduction in the naïve T-cell repertoire puts an individual at an increased risk for infection [17].

Some of the cellular changes that occur with immunosenescence include an accumulation of late-stage highly-differentiated T-cells compared to naïve or early-stage differentiated T-cells, an inverted CD4 : CD8 T-cell ratio (usually caused by an inflation of the highly-differentiated CD8+T-cell pool), poor T-cell proliferative responses to mitogens, and latent cytomegalovirus (CMV) infection [5]. Latent CMV infection, which affects about 60% of the general population and above 90% of those over age 80 in the US alone [22], exacerbates the accumulation of late-stage differentiated T-cells and, coupled with the age associated reduction in naïve T-cell numbers, dramatically reduces the diversity of the T-cell repertoire. This reduction in the diversity of the T-cell pool and the accumulation of memory T-cells facilitates T-cell exhaustion and contributes to impaired immunity [23]. Highly-differentiated T-cells also preferentially secrete pro-inflammatory cytokines such as IFN-γ and TNF-α, thereby also increasing chronic systemic inflammation [23]. Indeed, memory T-cells expressing a CD28- phenotype have been implicated in the pathogenesis of cardiovascular disease, osteoarthritis, and other age-associated inflammatory conditions [23]. Thus, cells of the adaptive immune system are susceptible to age-related change.

Aging and Innate Immunity

Aging is also associated with changes among the cells of the innate arm of the immune system. Monocytes, which can be divided into three distinct populations

based on their surface expression of CD14 and CD16 (classical: CD14++ CD16- ; intermediate: CD14++ CD16+ ; and non-classical: CD14+ CD16+)[24], have been reported to play a prominent role in a inflamm-inactivity and a variety of inflammatory-related diseases [8, 25]. For example, monocytes may be more involved than previously thought in the development and perpetuation of chronic diseases—including cardiovascular disease (CVD).

Traditionally, atherosclerotic plaque was thought to accumulate as a result of high levels of circulating oxidized low-density lipoproteins (LDL's) that promote the development of foam cells and fatty streaks within blood vessel walls, which in turn activates circulating monocytes, increasing their expression of adhesion molecules and recruitment of more monocytes, which extravasate the circulation into the neointima, and differentiate into macrophages that uptake lipids, resulting in the formation of additional foam cells and ultimately, atherosclerotic plaque [25]. More recently, however, an "inflammation-centric" model of CVD development has been proposed, which postulates that higher levels of pro-inflammatory cytokines are responsible for monocyte recruitment to the endothelium in the absence of hyperlipidemia. Those monocytes with higher levels of adhesion molecules (i.e. CD11b, CCR2, CX3CR1) extravasate into the neointima and differentiate into macrophages, where pathogen associated molecular patterns (PAMPs) interact with toll-like receptors (TLRs) on monocytes / macrophages to stimulate lipid uptake and thus, foam cell formation [25]. This inflammation-centric model of CVD development may explain why a variety of inflammatory diseases are associated with increased risk of CVD independent of hyperlipidemia.

The absolute number of circulating monocytes may increase [26] or remain unchanged [27] with age. Nevertheless, aging is associated with a shift in the proportions of monocyte subpopulations (i.e. an increase in the proportions of intermediate and non-classical monocytes) [27]. Both mouse and human models have demonstrated an impaired secretion of cytokines (IL-6, TNF-α) in response to TLR 1 and 2 stimulation [26], while the effects of aging on LPS-mediated cytokine secretion (via the TLR4 pathway) remains inconclusive [28]. However, it is well established that increased proportions of circulating intermediate (CD14++CD16+) and non-classical (CD14+ CD16+) monocytes are associated with increased pro-inflammatory cytokine production, as these cells produce copious amounts of pro-inflammatory cytokines (i.e. TNF-α, IL-6, IL1β) with little – no production of anti-inflammatory cytokines (i.e. IL-10) [29]. Furthermore, elevated proportions of non-classical monocytes are highly correlated with a variety of chronic inflammatory diseases [30], and increases in intermediate monocytes have been implicated in the prediction of cardiovascular events [31]. Thus, regardless of whether the number of total circulating monocytes increases or decreases with age, the age-associated decrease in the proportion of classical and increase in the proportion of non-classical monocytes may indicate an increased risk of health concerns for the elderly.

Taken together, aging is associated with a variety of innate and adaptive immunological changes that leave aged individuals at increased risk for opportunistic infections, inflammation-associated chronic diseases (like CVD), reduced vaccine efficacy, and malignancy. Thus, the importance of preventing or reversing these

immune and inflammatory changes is paramount to maintaining the length and quality of life of the aging population.

Effects of Psychological Stress on Immunity

Stress, defined by McEwen as “a real or interpreted threat to physiological or psychological integrity (i.e. homeostasis) of an individual that results in physiological or behavioral responses”, can occur in both acute and chronic situations [13]. Although exercise falls into this category and is often characterized as an acute stressor, this section will focus on psychological stress, as the outcomes of psychological stress compared to exercise-induced stress are not identical, and the effects of exercise on immunity are discussed separately below.

The duration and the course of psychological stress are large determinants of the type of immune response that occurs as a result [32]. Acute stress, lasting minutes to hours, elicits a physiological response similar to exercise, and is characterized by a sympathetic nervous system (SNS) activation, along with increased leukocyte trafficking [32, 33]. This “fight-or-flight” response is thought to be a protective mechanism, preparing the body to effectively combat an oncoming challenge [34].

Chronic stress, lasting several hours a day for a period of weeks or months, however, is reported to have deleterious effects on the immune system [34]. Indeed, chronic psychological stress has been associated with a variety of immunological detriments, including delayed wound healing, suppressed lymphocyte proliferative responses, increased duration and severity of infectious disease, latent viral reactivation, poorer vaccination efficacy, tumor development and progression, and

an accelerated progression of coronary artery disease [10, 32]. Furthermore, chronic psychological stress is also associated with dysregulated cortisol secretion, which may be partially responsible for many of the deleterious immune changes [34].

Under healthy circumstances, stress is associated with elevated levels of glucocorticoids as a result of hypothalamic-pituitary-adrenocortical (HPA)-axis and sympathetic-adrenal-medullary (SAM) activation [10, 35]. Traditionally, glucocorticoids are largely responsible for inflammation resolution [36]. However, under chronic, prolonged stress conditions, Miller et al (2002) and others have proposed a glucocorticoid resistance model, whereby continued elevations in these hormones elicit a counter-regulatory response resulting in the downregulation of glucocorticoid receptors on leukocytes and, therefore, a subsequent inability of glucocorticoids to inflict their anti-inflammatory functions [10, 11]. As a result of impaired inflammation-resolution, chronic systemic inflammation persists and elicits other deleterious physiological effects [11].

Several studies have shown support for the glucocorticoid resistance model. In a cohort of parents of pediatric cancer patients, IL-6 secretion by immune cells in response to *in vitro* stimulation with glucocorticoids (dexamethasone) was blunted across all concentrations of dexamethasone, compared to parents of healthy children [10]. Additionally, two studies from Cohen and colleagues have demonstrated that individuals who were under situations of chronic stress displayed markers of glucocorticoid resistance, while those who did not undergo a major stressful event did not. Glucocorticoid resistance in these studies was defined as a lack of a relationship between plasma cortisol levels and the neutrophil : lymphocyte

ratio, whereas a healthy physiological response was characterized by a positive correlation between plasma cortisol and neutrophil : lymphocyte ratio [11]. In other words, increased plasma cortisol levels in those who were under chronic psychological stress was not associated with increased neutrophil recruitment as it was among those who were not stressed, indicating an impaired immune response in stressed individuals. Furthermore, those without a correlation between plasma cortisol and leukocyte counts were more likely to develop a cold than those who did display a linear relationship, without any difference in plasma cortisol levels between the groups [11]. This adds additional support to the glucocorticoid resistance model by indicating that immune cells of those under chronic stress fail to respond to the stimulus of cortisol, indicating an impaired immune response and glucocorticoid resistance.

Building on the glucocorticoid resistance model, the Cohen group also measured inflammatory cytokine production *in vitro* (using dexamethasone) among the leukocytes of those with and without chronic stress and found that those with higher glucocorticoid resistance exhibited increased production of IL-6 and TNF [11]. Taken together, these findings indicate that stress is a cause of glucocorticoid resistance among immune cells and that increased glucocorticoid resistance is associated with increased inflammation, independent of cortisol levels.

The Cohen studies add to the glucocorticoid resistance model by emphasizing that the impact of chronic stress on the HPA axis may be more related to alterations in glucocorticoid receptors found on target tissues than to extreme long-term elevations in cortisol secretion [11]. This notion is supported by other

studies that have reported normal or lower than normal cortisol levels in individuals with posttraumatic stress disorder (PTSD), despite having an elevated concentration of corticotropin-releasing factor (CRF; a precursor to adrenocorticotropic hormone (ACTH), which is the precursor to cortisol) [37]. Thus, while chronic stress may interfere with circulating glucocorticoid levels, the more impactful effects likely arise as a result of the downregulation or insensitivity of glucocorticoid receptors on target tissues, contributing to the perpetuation of HPA axis dysregulation and chronic systemic inflammation.

Effects of Exercise on Immunity

The innate and the adaptive arms of the immune system are affected by participation in both acute and chronic exercise [38]. In response to an acute bout of dynamic exercise, a large leukocytosis is typically observed, which is characterized by a large mobilization of neutrophils and lymphocytes into the circulation along with a smaller contribution from monocytes, lasting for about 6-24 hours before returning to baseline levels [38]. This transient exercise-induced mobilization of cells is largely due to increases in shear stress and catecholamines that accompany exercise [39]. The stress hormone cortisol also plays a role, particularly after prolonged and / or exhaustive exercise, and is thought to be responsible for a secondary, delayed increase in neutrophil mobilization and the drop in lymphocytes (lymphocytopenia) below baseline levels, before returning to baseline levels about 6-24 hours after exercise cessation [40].

Lymphocyte and Monocyte Responses to Acute Exercise

More recent work in exercise and stress has shown that the acute exercise-induced immune response is not uniform, as certain cell types are much more responsive to the exercise stimulus than others [33, 41-43]. The cells preferentially mobilized into the circulation with exercise display phenotypes that reflect increased cytotoxicity, differentiation status, inflammation, tissue migration, and effector functions compared to those present in resting blood [38, 41, 43, 44]. Within the lymphocyte population, exercise causes a preferential mobilization of NK cells, CD8+ T-cells, and gamma-delta T-cells, with minimal changes in the numbers of circulating CD4+ T-cells and B-cells [33]. This can be broken down even further, as among CD8+ T-cells, those with greater differentiation status (i.e. effector memory (EM) and effector memory RA (EMRA) cells, expressing CD45RA-CD62L-, and CD45RA+CD62L-, respectively), which is associated with increased effector function, are preferentially mobilized over their less-differentiated counterparts (i.e. naïve (CD45RA+CD62L+) and central memory (CD45RA-CD62L+)) [41]. Furthermore, the loss of cell surface marker CD28, or the expression of cell surface marker CD57 are also indicative of high differentiation status [45, 46] and thus, CD8+ T-cells that are CD28- or CD57+ also demonstrate a preferential exercise-induced mobilization compared to their CD28+ or CD57- counterparts [41, 47].

In response to acute exercise, an immediate lymphocytosis occurs in the preferential manner discussed above. The increase in circulating lymphocytes typically lasts several minutes before dropping back down towards pre-exercise levels [48]. Depending on the intensity and duration of the exercise performed, the

lymphocyte number often decreases below baseline levels, causing a lymphocytopenia, which can last for several hours into recovery, with the most pronounced lymphocytopenia occurring following high-intensity, long-duration exercise [38]. The preferential mobilization of NK cells, highly-differentiated CD8+ T-cell subsets, and γδ T-cells into the blood compartment is transient, lasting only several minutes before these cells preferentially extravasate the circulation in the minutes following exercise cessation, returning to pre-exercise levels within 1h post-exercise [33, 41]

Monocytes, which are classified as classical, intermediate, and non-classical subpopulations, also exhibit a preferential response to acute exercise [43]. Monocytes that express CD16 are mobilized to a greater extent than classical monocytes, which do not express CD16 [43]. However, when the CD16+ monocytes are divided into intermediate (CD14++CD16+) and non-classical (CD14+CD16+) populations, it appears that while both increase, non-classical monocytes are mobilized to a greater extent [41, 49]. Consistent with the idea that exercise recruits cells with increased effector functions and inflammatory capacity, non-classical monocytes are known to exhibit pro-inflammatory properties in the absence of anti-inflammatory properties, as discussed above [29].

Compared to classical monocytes, non-classical monocytes are characterized by increased TLR2, TLR4, and HLA.DR, which suggests an increased capacity for antigen presentation to CD4+ T-helper cells and therefore the subsequent activation of the adaptive immune system [50, 51]. Non-classical monocytes also demonstrate higher levels of CCR5 (a chemokine receptor for multiple chemokines, including

macrophage inflammatory protein 1-alpha) and lower levels of CCR2 (monocyte chemoattractant protein-1 receptor) compared to classical monocytes, which suggests that classical and non-classical monocytes may exhibit different trafficking patterns and stimuli [49].

Similar to lymphocytes, acute exercise causes monocyte numbers to increase in the circulation for a short time (i.e. minutes) before returning to pre-exercise levels within the minutes – hours after exercise cessation [48]. This exercise-induced monocytosis has been observed in response to moderate-intensity aerobic exercise [48], short-duration high-intensity (anaerobic) exercise [43], and resistance training [16]. While acute exercise elicits a preferential mobilization of non-classical monocytes as discussed above, it also appears that these are the first to egress once the exercise stimulus has ceased [52].

It has been reported that acute exercise prompts a reduction in monocyte HLA.DR [51, 52]. Additionally, Simpson et al. (2009) showed that acute exercise results in a downregulation of TLR2 and TLR4 expression on classical monocytes, and an increase on non-classical monocytes [52], which adds to the previous literature describing an exercise-induced reduction in overall monocyte TLR2 and TLR4 expression [53]. Nevertheless, acute exercise effects on TLR receptors remains inconclusive, as Booth et al (2010) found and increase in monocyte TLR2 and TLR4 following exercise [51], while Oliveira and Gleeson (2010) found a reduction in TLR4 but not TLR2 following exercise [54]. Hong and Mills (2008) examined the effects of moderate intensity exercise on adhesion molecule CD62L and CD11b and found that exercise caused a differential response among various

monocyte subtypes [49], but the physiological underpinnings remain unknown. Therefore, although it is clear that exercise recruits monocytes into the circulation in a preferential manner, the specific characteristics of monocytes that are preferentially recruited have yet to be classified and may be differentially affected by the type of exercise performed.

Results from longitudinal studies consistently support the idea that exercise training status does not have a large effect on the proportions of overall leukocytes, lymphocytes, or monocytes in response to an acute bout of exercise. However, the absolute number of cells mobilized into the circulation following an acute exercise bout is often lower among those who are trained compared to untrained individuals [55]. Any differences between the immune response to acute exercise among trained compared to untrained individuals at a controlled intensity is more likely a product of alterations in β2-AR sensitivity, glucocorticoid receptor sensitivity, or cell adhesion molecules, which may be affected by regular exercise [55].

Lymphocyte and monocyte responses to chronic exercise

Regular physical activity across the lifespan is known to be advantageous to overall health, which includes immune functioning [5]. Nevertheless, there does not appear to be differences in the numbers of resting leukocyte numbers in athletes compared to healthy, age-matched controls [56]. Therefore, the majority of the literature examining chronic exercise changes to leukocyte populations has focused on aging populations and/or those with comorbidities that are known to alter or impair immunity.

In response to chronic exercise, there does not appear to be an effect of training status on NK cell numbers, although increases in NK cell function have been reported in previously inactive elderly individuals [57]. Cross-sectional data reported that fitness status appeared to be a better predictor of T-cell senescence than age, with those who had lower cardiorespiratory fitness demonstrating greater T-cell senescence compared to those with higher cardiorespiratory fitness [3]. Additionally, it has been reported that trained individuals display a reduced inflammatory response to LPS-stimulation and lower TLR4 surface expression on monocytes compared to those who are inactive, regardless of age [58-60]. Furthermore, two studies examining 12 weeks of combined (cardiovascular and resistance) exercise and resistance exercise only training in previously inactive older adults observed a reduction in the proportion of circulating non-classical monocytes after the training period [8, 9], indicating that exercise training is an effective tool in decreasing the proportion of circulating non-classical monocytes. In summary, while exercise training does not appear to have profound effects on the circulating numbers of broad leukocyte populations, it does seem to exert favorable effects on more discreet subpopulation in a way that becomes more apparent when examined in the context of age-related immune decline.

Mechanisms of Lymphocyte and Monocyte Mobilization

The large degree of exercise-induced leukocyte mobilization and subsequent egress to and from the circulation can be attributed to two primary mechanisms: increased hemodynamic shear stress and catecholamine secretion [61]. Hemodynamic shear stress, or the pressure of the blood against the endothelial

walls of the blood vessels, increases with exercise as a result of increased cardiac output and causes a demargination of cells that are typically adhered to the endothelial walls, thereby mobilizing them into the circulation [61]. Catecholamine secretion also increases with exercise, and is suggested to mediate leukocyte mobilization from marginal pools (i.e. spleen, lymph nodes, lungs, and skin) via β2-adrenergic receptors (β2-AR's) located in various concentrations on the cell surface of many leukocyte populations [39]. Indeed, the leukocyte subpopulations selectively recruited by exercise, such as cells with greater cytotoxic potential and higher cell differentiation status, are considered most instrumental in the body's 'fight or flight' response [34]. It has been shown that the mobilization of these cells (i.e. NK cells, highly differentiated CD8+ T-cells, gamma delta T-cells, and non-classical monocytes) is largely dependent on β2-AR mediated mechanisms independent of shear stress [41].

Cortisol is also a key stress hormone often released in response to acute exercise of high intensity and/or long duration [39]. A product of HPA-axis activation, cortisol is known to play a prominent role in inflammation and leukocyte trafficking [39, 62]. However, cortisol is known to exert delayed effects. Thus, the impact of cortisol on inflammation and leukocyte numbers is not typically observed until several hours into the recovery period following acute exercise [39]. Nevertheless, cortisol is a key player in inflammation-resolution and is thought to play a prominent role in the anti-inflammatory effects of exercise [63].

Exercise as an Anti-Aging, Anti-Stress Agent

There is an abundance of evidence that many “age-related” health conditions, such as cardiovascular disease (CVD), type 2 diabetes, metabolic dysregulation, high blood pressure, infectious disease, and malignancy are largely a product of immune dysfunction [5, 64]. It is well established that habitual physical activity can prevent or reduce the risk of developing many of these diseases, largely due to the effects of regular exercise on the immune system [5]. Indeed, it has been reported that adults who are physically active display longer telomeres (a marker of biological aging) on their immune cells compared to inactive counterparts, even when controlling for age, sex, BMI, smoking history, socioeconomic status, and physical activity at work [65]. Therefore, a multitude of cross-sectional and longitudinal studies have attempted to elucidate the mechanisms through which exercise may improve immune health of older adults.

Effects of Exercise Training on the Adaptive Immune System

When comparing sedentary older adults to active older adults and young adults, Yan et al. (2001) reported that the sedentary older adults displayed inverted CD4: CD8 ratios, which is an indicator of immune dysfunction [66]. They also found that this effect was not present in the active elderly group, as the CD4 : CD8 T-cell ratios were similar between these subjects and young adults [66]. Furthermore, Speilmann et al. (2011) reported a positive correlation between the proportion of senescent (highly-differentiated) CD4+ and CD8+ T-cells (KLRG1+/CD57+; KLRG1+;CD28-) and age [3]. However, once controlling for cardiorespiratory fitness level (VO_{2max}), the relationship between T-cell senescence and age no longer

existed. Rather, fitness level was significantly associated with CD4+ and CD8+ T-cell senescence even when controlling for age, BMI, and percent body fat [3]. Indeed, it has been suggested that regular exercise can prevent the accumulation of highly differentiated or senescent T-cells and the loss of low-differentiated or naïve T-cells, and hence proposed that implementing regular physical activity in older adults may elicit a shift in the T-cell repertoire in a way that facilitates the clearance to senescent T-cell clones and stimulates thymic output of naïve T-cells [17]. Nevertheless, evidence to support this using longitudinal exercise intervention studies in older adults are scarce [5, 7]

Effects of Exercise Training on the Innate Immune System

NK cell phenotype and function are also influenced by both aging and exercise [67, 68]. Additional cross-sectional data in older women showed that those who had higher levels of aerobic fitness had greater NK-cell function [69], although this result was not found in other studies of older adults [66]. Longitudinal studies have also reported mixed results, with some showing that exercise interventions can improve NK-cell function in a cohort of older adults [57, 70], and others showing no change [71, 72].

Monocytes also appear to respond to exercise training, although the specifics of their response are not yet well understood. Total monocyte TLR4 was shown to be lower in trained compared to untrained older adults [73]. Furthermore, 12 weeks of a combined aerobic and resistance training and a resistance training only program for older adults both showed that exercise training in previously inactive older adults was effective in reducing the number of circulating non-classical monocytes to the

same level as physically active counterparts [8, 9]. Additionally, Shimizu et al. (2011) showed that 12 weeks of exercise training in older adults resulted in increased co-stimulatory molecule CD80 expression on monocytes [7]. As CD80 signaling is responsible for T-cell activation, and is often reduced with aging [28], it is possible that exercise can enhance immune function in older adults through this pathway.

Taken together, it appears that the detriments in the immune system that are oftentimes attributed to aging are in fact more related to physical inactivity. Indeed, a variety of studies have shown that older adults who are physically active demonstrate immune characteristics that are similar to younger counterparts [5]. Therefore, exercise has the potential to serve as a cost-effective tool in combating the immune detriments that are traditionally associated with age.

Effects of Exercise on the Stress Response

One way in which exercise may offset the negative immunological changes often associated with aging is via the body's ability to cope with psychological stress. Indeed, Puterman and colleagues (2010) reported an inverse relationship between perceived psychological stress and telomere length such that higher stress was associated with shorter telomeres [15]. Interestingly, however, physical activity (42 minutes of vigorous activity over 3 days) was able to protect against the detrimental effects of stress on telomere length, as it served as a significant moderator on the relationship even after controlling for age, BMI, and education [15]. However, human studies examining the effects of exercise on immune parameters of those under chronic stress are severely lacking.

When examining the effect of exercise training on an acute stress response, it has been reported that training is effective in reducing the cardiovascular reactivity as measured by mean arterial pressure and relative heart rate to the acute stressor [74]. This is consistent with the cross-stressor adaptation hypothesis, which postulates that repeated exercise training habituates the body to handle acute stressors (i.e. the stress of exercise) such that when the nature of the stressor changes (i.e. from physical exercise to a psychological stressor), the body is able to cope with it in a way that minimizes the disturbances to homeostasis [75]. Indeed, Rimmele et al (2007) exposed exercise-trained and exercise-untrained subjects to a standardized laboratory psychological stressor and found that trained subjects demonstrated a lower salivary cortisol and heart rate response to the stressor when compared with their untrained counterparts [14]. The trained subjects also demonstrated higher calmness and better mood than the untrained counterparts [14]. Thus, it is suggested that regular exercise training may improve the body's ability to cope with acute psychological stressors.

There is also evidence to support the potential role of exercise as an intervention strategy to improve chronic stress conditions. Highlighting the paradoxical role of exercise as both a stressor and a stress reliever, it is suggested that repeated exposure to acute exercise via habitual exercise training elicits hormonal perturbations from which the body learns to recover more efficiently and enables the body to better cope with situations of regularly-occurring psychological stressors [13]. Experimental evidence to support this theory, however, is lacking. Nevertheless, the ability of exercise training to improve anxiety is widely reported

[76, 77], and thus the mechanisms underpinning these effects may be related to the ability of exercise to modulate the body's stress response.

Nevertheless, the existing evidence supports the notion that regular exercise may increase the glucocorticoid receptor sensitivity such that less hormone is needed in order to achieve the desired results [13]. As such, exercise could contribute to the aforementioned glucocorticoid resistance model by "undoing" the effects of chronic stress on the sensitivity of glucocorticoid receptors on immune cells. In other words, although chronic stress may reduce glucocorticoid receptor sensitivity on immune cells and thus impair the anti-inflammatory effects of glucocorticoid secretion, it is possible that habitual exercise can "re-sensitize" the immune system to responding to glucocorticoids, and therefore serve as a potential mechanism through which exercise can counter the deleterious effects of stress on immunity.

Gaps in the Current Literature

Resistance Exercise and Immunity

The majority of the literature focusing on exercise and the immune response is derived from studies examining aerobic, cardiorespiratory-type exercise, with much less focus on resistance training [16]. Thus far, the consensus among the exercise immunology community for the effects of resistance training on the acute immune response state that, similar to aerobic training, exercise dose determines the magnitude of the immune response, with higher exercise doses eliciting the greatest immune perturbations, with either delayed or non-existent immunological changes observed with low-dose resistance exercise [16]. Furthermore, the

similarities and differences between the immune response to aerobic compared to resistance training are not well characterized and, to our knowledge, have not been conducted in a crossover fashion within a single experimental design. The present study aims to address this by comparing the immune response of subjects under both cardiorespiratory and resistance training conditions using a crossover design.

Training Status and Immunity

The effects of training status on the immune response and function are also not well understood. Preliminary research comparing a limited number of immune parameters in trained compared to untrained individuals exists, but needs to be replicated and elaborated upon as well as examined in longitudinal exercise interventions. The present study will compare the immune responses of trained and untrained individuals to acute exercise. Thus, the present study will aim to replicate some of the existing research findings [3, 8], while also examining additional aspects of immunity and immune functioning.

Age, Exercise, and Immunity

The effect of exercise on immunity in older adults is a growing body of research. There is evidence to support that older adults who are regularly physically active exhibit both lower proportions of non-classical monocytes as well as higher proportions of naïve T-cells/ lower proportions of highly-differentiated T-cells compared to their physically inactive counterparts [3, 8, 78], but this needs to be replicated in future research in order to become well-established. It also remains to be determined whether there are certain immune characteristics that are indicative of accelerated aging and whether they can be modified via lifestyle choices. The

body of evidence examining the immune response to exercise among older adults is still relatively small, and holds much potential for uncovering exercise as a simple, cost-effective intervention that may have profound health effects for an aging population.

Summary

Exercise has been proposed as an attractive, cost-effective intervention to prevent or reverse a variety of age-associated diseases, including cardiovascular disease, hypertension, cancer, arthritis, type 2 diabetes, osteoporosis, and certain cancers [2, 5]. The potential for exercise to have a positive effect on health and disease prevention in the aged in largely due to its effects on immunity and inflammation [2]. Indeed, exercise has been shown to profoundly affect the immune system and has been proposed as a means of offsetting age-associated immune declines [5]. Nevertheless, much remains to be determined about the effects of exercise modality, training status, and age on the immune response to exercise. Such knowledge can be used as a means of informing exercise prescription guidelines for aging individuals, as well as adding to the body of literature that aims to compare and to characterize the immune response across various factors (i.e. modality, or training status). The present study aims to address these criteria using a crossover design, which will serve as a novel approach towards shedding light on many of these nuances.

CHAPTER THREE

Manuscript 1

Title: T-cell responses to acute cardiorespiratory and resistance exercise in cohorts of physically active or physically inactive older adults

Abstract

Aging is associated with many chronic diseases that are maintained and perpetuated by immune dysregulation and chronic systemic inflammation. T-cells often undergo age-related changes, including an accumulation of memory cells, which places individuals at increased risk for infection and may predispose them to increased inflammation. Regular exercise training has been suggested to offset age-related changes in T-cells, but the mechanisms behind this effect are not fully understood. Furthermore, the majority of literature is derived from cardiorespiratory exercise (CRE) studies, with much less understood about the T-cell response to resistance exercise (RE). The purpose of this study was to examine the effects of acute CRE and acute RE on the T-cell response among a cohort of physically active older adults (OPA) compared to a cohort of physically inactive older adults (OPI). **METHODS**: Twenty-four healthy older adults (OPA n=12; OPI n=12; mean ± SD; age (yrs) OPA 62 ± 5, OPI 64 ± 5; height (cm) OPA 170.9 ± 6.9, OPI 162.9 ± 8.0; weight (kg) OPA 69.3 ± 10.2, OPI 68.2 ± 12.8; BMI (kg/m^2) OPA 23.9 ± 3.0, OPI 25.6 ± 3.5) completed one bout of CRE and one bout of RE in a randomized order, both at a moderate intensity, and separated by at least 7 days. Blood samples were taken at pre-exercise, post-exercise, and 1h post-exercise (recovery) and analyzed for CD4+ and CD8+ T-cells and their differentiation status using surface markers CD45RA, CD62L, and CD57, as well as for Th17 cells (CD4+ CD161+ CD196+) using flow cytometry. **RESULTS**: OPI had higher numbers of circulating CD57+ EMRA CD4+ T-cells (OPA, mean ± SE, 1 ± 2 cells/uL; OPI, 6 ± 2 cells/uL; p=0.01; z=2.32) than OPA at pre-exercise. Both CRE and RE elicited a significant

mobilization of highly-differentiated (CD45RA+ CD62L-; CD57+ CD45RA+ CD62L-) CD8+ T-cells into the circulation post-exercise in both OPA and OPI groups. Furthermore, CRE resulted in a decrease in the number of circulating Th17 cells post-exercise, while RE increased Th17 cell mobilization compared to the CRE response. **CONCLUSION**: Taken together, T-cells in OPA and OPI respond similarly to acute exercise and support previously reported data showing a significant mobilization of highly differentiated T-cells. The present study confirms that moderate intensity RE also elicits this response, but highlights potential differences between CRE and RE on the immune responses of T-cells, particularly in OPI individuals.

Introduction

Aging is associated with a host of chronic diseases, such as cardiovascular disease, hypertension, cancer, osteoarthritis, type 2 diabetes, and osteoporosis [1]. Age-associated Immune system dysregulation (i.e. immunosenescence) and chronic systemic inflammation serve as common underlying conditions that contribute to the development and the perpetuation of these diseases and are often exacerbated by physical inactivity [2].

Within the lymphocyte population, T-cells serve as one of the lymphocyte subtypes that undergo age-related change. Indeed, aging has been linked to a reduction in the naïve T-cell repertoire, which is often characterized by an accumulation of memory T-cells and thereby an elevated proportion of highly-differentiated T-cells [23]. Such a situation reduces the diversity of the T-cell pool and places individuals at greater risk for impaired immunity [5]. Furthermore, highly-differentiated T-cells preferentially secrete pro-inflammatory cytokines such as TNF and IFN-γ, which may also contribute to chronic inflammation and poorer immunity [23].

Physical exercise has been shown to alter various lymphocyte subpopulations, particularly those with phenotypes associated with higher effector functions, migration potential, and inflammatory properties such as NK cells, CD8+ T-cells, and gamma-delta T-cells [79, 80]. Within the CD8+ T-cell population, those with a highly-differentiated phenotype are mobilized into the circulation at preferentially higher proportions than their lower-differentiated counterparts, due largely to increases in hemodynamic shear stress and catecholamine secretion that

accompany acute exercise [41]. For example, CD8+ T-cells can be classified by differentiation status according to their expression of surface receptors CD45RA and CD62L as naïve (CD45RA+CD62L+), central memory (CM; CD45RA-CD62L+), effector memory (EM; CD45RA-CD62L-), and effector memory RA (EMRA; CD45RA+CD62L-). In response to acute exercise, the proportions of EMRA CD8+ T-cells exhibited the largest increase, followed by EM, CM, and naïve CD8+ T-cells, respectively without any significant exercise induced changes among naïve, CM, EM, or EMRA CD4+ T-cells [41].

The effects of chronic exercise on lymphocyte and lymphocyte subpopulations are less understood. Cross-sectional reports indicate that fitness status is a better predictor of senescent T-cell proportions than age [3], which is supported by a longitudinal study that demonstrated that 12 weeks of exercise training increased the number of T-cells that express the cell surface marker CD28 (a marker of low-differentiation status) [7]. Nevertheless, more research is needed in order to determine whether engaging in regular physical activity can reduce or prevent the accumulation of highly-differentiated T-cells and allow for the production or maintenance of the naïve and low-differentiated T-cell pools.

Furthermore, the majority of the literature to date on the effects of exercise on T-cells and T-cell subpopulations is derived from cardiovascular exercise studies, with little known about the effects of both acute and chronic resistance training [16]. It is believed that resistance training exerts similar effects to cardiovascular training on immune parameters [16], but to our knowledge, cardiovascular and resistance conditions have not been directly compared in a crossover study design.

Results from longitudinal studies indicate that chronic exercise training does not exert substantial effects on the number of circulating leukocytes. Nevertheless, in response to acute exercise at any given intensity, the absolute number cells mobilized into the circulation is often lower among trained individuals compared to untrained individuals. The smaller absolute response of leukocytes in trained individuals is likely a result of differences in β2-adrenergic receptor sensitivity, glucocorticoid receptor sensitivity, or cell adhesion molecules, which may be affected by regular exercise [55].

Therefore, the purpose of the present study was to examine the effects of acute cardiorespiratory and acute resistance exercise on the T-cell response among a cohort of physically active older adults (OPA) compared to a cohort of physically inactive older adults (OPI). We hypothesized that both cardiorespiratory and resistance exercise bouts will elicit a T-cell mobilization along with a preferential mobilization of highly-differentiated CD8+ T-cells, but that the magnitude of this mobilization would be higher following cardiorespiratory exercise. Furthermore, we hypothesized that the magnitude of T-cell and T-cell subpopulation mobilization would be higher among physically inactive older adults compared to physically active older adults. We report here that older adults who are physically active have lower numbers of circulating senescent CD4+ T-cells compared to age-matched physically inactive counterparts, and that moderate-intensity cardiorespiratory exercise (CRE) and resistance exercise (RE) elicit similar T-cell and T-cell subset responses in physically active and physical inactive older adults.

Methods

Subjects

Two groups of 12 older adults (55-75 years old) who were either physically active (OPA) or physical inactive (OPI) were recruited for participation (Figure 1.1). Physically active and physically inactive were delineated using the guidelines set forth by the American College of Sports Medicine. Briefly, in order to be considered physically active, participants had to be exercise trained, as defined by participating in at least 30 minutes of moderate to vigorous intensity cardiovascular activity for at least 3 days per week and engaging in resistance training for all of the major muscle groups approximately 2 days / week for the past 3 months. Participants classified as physically inactive were exercise untrained, which was defined as participating in less than 30 minutes of moderate to vigorous intensity cardiovascular activity twice per week and no regular resistance training for at least the last 3 months [81].

Recruits were excluded if: they had any contraindications to moderate-vigorous exercise; if they have had any recent illness or have been instructed not to exercise by their healthcare provider; if they had range of motion restrictions that would prevent them from participating in aerobic or resistance training with proper form (they must be ambulatory); if they were taking medications (prescription or over the counter) known to influence immune function (including daily NSAID's and beta blockers), cholesterol-lowering medications (statins), drugs that increase bone mass (bisphosphonates), or steroids; if they had known cardiovascular, respiratory, metabolic, or renal disease, with the exception of controlled hypertension (as defined

by resting BP below 140/90) and/or controlled asthma (self-reported); if they fell outside of a BMI range of (18.5 – 30 kg•m^{-2}); if they consumed alcohol or recreational drugs within 24h prior to visits; if women were pre-menopausal (must not have had a menses for at least 12 months); and if they had scheduling conflicts that would prevent them from reporting to the laboratory of integrated physiology 4 times over the course of the study.

All participants provided a signed written statement of informed consent prior to participation in the study. All study procedures were approved by the Institutional Review Board at the University of Houston.

Participants were assigned to OPA or OPI groups based on the results of the Exercise Frequency Questionnaire, where participants were required to detail the types of weekly activity that they participate in and the length of time that each activity is performed. To confirm activity status, participants were given an ActiGraph accelerometer (wGT3X-BT, ActiGraph ® Corp, Pensacola, FL, USA) along with a set of written instructions, and asked to wear it for seven full days. Participant characteristics are presented in Table 1.1.

Experimental Design

The present study was a randomized, complete cross-over design with three time points (pre-exercise, post-exercise, and recovery), two activity levels (OPA and OPI), and two exercise modalities (cardiorespiratory exercise (CRE), and resistance exercise (RE)). Participants reported to the Laboratory of Integrated Physiology 3 times over the course of the study following an overnight fast.

Visit 1: During visit 1, participants underwent a health screening, during which they filled out a PAR-Q+ and a medical history questionnaire, provided a written list of all medications, and underwent heart rate and blood pressure screening to ensure that they met the inclusion / exclusion criteria. Following this, height and weight were also measured to ensure that participants fell within the BMI range of 18.5-30 $kg \bullet m^{-2}$. All eligible participants were then classified as falling into OPA or OPI, and underwent a Dual Energy X-Ray Absorptiometry (DEXA; Hologic Discovery A DXA, Marlborough, MA, USA) scan to assess body composition and a heart rate variability (HRV) assessment using a heart rate monitor (Polar V800, Polar Electro, Bethpage, NY, USA). For the HRV assessment, participants were fitted with a heart rate monitor and asked to lay in the supine position. After the first 5 minutes, heart rate was recorded for an additional 10 minutes for heart rate variability analyses. During this time, participants were asked to minimize talking and movement. The lowest HR obtained during this assessment was recorded and used to calculate aerobic exercise intensity for subsequent laboratory visits using the Karvonen formula [82], with maximum heart rate (MHR) calculated using the formula MHR = 220 - age. Following these assessments, participants were offered a small snack and proceeded with exercise testing to measure muscular strength.

8-RM Testing: Following a warm up on a motorized treadmill, participants went to the weight room where they were familiarized with 8 different machine-based exercises (leg press, chest press, leg curl, lateral pull down, weighted calf raise, triceps extension, leg extension, and seated row) using stacked weight machines. All equipment was adjusted for each participant by a trained technician, who also

instructed each participant on proper form and technique throughout each exercise. All participants performed each exercise with minimal weight until they were comfortable with the movement and technique. Following this, an 8 repetition maximum (8-RM) was measured on each piece of equipment for each participant based on the protocols in the American College of Sports Medicine (ACSM) guidelines 10th edition [81]. Briefly, each participant performed one set of several repetitions of each exercise with a light load, after which the resistance was gradually increased until an 8-10 RM is achieved. The amount of load increase will depend upon each participants' self-perceived capacity. Two to five minutes of rest were implemented in between subsequent attempts until an 8-RM was achieved. The Epley formula [83] was used to estimate 1-RM from the 8-RM, and the workload for the RT visit was calculated by taking 70% of predicted 1-RM for each exercise for each participant.

Following Visit 1, each participant was randomized stratified for group and sex to complete either CRE first and RE second, or RE first and CRE second. *Visit 2:* The second laboratory visit consisted of either CRE or RE. Whichever was not performed during visit 2, was performed during visit 3. When participants arrived for the CRE session, study personnel verified that they were in a fasted state (at least 8h), that they did not consume alcohol or exercise within the last 24 hours, and that they had not taken any additional medications to the ones they had previously reported.

Participants were then fitted with a heart rate monitor and asked to rest in the seated position for 10 minutes, after which time resting heart rate and blood

pressure were recorded and a resting blood sample was taken by a trained technician from a vein in the antecubital space into sodium heparin-containing (green-top), and silica for serum determination-containing (red-top) blood collection tubes for blood and serum analyses, respectively. The participant then began the cardiorespiratory fitness exercise session, which consisted of a 5-minute warm-up of self-paced slow walking on the treadmill, followed by an increase in the speed and/or grade of the treadmill such that each participant's heart rate was within 60-70% of heart rate reserve, which the participant maintained for an addition 30 minutes.

After the 30 minutes of moderate-vigorous intensity aerobic exercise, participants were quickly seated and an immediately post-exercise blood sample was drawn from a vein in the antecubital space. Participants were then asked to get back on the treadmill to perform a cool down at a slow, self-selected pace for an additional 2 minutes. Participants were then instructed to rest for an additional 60 minutes following exercise completion, during which time HRV was measured once again using the aforementioned methods, and at 60 minutes post-exercise a third and final blood sample was taken.

Visit 3: Visit 3 consisted of procedures identical to that of Visit 2, with the only difference being the type of exercise performed. When participants arrived for the RT session, all procedures were exactly the same through the completion of a 5-minute cardiovascular warm-up on a motorized treadmill at a self-selected pace. Following the warm-up, participants completed 3 sets of 8-12 repetitions at 70% of 1-RM with 30-120 seconds of rest in between subsequent sets and 1-2 minutes of rest between subsequent exercises. All participants completed each exercise in the

same order: leg press, chest press, leg curl, lateral pull down, weighted calf raise, triceps extension, leg extension, and seated row. All sets on each exercise were completed before the participant moved onto the next exercise. Following the completion of the final set of the last exercise, an immediately post-exercise blood sample was taken and participants were then asked to get back on the treadmill to perform a cool down at a slow, self-selected pace for an additional 2 minutes. Just as in Visit 2, participants were then instructed to rest for an additional 60 minutes following exercise completion, during which time HRV was measured once again using the aforementioned methods, and at 60 minutes post-exercise a third and final blood sample was taken.

Flow Cytometry

Multi-color flow cytometry was used to identify lymphocytes and lymphocyte subsets in whole blood. All monoclonal antibodies were purchased from Miltenyi Biotech (Bergisch Gladbach, Germany) unless otherwise stated, and the MACSQuant ® Analyzer 10 (Miltenyi Biotech, Gladback, Germany) flow cytometer was used to run each sample. Lymphocyte count was determined using 50 ul whole blood labeled with VioBlue-conjugated anti CD45 mAb and incubated for 30 minutes. Red blood cell lysis buffer was added in a 1:11 ratio and samples incubated for another 20 minutes. One minute prior to running each sample of the flow cytometer, PI was added to each sample in order to identify any dead cells. Lymphocyte count was determined using forward and side-scatter plots, which were gated electronically. Cells within the lymphocyte gate that were CD45+ and negative for PI

were counted and multiplied by the dilution factor of 11 to obtain the number of lymphocytes per ul of blood.

T-cells and T-cell subsets were identified using 100 ul whole blood labeled with VioBlue-conjugated anti-CD45RA, VioGreen-conjugated anti-CD3 mAb, FITC-conjugated anti-CD4 mAb, PE-conjugated anti-CD196 mAb, PerCP-conjugated anti-CD8 mAb, PE-Vio770-conjugated anti-CD62L mAb, APC-conjugated anti-CD161 mAb, and APC-Vio770-conjugated anti-CD57 mAb. Following a 30 minute incubation, samples were incubated with red blood cell lysing buffer for 20 minutes, washed, and then run on the flow cytometer. T-cells were identified as those within the lymphocyte gate that expressed the CD3 surface antigen. The CD4+ T-cells (CD3+CD4+) and CD8+ T-cells (CD3+CD8+) were gated within the CD3+ lymphocytes and differentiation status was assessed based on their expression of CD45RA/CD62L and CD57. Th17 cells were identified as those CD4+T-cells that were also CD196+ and CD161+. The total number of T-cell and T-cell subtypes were identified by multiplying the percentage of the cells of interest by the total lymphocyte count.

Statistical Analysis

Independent samples t-tests were used to determine differences between OPA and OPI subject characteristics and fitness measurements.

To assess baseline differences in immune parameters (differences between OPA and OPI at pre-exercise), a linear mixed effects model was fit, modeling *condition* (CRE or RE) and *group* (OPA and OPI) and their

interaction. Subject was included as a random intercept term. Specific comparisons were tested using contrasts.

To determine whether the condition (CRE or RE) influenced the mobilization of T-cells and T-cell subsets as well as to determine whether physical activity status influenced these results, a linear mixed effects model was fit on the change from pre-exercise values with factors *group* (OPA and OPI), *condition* (CRE and RE), and *time* (pre-exercise, post-exercise, and recovery), along with their interactions. Pre-exercise values served as a covariate, and the intercept was tested in order to determine the differences from the pre-exercise time point. Contrasts were used to assess the difference between each group and condition of exercise. Data was transformed using a Box-Cox transformation prior to running in order to improve model fit (i.e. normality and equality of variances). Statistical significance was determined *a priori* at $p<0.05$. All statistical analyses were run using R version 3.6.2 and R Markdown to generate data analysis reports.

Results

Baseline Differences between OPI and OPA

All participants successfully completed both CRE and RE exercise trials. Participant physical characteristics are displayed in Table 1.1, and exercise data is displayed in Table 1.2.

OPA group has lower body fat % and RHR, and higher MVPA and muscular strength compared to OPI

The OPA group had lower relative body fat ($p<0.01$) and resting heart rate (RHR; $p=0.01$), and were taller in height ($p=0.03$) compared to OPI. The results from the ActiGraph ® confirmed that OPA spent significantly more time per day on average engaging in moderate to vigorous physical activity (MVPA) compared to OPI ($p=0.02$). Furthermore, 1RM's for OPA were greater than OPI for leg press ($p=0.02$), chest press ($p=0.01$), leg curl ($p<0.01$), lat pull down ($p= 0.01$), weighted calf raise ($p<0.01$), triceps extension ($p<0.01$), and seated row ($p=0.01$). No group differences were observed between HR at 60% or 70% HRR, leg extension 1RM, or average exercise HR during the CRE trial.

CD57+ EMRA CD4+T-cells are the only T-cells with consistent pre-exercise differences between OPA and OPI groups

Main effects for group at pre-exercise were found for total lymphocytes ($p=0.03$, $z=1.28$), CD57+ EMRA CD4+ T-cells ($p=0.01$, $z=2.32$), and Naïve CD8+ T-cells ($p=0.04$, $z=1.91$). However, the only cell type where pre-exercise values were significantly higher in OPI compared to OPA during both CRE ($p<0.01$; $z=2.54$) and RE ($p=0.02$; $z=1.47$) trials was CD57+ EMRA CD4+ T-cells.

Differences between CRE and RE

Exercise mobilized all T-cell subpopulations into the circulation immediately post-exercise

Regardless of CRE or RE exercise, a significant mobilization of all measured subpopulations (total lymphocytes (OPA: $p<0.01$, $z=7.36$; OPI: $p<0.01$, $z=6.87$), T-cells (OPA: $p<0.01$, $z=5.25$; OPI: $p<0.01$, $z=5.63$), CD4+ T-cells (OPA: $p<0.01$,

z=4.07 ; OPI: p<0.01 , z=5.84), CD8+ T-cells OPA: p<0.01, z=5.37 ; OPI: p<0.01, z=4.96), naïve CD4+ T-cells (OPA: p=0.04, z=2.09; OPI: p<0.01, z=4.42), naïve CD8+ T-cells (OPA: p=0.02, z=2.38; OPI: p<0.01, z=2.97), CM CD4+ T-cells (OPA: p<0.01, z=3.19; OPI: p<0.01, z=5.19), CM CD8+ T-cells (OPA: p<0.01, z=3.23; OPI: p<0.01, z=3.68), EM CD4+ T-cells (OPA: p<0.01, z=4.44; OPI: p<0.01, z=5.27), EM CD8+ T-cells (OPA: p<0.01, z=3.96; OPI: p<0.01, z=3.90), EMRA CD4+ T-cells (OPA: p<0.01, z=4.00; OPI: p<0.01, z=6.84), EMRA CD8+ T-cells (OPA: p<0.01, z=6.28; OPI: p<0.01, z=5.90), CD57+ EMRA CD4+ T-cells (OPA: p=0.04, z=2.16; OPI: p<0.01, z=3.79), CD57+ EMRA CD8+ T-cells (OPA: p<0.01, z=7.02; OPI: p<0.01, z=5.86), and Th17 cells (OPA: p<0.01, z=3.62; OPI: p<0.01, z=3.98) occurred from pre- to post-exercise within both OPA and OPI groups.

Highly differentiated CD8+ T-cells are mobilized with both CRE and RE exercise in both OPA and OPI groups

EMRA CD8+ T-cells (OPA CRE p<0.01, z=-4.82, OPA RE p<0.01, z=-4.68; OPI CRE p<0.01, z=-3.01, OPI RE p<0.01, z=-4.72) and CD57+ EMRA CD8+ T-cells (OPA CRE p<0.01, z=5.75, OPA RE p<0.01, z=4.88; OPI CRE p<0.01, z=3.71, OPI RE p<0.01, z=5.29) were significantly mobilized from pre-exercise to post-exercise for both CRE and RE trials in both OPA and OPI groups (Figure 1.2). There were no differences between groups or types of exercise.

Group Differences in T-cell Responses

RE mobilized more T-cell subpopulations than CRE in OPI group only

Within OPI, all T-cells and T-cell subpopulations significantly increased in number from pre-exercise to post-exercise with RE only (total lymphocytes: $p<0.01$, $z=7.16$; T-cells: $p<0.01$, $z=5.24$; CD4+ T-cells: $p<0.01$, $z=5.12$; CD8+ T-cells: $p<0.01$, $z=3.89$; naïve CD4+ T-cells: $p<0.01$, $z=3.82$; naïve CD8+ T-cells: $p<0.01$, $z=3.26$; CM CD4+ T-cells: $p<0.01$, $z=4.60$; CM CD8+ T-cells: $p<0.01$, $z=3.34$; EM CD4+ T-cells: $p<0.01$, $z=5.51$; EM CD8+ T-cells: $p<0.01$, $z=3.09$; EMRA CD4+ T-cells: $p<0.01$, $z=5.30$; EMRA CD8+ T-cells: $p<0.01$, $z=-4.72$; CD57+ EMRA CD4+ T-cells: $p<0.01$, $z=4.25$; CD57+ EMRA CD8+ T-cells: $p<0.01$, $z=5.28$; Th17 cells: $p<0.01$, $z=3.06$) . Conversely CRE significantly mobilized total lymphocytes ($p<0.01$, $z=6.65$), EMRA CD4+ T-cells ($p<0.01$, $z=5.06$), CD8+ T-cells ($p=0.04$, $z=2.04$), EMRA CD8+ T-cells ($p=0.02$, $z=-3.01$), and CD57+ EMRA CD8+ T-cells ($p<0.01$, $z=3.71$) within OPI.

Within OPA, RE significantly mobilized total lymphocytes ($p<0.01$, $z=5.85$), T-cells ($p=0.01$, $z=2.75$), EM CD4+ T-cells ($p<0.01$, $z=2.90$), CD8+ T-cells ($p<0.01$, $z=3.37$), EM CD8+ T-cells ($p=0.02$, $z=2.33$), EMRA CD8+ T-cells ($p<0.01$, $z=-4.63$), and CD57+ EMRA CD8+ T-cells ($p<0.01$, $z=4.88$). CRE significantly mobilized total lymphocytes ($p<0.01$, $z=7.15$), T-cells ($p= 0.04$, $z=2.09$), EM CD4+ T-cells ($p=0.04$, $z=2.08$), EMRA CD4+ T-cells ($p<0.01$, $z=3.61$), CD57+ EMRA CD4+ T-cells ($p=0.02$, $z=2.37$), CD8+ T-cells ($p<0.01$, $z=2.82$), EMRA CD8+ T-cells ($p<0.01$, $z=-4.82$), and CD57+ EMRA CD8+ T-cells ($p<0.01$, $z=5.77$).

Some T-cell subtypes remain elevated at recovery time point in OPI RE Only

Within the OPI group only, EM CD4+ T-cells ($p<0.01$, $z=2.82$) and CM CD8+ T-cells ($p=0.02$, $z=2.92$) remained significantly elevated above pre-exercise values at the recovery time point. Recovery was not higher than immediately post-exercise. All T-cells and T-cell subtypes returned to pre-exercise counts at the recovery time point within the OPI group with CRE exercise.

Conversely, in the OPA participants, RE caused a decrease from pre-exercise during the recovery time point for total lymphocytes ($p<0.01$, $z=-3.25$) and EMRA CD4+ T-cells ($p=0.03$, $z=-2.15$), while CRE decreased recovery total lymphocytes ($p<0.01$, $z=-3.70$), T-cells ($p=0.04$, $z=-2.12$), CD4+ T-cells ($p=0.03$, $z=-2.22$), and naïve CD4+ T-cells ($p<0.01$, $z=-2.88$) below pre-exercise.

Significant differences between CRE and RE exist within OPI group only, with the exception of Th17 cells

Within the OPI group only, the pre-exercise to post-exercise mobilization of cells was significantly greater for RE compared to CRE for T-cells ($p=0.03$, $z=2.16$), CD4+ T-cells ($p=0.01$, $z=2.79$), naïve CD4+ T-cells ($p=0.04$, $z=2.10$), CM CD4+ T-cells ($p=0.02$, $z=2.40$), EM CD4+ T-cells ($p<0.01$, $z=2.90$), CD57+ EMRA CD4+ T-cells ($p=0.04$, $z=2.02$), and CM CD8+ T-cells ($p=0.04$, $z=1.99$). Furthermore, at the recovery time point, CD57+ EMRA CD8+ T-cells were significantly higher after RE compared to CRE ($p=0.03$, $z=2.21$). There were no differences between CRE and RE within the OPA group for any cell type or at any time point except for Th17 cells.

Th17 cells differed significantly between CRE and RE at post-exercise within both OPA ($p=0.04$, $z=2.07$) and OPI groups ($p=0.01$, $z=2.58$). In both groups, RE

appeared to mobilize Th17 cells within the circulation, whereas CRE did not, regardless of physical activity status (Figure 1.3).

Discussion

This is the first study to compare the effects of acute moderate-intensity cardiorespiratory exercise (CRE) and resistance exercise (RE) on T-cell responses of older adults in a crossover study design. We did this by recruiting older physically active (OPA) and older physically inactive (OPI) adults and having all participants complete an acute bout of moderate-intensity cardiorespiratory exercise, and on a separate occasion an acute bout of resistance training in a randomized order while taking blood samples prior to exercise (pre), immediately following exercise cessation (post), and 1h following exercise cessation (recovery). We showed that older adults who are physically active have lower numbers of circulating senescent CD4+ T-cells compared to age-matched physically inactive counterparts, that moderate-intensity CRE and RE both facilitate a significant mobilization of highly-differentiated CD8+ T-cells into the circulation regardless of physical activity status, that RE mobilized more T-cell subpopulations than CRE within OPI only, and that Th17 cells responded differently to CRE compared to RE regardless of physical activity status.

Aging has been associated with immunosenescence, a canopy term used to describe the age-related decline in immune competency. More recently, however, physical activity has emerged as a potentially more potent mediator of the relationship between aging and immune decline [2]. This notion is supported by several cross-sectional and longitudinal studies that have reported marked

differences in immune characteristics of those that engage in regular physical activity compared to those who do not, as well as changes in immune properties following a period of regular exercise training in previously sedentary individuals [3, 5, 7, 79]. One of the characteristics of age-related immune decline is an accumulation of highly-differentiated T-cells accompanied by a reduction in the naïve T-cell pool.

Results from the present study partially support these findings, as highly differentiated CD4+ T-cells (CD57+ EMRA CD4+ T-cells) were found to be higher in OPI compared to OPA at rest (pre-exercise). Nevertheless, the present study did not find consistent significant differences between the groups for naïve T-cells within either the CD4+ nor the CD8+ subpopulations. This largely reflects what is found in the literature, as there is abundant evidence to suggest that physical activity can improve the decline in several parameters of immune competency that often accompany aging, however more research is necessary in order to delineate the precise frequency, intensity, modality, and duration of activity necessary to facilitate these improvements [79]. This is illustrated in a study where older adults (ages 65-85) were categorized as never trained (NT), leading a moderate training lifestyle (MT), and leading an intense training lifestyle (IT). Significant differences between the number of senescent T-cells (using cell surface markers CD45RA and CCR7) were found only between the NT and IT groups [84]. Furthermore, when using cell surface marker CD28 as a marker of T-cell senescence, no differences were observed between any of the groups [84]. These results suggest both that there may be a dose-type response in which regular physical activity exerts anti-aging

immunological changes and also that the benefits of regular exercise on immunity may not be readily apparent among all ways of characterizing markers of immunosenescence.

Indeed, the majority of exercise and vaccination studies conducted in human subjects utilize biomarkers such as antibody titers as a barometer for enhanced immunity following vaccination [79]. As such, the effectiveness of exercise programming in improving vaccine efficacy is often judged based on the quantification of antibody titers. However, evidence using murine models has demonstrated that chronic exercise in the months prior to, or acute exercise in the hours and days following, exposure to influenza elicited improvements in viral clearance, infection severity, inflammation, and survival [85, 86]. Furthermore, an additional murine model showed that despite lower antibody responses in mice who exercised prior to influenza exposure compared to those who did not exercise, the exercising mice were not at any greater risk of secondary infection [87], indicating that while some quantifiable characteristics may be different between exercising and non-exercising groups, their ability to optimize health and immunity may not be accurately reflected. Hence, this highlights the necessity for future research to examine the functionality of T-cells among older adults of various physical activity statuses, as changes in numbers of discrete T-cell populations at rest may not be solely indicative of changes in immune functioning and competency.

The present study also confirms previously reported data showing that exercise significantly mobilizes highly-differentiated CD8+ T-cells, which are known to have heightened effector functions compared to their lower-differentiated and

CD4+ T-cell counterparts [33, 41, 42]. Adding to this, however, the present study shows that the significant mobilization of highly-differentiated CD8+ T-cells can occur with moderate intensity CRE and RE in a population of older adults who are both physically active and physically inactive.

There are several potential benefits associated with the significant mobilization of highly-differentiated CD8+ T-cells, along with other leukocytes with increased effector functions, inflammatory properties, and cell adhesion markers (i.e. NK cells, gamma delta T-cells, and non-classical monocytes). In an acute setting, the exercise-induced mobilization and rapid egress of these cells is thought to heighten the body's ability to fight infection [79]. In other words, there is an emerging theory that suggests that exercise serves as the initial stimulus to recruit these immune cells with specific properties into the circulation, and then rapidly redistribute them to mucosal surfaces where they will facilitate heightened immune surveillance, thereby enhancing the body's ability to launch an immune response against an invading antigen [79]. This is in contrast to the widely discussed "open-window theory", which postulates that the egress of leukocytes within the hours and days following acute exercise serves as a period of immunosuppression during which the host is at an increased risk for opportunistic infection [88]. The evidence to support this theory, however, is lacking and the evidence supporting an elevated state of immunity is mounting [79]. Thus, regular exercise may serve to heighten immunity by allowing the individual to exist within the state of heightened immunity more frequently than an individual who does not exercise.

Regular exercise and therefore frequent mobilization and subsequent egress of highly-differentiated CD8+ T-cells may provide additional benefits to the overall properties of the immune system. A hallmark of immunosenescence is an accumulation of highly-differentiated T-cells accompanied by a reduction in the naïve T-cell pool, which theoretically places an individual at a heightened risk of infection due to a reduction in the ability of the immune system to respond to a novel antigen [17]. It has been proposed that the exercise-induced recruitment of highly-differentiated T-cells both facilitates an increased opportunity for apoptosis (i.e. the removal of excess memory T-cell clones) and simultaneously stimulates thymic output, resulting in the production of naïve T-cells [17]. Indeed, several studies have shown that apoptosis following acute exercise is increased [89], and also that although thymic involution begins earlier in life (i.e. around puberty), the thymus continues to produce new T-cells well into advanced aging [90] and exercise may serve as a means of stimulating an increase in thymic output [17]. In summary, the immunological disturbances to the T-cell pool induced by regular bouts of exercise may serve to improve immunity in older adults via several physiological pathways.

The present study also reported a differential response of Th17 cells, whereby circulating numbers of these cells were increased with RE and decreased with CRE. Th17 cells are a subset of T-helper (CD4+) cells that produce large amounts of IL-17, a pro-inflammatory cytokine that is implicated in allergic reactions and autoimmunity [91]. Concentrations of Th17 cells are also reported to increase with age [92], and may be mitigated with regular physical activity [93]. However, very little is known about the response of Th17 cells to acute exercise, especially in

humans. Limited data suggests that Th17 cells increase in response to ultra-endurance or exhaustive exercise events [94, 95], and that this response may be stimulated by changes in serum cytokine levels [94]. However, it must be noted that methodological differences in the ways of identifying Th17 cells make it difficult to compare these results to the present study. Nevertheless, this serves as the first study to report the exercise response of Th17 cells to moderate intensity CRE and RE in a cohort of older adults. Future research should aim to determine the mechanisms of mobilization of these cells and potential reasons why the response may be different among different training modalities. This is particularly intriguing considering most other immune cell subsets appear to respond similarly between CRE and RE.

While the immune response to CRE and RE in the present study appear to be similar, some marked differences were found within the OPI group in particular. All T-cells and T-cell subtypes measured were significantly elevated post-exercise following RE but not CRE. This is likely because RE served as a novel stimulus for those in the OPI group, so even though it was performed at a moderate intensity, it still served as a physiological stimulus that the body was unaccustomed to responding to, which resulted in a more robust reaction. Indeed, research comparing trained vs. untrained individuals has demonstrated that the absolute number of cells mobilized into the circulation following an acute bout of exercise is lower in those who are trained compared to untrained individuals [55]. As the CRE exercise consisted of treadmill walking, it makes sense that the immune response of the OPI group was closer to that of their OPA counterparts because even though regular,

structured physical activity was not a lifestyle habit employed by those in the OPI group, walking did not serve as a foreign stimulus to these individuals.

This idea is reinforced by the data from the recovery time point, whereby a few T-cell subsets (EM CD4+ T-cells, and CM CD8+ T-cells) remained significantly elevated above pre-exercise levels following RE in the OPI group only. This is in contrast to the OPA group, where all T-cell subtypes were either back at pre-exercise or below pre-exercise values at the recovery time point. Such differences suggest a reduction in the efficiency of the immune response between OPA and OPI, and may be due to differences in β2-adrenergic receptor sensitivity, glucocorticoid receptor sensitivity, or cell adhesion molecules [55].

Several methodological aspects of the present study must also be addressed. The inclusion / exclusion criteria for the study were quite rigorous, such that the OPI group consisted of a “healthy” group of older individuals, without many of the comorbidities that often accompany aging (i.e. diabetes, obesity, cardiovascular conditions, and those who were taking beta blockers or statins for high blood pressure or high cholesterol). This was done in an attempt to focus on the effects of regular physical activity on immune parameters in the absence of glaring confounders and serves as a major strength of the study. Furthermore, we used the ACSM guidelines for physical activity and participants’ self-reported activity in order to stratify between our physically active and inactive groups. This means that the gap between physically active and inactive was not tremendous (i.e. the active group did not consist of competitive athletes, while the inactive group was otherwise quite healthy and while they did not engage in regular, structured exercise, they did lead

ambulatory lifestyles), and thus more research is needed in order to determine whether there are further differences in immune characteristics and responses in those who engage in higher volumes and intensities of physical activity compared to those who are even more sedentary. Additionally, latent CMV infection was not controlled for in the present study and is known to affect the composition of the T-cell pool and T-cell responses to acute exercise. However, this was addressed statistically by using each participant's pre-exercise value as a covariate in the model. Lastly, the OPA group consisted of an equal number of male and female participants, whereas OPI consisted primarily of female participants, which may have influenced group differences [96].

In conclusion, this is the first study to compare moderate intensity CRE and RE in a cohort of physically active and inactive older adults of otherwise similar health status. Our findings indicate that CRE and RE have similar effects on T-cell subset mobilization, including a significant mobilization of highly-differentiated CD8+ T-cells. As the majority of existing literature on exercise and the immune response is derived from cardiorespiratory exercise studies, these findings provide insight as to the effects of resistance training on the immune response to exercise.

Table 1.1: Subject Characteristics. Older Physically Active (OPA; n=12; 6 males, 6 females). Older Physically Inactive (OPI; n=12; 1 male, 11 females).

	OPA (Mean ± SD)	**OPI** (Mean ± SD)
Age (yrs)	62 ± 5	64 ± 5
Height (cm)	170.9 ± 6.9	162.9 ± 8.0*
Weight (kg)	69.3 ± 10.2	68.2 ± 12.8
BMI (kg/m^2)	23.9 ± 3.0	25.6 ± 3.5
Body Fat (%)	26.6 ± 7.1	35.9 ± 6.3*
RHR (bpm)	51 ± 6	61 ± 10*
Resting Systolic BP (mmHg)	120 ± 13	127 ± 7
Resting Diastolic BP (mmHg)	82 ± 5	84 ± 4
Total MVPA (avg min/day)	101.0 ± 29.3	72.0 ± 27.1*

* denotes significant difference between OPI and OPA groups ($p<0.05$)
Body Mass Index (BMI); Resting Heart Rate (RHR); Blood Pressure (BP); Moderate – Vigorous Physical Activity (MVPA)

Table 1.2: Exercise Data from Older Physically Active (OPA; n=12) and Older Physically Inactive (OPI; n=12) groups. One Repetition Maximum (1RM) values were calculated using the Epley (1985) formula from 8 RM scores obtained during Visit 1.

	OPA (Mean ± SD)	**OPI** (Mean ± SD)
HR @ 60% HRR (bpm)	115 ± 4	118 ± 5
HR @ 70% HRR (bpm)	126 ± 4	128 ± 5
Leg Press 1RM (lbs)	199.9 ± 52.7	153.5 ± 39.0*
Chest Press 1 RM (lbs)	68.2 ± 33.3	36.2 ± 17.3*
Leg Curl 1 RM (lbs)	90.6 ± 28.5	59.1 ± 15.8*
Lat Pull Down 1 RM (lbs)	109.7 ± 31.6	78.5 ± 23.3*
Calf Raise 1 RM (lbs)	190.6 ± 50.3	133.5 ± 34.1*
Triceps Extension 1 RM (lbs)	38.8 ± 150.0	20.8 ± 14.1*
Leg Extension 1 RM (arbitrary units)	8.4 ± 2.8	8.0 ± 10.4
Seated Row 1 RM (lbs)	86.5 ± 32.0	56.0 ± 19.5*
Estimated VO2max (ml/kg/min)	45.3 ± 16.8	35.0 ± 8.1
Average CRE Exercise HR (bpm)	117 ± 11	118 ± 10
Average CRE Exercise RPE	13 ± 1	13 ± 1
RE Training Volume (sets x reps x weight)	15,971 ± 3893	10,609 ± 2832*

* denotes significant difference between OPI and OPA groups ($p<0.05$)
Heart Rate (HR); Rating of Perceived Exertion (RPE; using Borg Scale 6-20)

Table 1.3: The effects of an acute bout of cardiorespiratory (CRE) or resistance (RE) exercise on circulating numbers of lymphocytes and lymphocyte subsets in older physically active (OPA) and older physically inactive (OPI) participants. Blood samples were taken immediately prior to exercise (pre-exercise), immediately upon exercise cessation (post-exercise) and after 1h of rest following exercise cessation (recovery) during both CRE and RE trials.

		OPA		OPI	
		CRE (Mean ± SE)	**RE** (Mean ± SE)	**CRE** (Mean ± SE)	**RE** (Mean ± SE)
Lymphocytes (x10^3 cells/uL)					
	Pre-exercise	1.2 ± 0.2	1.3 ± 0.2	1.6 ± 0.2	1.4 ± 0.2
	Post-exercise	1.7 ± 0.2*	1.7 ± 0.2*	2.0 ± 0.2*	1.9 ± 0.2*
	Recovery	1.1 ± 0.2*	1.1 ± 0.2*	1.5 ± 0.2	1.4 ± 0.2
T-cells (x10^3 cells/uL) (CD3+ Lymphocytes)	Pre-exercise	0.7 ± 0.1	0.7 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
	Post-exercise	0.9 ± 0.1*	0.8 ± 0.1*	1.1 ± 0.1	1.1 ± 0.1*^
	Recovery	0.7 ± 0.1*	0.7 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
CD4+ T-cells (cells/uL) (CD3+ CD4+)	Pre-exercise	523 ± 87	447 ± 87	796 ± 87	587 ± 87
	Post-exercise	541 ± 87	505 ± 87	813 ± 87	783 ± 87*^
	Recovery	455 ± 87*	444 ± 87	729 ± 88	674 ± 89
CD8+ T-cells (cells/uL) (CD3+ CD8+)	Pre-exercise	180 ± 29	179 ± 29	233 ± 29	173 ± 29
	Post-exercise	238 ± 29*	239 ± 29*	261 ± 29*	245 ± 29*
	Recovery	160 ± 29	173 ± 29	219 ± 29	206 ± 30
Naïve CD4+ T-cells (cells/uL) (CD45RA+ CD62L+)	Pre-exercise	141 ± 46	115 ± 46	316 ± 46	213 ± 46
	Post-exercise	138 ± 46	123 ± 46	308 ± 46	270 ± 46*^
	Recovery	117 ± 46*	112 ± 46	268 ± 46	225 ± 46
CM CD4+ T-cells (cells/uL) (CD45RA- CD62L+)	Pre-exercise	233 ± 35	205 ± 35	313 ± 35	240 ± 35
	Post-exercise	229 ± 35	223 ± 35	318 ± 35	318 ± 35*^
	Recovery	205 ± 35	207 ± 35	291 ± 35	279 ± 35
EM CD4+ T-cells (cells/uL) (CD45RA- CD62L-)	Pre-exercise	141 ± 24	120 ± 24	154 ± 24	125 ± 24
	Post-exercise	160 ± 24*	150 ± 24*	166 ± 24	176 ± 24*^
	Recovery	126 ± 24	119 ± 24	157 ± 24	159 ± 24*
EMRA CD4+ T-cells (cells/uL) (CD45RA+ CD62L-)	Pre-exercise	8 ± 3	7 ± 3	13 ± 3	9 ± 3
	Post-exercise	13 ± 3*	9 ± 3	21 ± 3*	18 ± 3*
	Recovery	7 ± 3	6 ± 3*	13 ± 3	11 ± 3
CD57+ EMRA CD4+ T-cells (cells/uL) (CD57+ CD45RA+ CD62L-)	Pre-exercise #	1 ± 2	1 ± 2	7 ± 2	5 ± 2
	Post-exercise	2 ± 2*	2 ± 2	9 ± 2	10 ± 2*^◆
	Recovery	1 ± 2	1 ± 2	6 ± 2	6 ± 2

* denotes significant within-group difference compared to pre-exercise ($p<0.05$)
^ denotes significant within-group differences from the same time point in CRE compared to RE while controlling for pre-exercise values ($p<0.05$)
# denotes significant difference at pre-exercise between OPA and OPI groups ($p<0.05$)
◆ denotes significant difference between the number of cells mobilized post-exercise during CRE compared to the number of cells mobilized during RE while controlling for pre-exercise in OPA compared to OPI groups

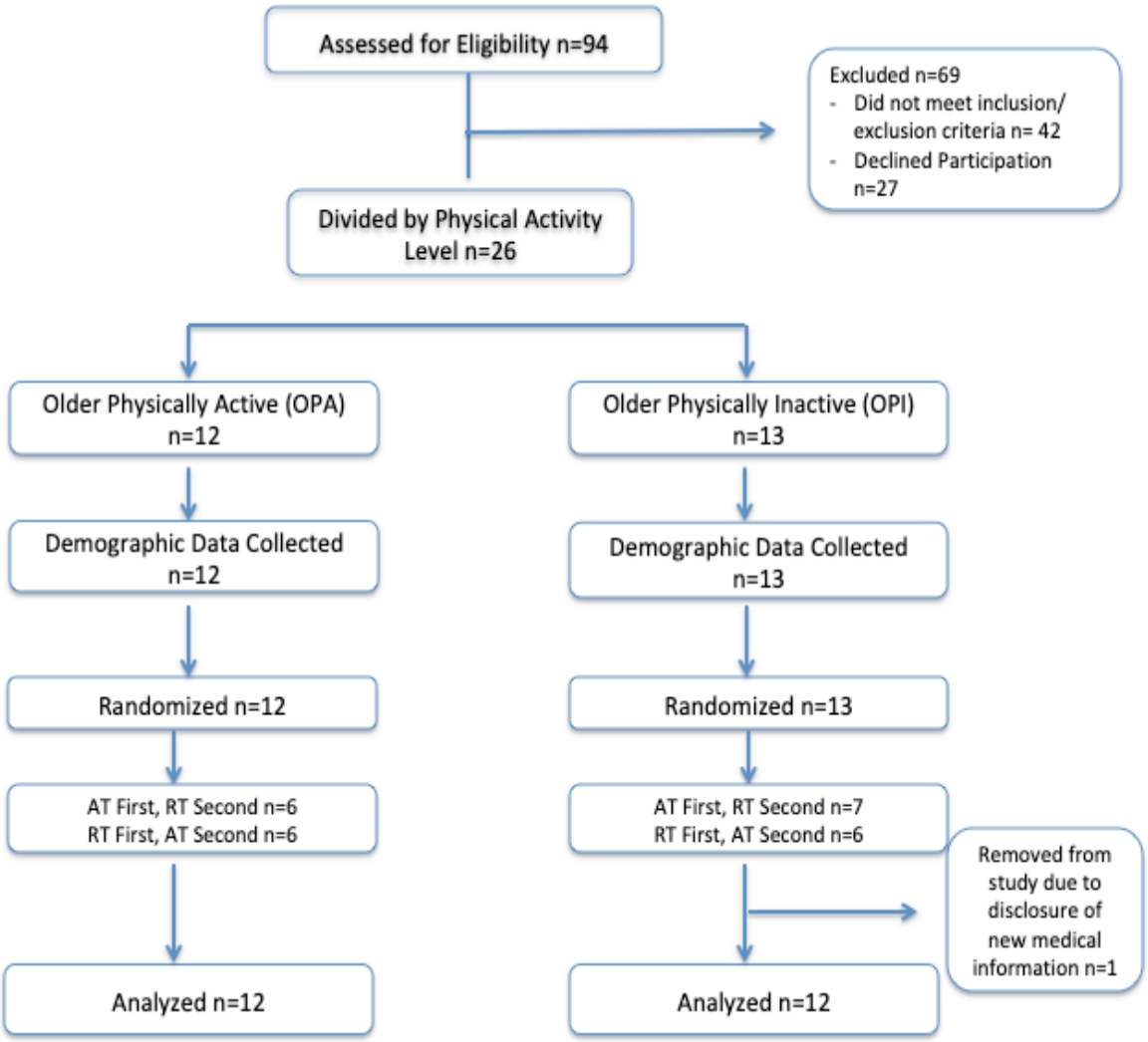


Figure 1.1: Recruitment and Retention Flow Chart

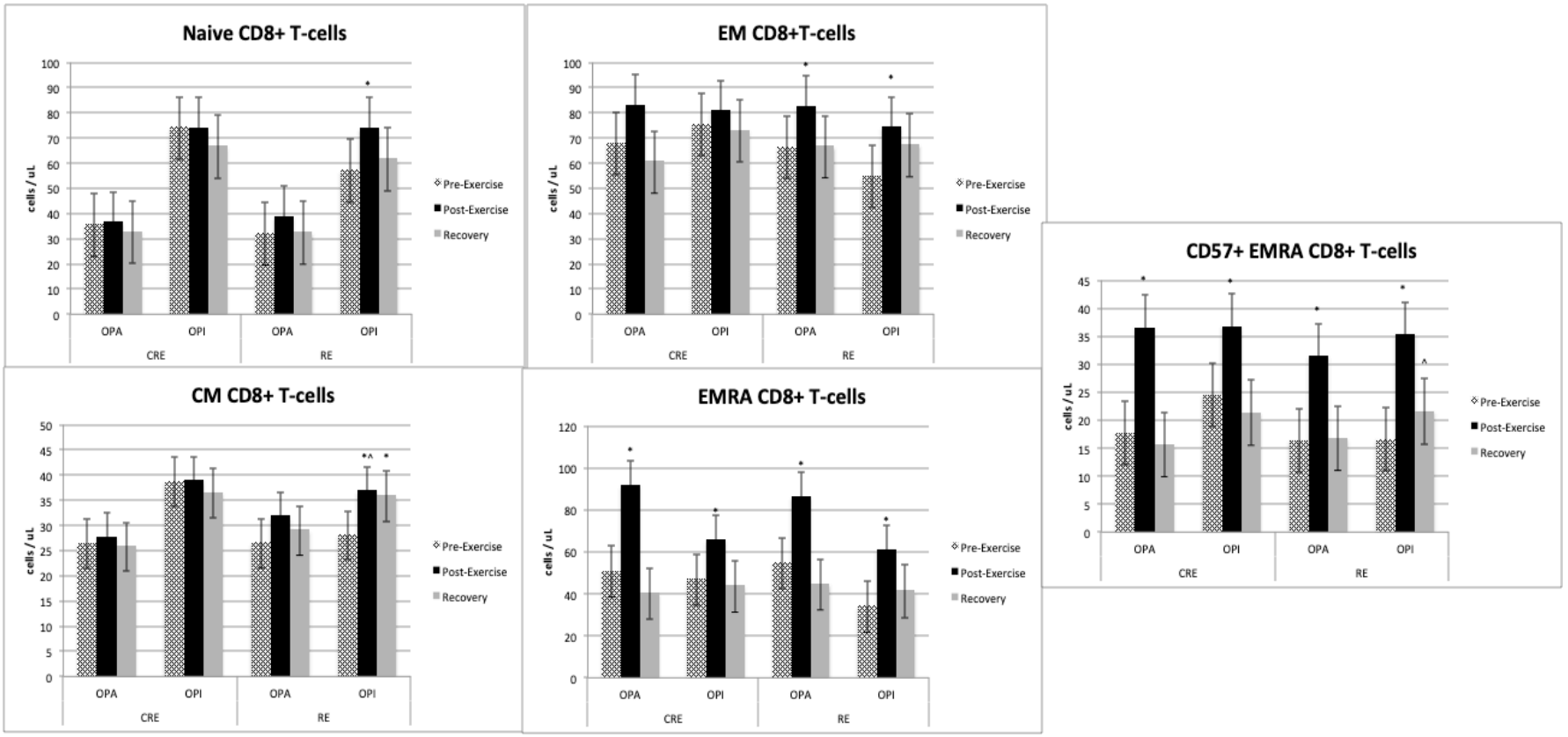


Figure 1.2: CD8+T-cell subsets at pre-exercise, post-exercise, and recovery in response to cardiorespiratory (CRE) and resistance (RE) exercise in older physically active (OPA) and older physically inactive (OPI) groups.

* denotes significant difference compared to pre-exercise ($p<0.05$)
^ denotes significant within-group differences from the same time point in CRE while controlling for pre-exercise values ($p <0.05$)

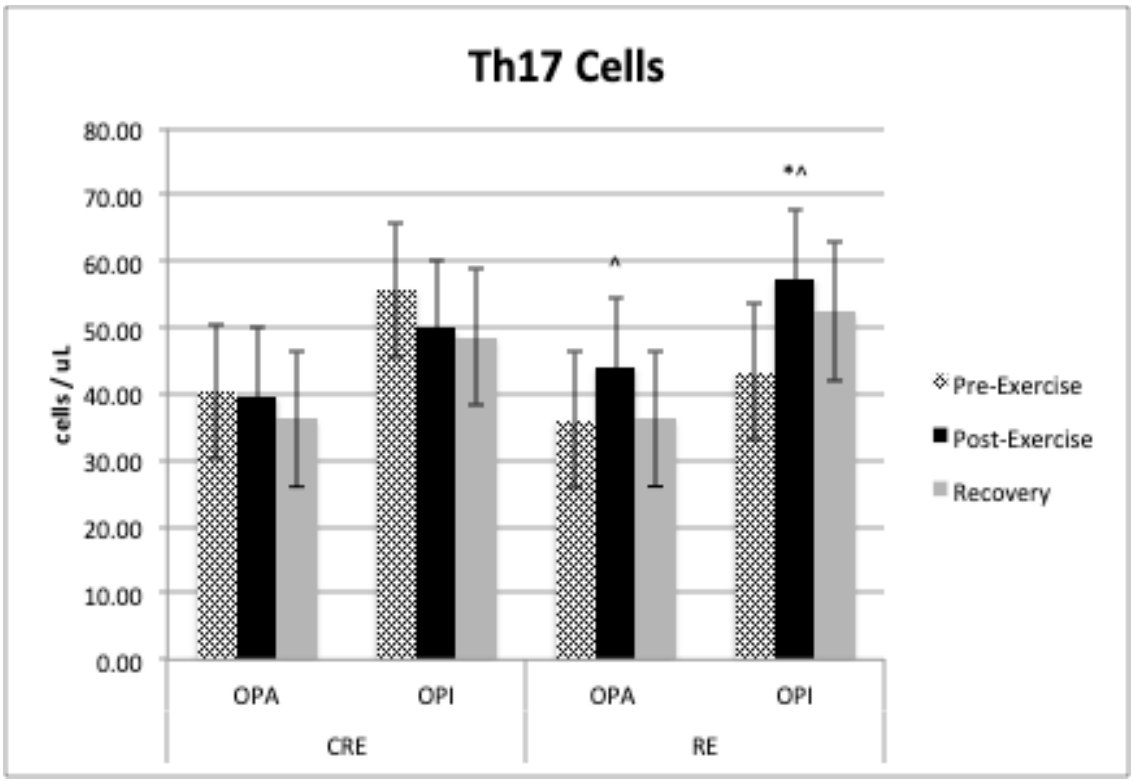


Figure 1.3: Th17 Cells at pre-exercise, post-exercise, and recovery in response to cardiorespiratory (CRE) and resistance (RE) exercise in older physically active (OPA) and older physically inactive (OPI) groups

* Indicates significant difference compared to pre values ($p < 0.05$)
^ indicates significant within-group differences from post-exercise CRE while controlling for pre-exercise values ($p < 0.05$)

CHAPTER FOUR

Manuscript 2

Title: Monocyte response to acute cardiorespiratory and resistance exercise in cohorts of physically active and physically inactive older adults

Abstract

Monocytes undergo shifts in their subpopulation proportions as well as functional changes with aging and physical inactivity. These detrimental changes have been implicated in the perpetuation of chronic systemic inflammation and cardiovascular disease (CVD) and may be exacerbated by prolonged psychological stressors. Regular physical activity may attenuate or prevent these negative immunological and inflammatory changes in monocytes, but the mechanisms that underlie this hypothesis remain unknown. Furthermore, the majority of literature is derived from cardiorespiratory exercise (CRE) studies, with much less understood about the monocyte response to resistance exercise (RE). The purpose of this study was to examine the effects of acute cardiorespiratory (CRE) and acute resistance exercise (RE) on the cellular and functional monocyte response between a cohort of physically active older adults (OPA) and a cohort of physically inactive older adults (OPI). **METHODS**: Twenty-four healthy older adults (OPA n=12; OPI n=12; mean ± SD; age (yrs) OPA 62 ± 5, OPI 64 ± 5; BMI ($kg•m^{-2}$) OPA 23.9 ± 3.0, OPI 25.6 ± 3.5).) completed one bout of CRE and one bout of RE in a randomized order, both at a moderate intensity, and separated by at least 7 days. Blood samples were taken at pre-exercise, post-exercise, and 1h post-exercise (recovery) and analyzed for classical (CD14++ CD16-), intermediate (CD14++ CD16+) and non-classical (CD14+ CD16+) monocytes, as well as surface expression of surface receptors CD11b, CCR5, CX3CR1, and CCR2 using flow cytometry. *In vitro* analyses examined LPS-stimulated cytokine (interleukin (IL)-6 and tumor necrosis factor (TNF)) secretion and glucocorticoid resistance. **RESULTS**: OPA and OPI had similar

pre-exercise and monocyte responses to exercise; particularly, both CRE and RE significantly mobilized monocytes and monocyte subtypes that expressed surface markers CD11b, CCR5, CX3CR1, and CCR2 into the circulation. However, RE mobilized more monocyte subtypes immediately post-exercise, whereas CRE elicited greater differences compared to pre-exercise at the recovery time point. Furthermore, with LPS stimulation, CRE resulted in an increase in pro-inflammatory TNF post-exercise, whereas RE did not. **CONCLUSION**: Monocytes appear to respond similarly to CRE and RE in both OPA and OPI individuals. However, differences between CRE and RE may exist. These findings support that moderate-intensity resistance exercise may operate via different physiological mechanisms compared to cardiorespiratory-based exercise to influence the pro and anti-inflammatory responses to exercise.

Introduction

Unfavorable immune changes and increased systemic inflammation that have traditionally been associated with aging may instead be more related to physical inactivity. Indeed, “inflamm-inactivity” is a term that is increasingly utilized to describe the elevations in chronic systemic inflammation and shifts in the proportions and functional properties of immune cells often observed with aging and physical inactivity [2].

Monocytes are a broad leukocyte subtype that exhibit a variety of changes in both composition and functional properties with aging and/or physical inactivity, and have been implicated in the perpetuation of chronic systemic inflammation and cardiovascular disease (CVD) [8, 25]. Specifically, although the total number of monocytes often remains unchanged, there is an age-associated shift in the proportions of monocyte subsets. This is characterized by an increase in circulating intermediate (CD14++ CD16+) and non-classical (CD14+ CD16+) monocytes [27]. CD16+ monocytes (intermediate and non-classical monocytes) have been linked to increased inflammatory properties via the secretion of a host of inflammatory cytokines such as TNF and IL-6, and are known to secrete more of these (and virtually no anti-inflammatory cytokines) compared to their classical counterparts [29].

Changes in circulating cortisol or glucocorticoid receptor sensitivity may also serve as a means of exacerbating the cellular proportional shifts and pro-inflammatory changes to monocytes that are often associated with aging and physical inactivity. In addition to disruptions in the hypothalamic-pituitary-adrenal

(HPA)-axis and thereby glucocorticoid (cortisol) secretion, chronic stress has been associated with glucocorticoid resistance and increased production of TNF and IL-6 [11]. It has also been suggested that circulating cortisol increases with age [97], and this may impact the sensitivity of its receptors. Glucocorticoid resistance, which is characterized by either a reduction in the sensitivity, or a downregulation of the number of glucocorticoid receptors on target tissues, is another potential source of increased chronic systemic inflammation, as glucocorticoids are known to possess potent properties of inflammation resolution [36]. Thus, the body's resistance towards the effects of glucocorticoids as a result of aging or chronic stress may be an important source of immunological alterations and increased systemic inflammation.

Regular physical activity, however, may be able to counter the deleterious effects of both aging and chronic stress on the aforementioned monocyte changes and increases in inflammation [2, 13]. Several researchers have reported that older adults who are physically active have lower percentage of non-classical monocytes compared to their inactive counterparts and that a period of regular exercise training in older adults who were previously inactive can indeed facilitate a shift in baseline monocyte proportions towards that of their active peers [8, 9]. Additionally, repeated exposure to habitual exercise (repeated bouts of an acute physical stressor i.e. exercise training) elicits a variety of hormonal perturbations from which the body learns to recover more efficiently. This exercise training leads to improvements in the body's ability to cope with and resolve repeated psychological stressors [13]. Therefore, the purpose of the present study was to examine the independent effects

of acute cardiorespiratory (CRE) and acute resistance exercise (RE) on the circulating cellular and functional monocyte response among a cohort of physically active older adults (OPA) compared to a cohort of physically inactive older adults (OPI). We hypothesized that both cardiorespiratory and resistance exercise bouts will elicit a monocyte mobilization particularly among the non-classical monocytes, but that the magnitude of this mobilization would be higher following cardiorespiratory exercise and in physically inactive older adults. Furthermore, we hypothesized that exercise would elicit an increase in inflammatory markers when stimulated with LPS and treated with dexamethasone (dex) *in vitro* and that the magnitude of the inflammatory response would be greater following CRE, particularly in physically inactive individuals.

Methods

Subjects

Two groups of 12 older adults (56-75 years) who were either physically active (OPA) or physical inactive (OPI) were recruited for participation. Physically active and physically inactive were delineated using the guidelines set forth by the American College of Sports Medicine [81]. Briefly, physically active participants had to be exercise trained, as defined by participating in at least 30 minutes of moderate to vigorous intensity cardiovascular activity for at least 3 days per week and engaging in resistance training for all of the major muscle groups approximately 2 days / week for at least the past 3 months. Participants classified as physically inactive were exercise untrained, which was defined as, for at least the last 3

months, participating in less than 30 minutes of moderate to vigorous intensity cardiovascular activity twice per week and no regular resistance training [81].

Inclusion / exclusion criteria are also described in Chapter 3. Potential participants were excluded if they had any contraindications to moderate-vigorous exercise; if they had any recent illness or have been instructed not to exercise by their healthcare provider; if they had range of motion restrictions that would prevent them from participating in aerobic or resistance training with proper form (they must be ambulatory); if they were taking medications (prescription or over the counter) known to influence immune function (including daily NSAID's and beta blockers), cholesterol-lowering medications (statins), drugs that increase bone mass (bisphosphonates), or steroids; if they had known cardiovascular, respiratory, metabolic, or renal disease, with the exception of controlled hypertension (as defined by resting BP below 140/90) and/or controlled asthma (self-reported); if they fell outside of a BMI range of (18.5 – 30 $kg \bullet m^{-2}$); if they consumed alcohol or recreational drugs within 24h prior to visits; if women were pre-menopausal (must not have had a menses for at least 12 months); and if they had scheduling conflicts that would prevent them from reporting to the laboratory of integrated physiology 4 times over the course of the study. All participants provided a signed written statement of informed consent prior to participation in the study. All study procedures were approved by the Institutional Review Board at the University of Houston.

Participants were assigned to OPA or OPI groups based on self-report. Each participant filled out an Exercise Frequency Questionnaire, where they were required

to detail the types of weekly activity that they participate in and the length of time that each activity is performed. ActiGraph accelerometers (wGT3X-BT, ActiGraph ® Corp, Pensacola, FL, USA) were used to verify activity status, and confirmed that OPA participated in more moderate to vigorous physical activity (MVPA) compared to OPI (Table 1.1).

Experimental Design

The present study was a randomized, complete cross-over design with three time points (pre-exercise, post-exercise, and recovery), two activity levels (OPA and OPI), and two exercise modalities (cardiorespiratory exercise (CRE), and resistance exercise (RE)). Participants reported to the Laboratory of Integrated Physiology three times over the course of the study following an overnight fast, as previously described (Chapter 3).

Flow Cytometry

Multi-color flow cytometry was used to identify monocytes and monocyte subsets in whole blood. All monoclonal antibodies were purchased from Miltenyi Biotech (Bergisch Gladbach, Germany) unless otherwise stated, and a MACSQuant ® Analyzer 10 (Miltenyi Biotech, Gladback, Germany) flow cytometer was used to analyze each sample. Monocyte count was determined using 50 µL whole blood labeled with VioBlue-conjugated anti-CD45 mAb and FITC-conjugated anti-CD14 mAb and incubated for 30 minutes. Red blood cell lysis buffer was added in a 1:11 ratio and samples incubated for another 20 minutes. One minute prior to analyzing each sample of the flow cytometer, PI was added to each sample in order to identify any dead cells. Monocyte count was determined using forward and side-scatter

plots, which were gated electronically. Cells within the monocyte gate that were CD45+, CD14+, and negative for PI were counted and multiplied by the dilution factor of 11 to obtain the number of monocytes per μL of blood.

Monocyte subsets were identified using 100 μL whole blood labeled with VioGreen-conjugated anti-CD11b mAb, FITC-conjugated anti-CD14 mAb, PE-conjugated anti-CD16 mAb or PE-Vio615-conjugated anti-CD16 mAb, PE-Vio770-conjugated anti-CCR5 mAb, APC-conjugated anti-CX3CR1 mAb, and APC-Vio770-conjugated anti-CCR2 mAb. Following a 30 minute incubation, samples were incubated with red blood cell lysing buffer for 20 minutes, washed, and then analyzed on the flow cytometer. Monocytes were identified as those within the monocyte gate that expressed the CD14 surface antigen. Classical, intermediate, and non-classical monocytes were gated within the CD14+ monocytes and determined based on their expression of CD14 and CD16 surface markers (classical monocytes (CD14++ CD16-); intermediate monocytes (CD14++ CD16+); non-classical monocytes (CD14+ CD16+)). Surface expression of CD11b, CCR5, CX3CR1, and CCR2 were determined by gating on the positive cell populations for each of these markers within each of the classical, intermediate, and non-classical gates. The total number of monocyte subtypes were identified by multiplying the percentage of the cells of interest by the total monocyte count, and median fluorescence intensity (MFI) was recorded.

Serum Analyses

Cortisol was measured using blood serum samples collected at pre-exercise, post-exercise, and recovery time points, which were stored at -80°C, thawed, and

measured in batch analysis in duplicate using commercially available ELISA kits (Rocky Mountain Diagnostics, Colorado Springs, CO, USA) according to manufacturer's instructions.

LPS Stimulations

Using whole blood cultures, 100 µL/mL of heparinized whole blood was added to five wells of 1800 µL-supplemented RPMI 1640 media (2 nM/mL glutamine; 100 U/mL penicillin; 100 µg/mL streptomycin) on a sterile 24-well cell culture plate for each time point. Each well contained 1ng/mL LPS (*S. enteriditis*; Sigma-Aldrich, St. Louis, MO, USA), 1 ng/mL LPS + 10 µg/mL polymixin-B (PMB; Sigma-Aldrich), 25 µg/mL LPS, 25 µg/mL LPS + 10 µg/mL PMB, and no LPS or PMB. PMB prevents LPS from signaling through TLR4 by binding to the lipid A portion of LPS. Thus, PMB was used to verify that the inflammatory response of monocytes is a result of LPS signaling through the TLR4 pathway. Prior to adding the whole blood, plates were incubated in a 37°C, 5% CO_2, humidified environment for a minimum of 15 minutes in order to allow PMB/LPS binding. Whole blood was then added and the plate was incubated in a 37°C, 5% CO_2, humidified environment for 24h. Following incubation, the plates were centrifuged at 800 g for 10 minutes at 4°C and the supernatants were removed, syringe-filtered, and frozen at -80°C until batch analysis. IL-6 and TNF concentrations were measured in duplicate using commercially-available ELISA kits (BD OptEIA, BD Biosciences Pharminogen, San Diego, CA, USA) according to the manufacturer's instructions. Wells with high concentrations of IL-6 and TNF were diluted per the manufacturer's instructions such that the cytokine concentrations remained within the range of the standard curve.

Glucocorticoid Stimulations

Monocyte function was additionally quantified *in vitro* under conditions of physiological stress. Using whole blood cultures, 40 µL of heparinized whole blood was added to six wells of 910 µL -supplemented RPMI 1640 media (2 nM/mL glutamine; 100 U/mL penicillin; 100 µg/mL streptomycin) and 50 µL of dexamethasone (dex; Sigma-Aldrich, St. Louis, MO, USA) diluted to various concentrations on a sterile 48-well cell culture plate. Each well contained 0.1 ng/mL LPS and 0, 10^{-6}, 10^{-7}, 10^{-8} M dex, with two additional control wells containing only whole blood + media, and whole blood + 50 µL of 10^{-6} M dex without LPS. Plates were incubated in a 37°C, 5% CO_2, humidified environment for 20h, after which they were centrifuged at 800 g for 10 minutes at 4°C, the supernatants were removed, syringe-filtered, and frozen at -80°C until batch analysis. IL-6 and TNF concentrations were measured in duplicate using the same commercially-available ELISA kits and procedures as described above.

Statistical Analyses

To assess baseline differences (differences between OPA and OPI at pre-exercise), a linear mixed effects model was fit, comparing *condition* (CRE or RE) and *group* (OPA and OPI) and their interaction. Subject was included as a random intercept term. Specific comparisons were tested using contrasts.

To determine whether the condition (CRE or RE) influenced the mobilization of monocytes, monocyte subsets, and cytokine responses as well as to determine whether physical activity status influenced these results, a linear mixed effects model on the change from pre-exercise was fit on each factor (*group* (OPA and

OPI), *condition* (CRE and RE), and *time* (pre-exercise, post-exercise, and recovery)) and their interactions. Pre-exercise values served as a covariate, and the intercept was tested in order to determine the differences from the pre-exercise time point. Contrasts were used to assess the differences between each group and condition of exercise. Data was transformed prior to running in order to improve model fit (i.e. normality and equality of variances), and contrasts were used to compare specific time points across the factors. However, for ease of comparison to other literature, the data presented in the manuscript are the actual values (not transformed), even though the p-values are derived from using the transformed data. Statistical significance was determined *a priori* at $p<0.05$. All statistical analyses were run using R version 3.6.2 and R Markdown to generate data analysis reports.

Results

Baseline Differences between OPA and OPI

All participants successfully completed both CRE and RE exercise trials. Participant physical characteristics and exercise data are displayed in chapter 3 (age: OPA 62 ± 5, OPI 64 ± 5 yrs; height: OPA 170.9 ± 6.9, OPI 162.9 ± 8.0 cm; weight: OPA 69.3 ± 10.2, OPI 68.2 ± 12.8 kg; BMI: OPA 23.9 ± 3.0, OPI 25.6 ± 3.5 $\bullet m^{-2}$). OPA participants demonstrated significantly higher muscular strength compared to OPI for leg press ($p=0.02$), chest press ($p<0.01$), leg curl ($p<0.01$), lat pull down ($p=0.01$), weighted calf raise ($p<0.01$), triceps extension ($p<0.01$), and seated row ($p=0.01$). No significant differences between groups were found for leg extension or estimated $\dot{V}O_{2max}$.

OPI exhibits higher CCR2 expression on non-classical monocytes compared to OPA

Main effects for group at pre-exercise were found for the numbers of CCR5+ classical monocytes, CX3CR1+ classical, intermediate, and non-classical monocyte MFI, the numbers of CCR2+ intermediate monocytes, CCR2+ non-classical monocyte MFI, and unstimulated IL-6 dex per monocyte ($p<0.05$). Only CCR2+ non-classical monocyte MFI was significantly different during both CRE ($p<0.01$, $z=2.64$) and RE ($p=0.03$, $z=2.34$) trials for OPI and OPA.

Effect of physical activity and exercise modality on monocyte responses

Exercise mobilized classical and non-classical monocyte subtypes into the circulation immediately post-exercise

Regardless of CRE or RE, exercise mobilized total ($p<0.01$, $z=6.86$), classical ($p<0.01$, $z=6.78$), and non-classical ($p<0.01$, $z=-7.83$) monocytes into the circulation (Figure 2.1), in addition to CD11b+, CCR5+, CX3CR1+, and CCR2+ classical monocytes, CCR5+ and CX3CR1+ intermediate monocytes, and CD11b+, CCR5+, CX3CR1+, and CCR2+ non-classical monocytes ($p<0.05$). There was no significant mobilization of intermediate monocytes, CD11b+, and CCR2+ intermediate monocytes. The recovery time point was significantly lower than pre-exercise for intermediate monocytes, CD11b+, CCR5+, CX3CR1+, and CCR2+ intermediate monocytes, and CCR5+ classical monocytes ($p<0.05$). Monocyte cell counts are presented in Table 2.1 and Figures 2.1 and 2.2.

RE mobilized more monocyte subtypes from pre-exercise to post-exercise than CRE in both OPA and OPI groups. OPA and OPI demonstrate similar response

In response to RE, OPA demonstrated a significant mobilization of all monocytes and monocyte subtypes (total; classical; intermediate; non-classical; CD11b+, CCR5+, CX3CR1+, and CCR2+ classical; CD11b+, CCR5+, CX3CR1+, and CCR2+ intermediate; and CD11b+, CCR5+, CX3CR1+, and CCR2+ non-classical; $p<0.05$) and OPI demonstrated a significant mobilization of all the aforementioned cells except CD11b+ intermediate monocytes. Conversely, CRE resulted in a significant mobilization of total, classical, and non-classical monocytes, as well as CD11b+, CX3CR1+, and CCR2+ classical, and CD11b+ and CX3CR1+ non-classical monocytes within the OPA group ($p<0.05$). The same cell types were significantly mobilized with CRE within the OPI group with the addition of CCR5+ non-classical monocytes ($p<0.05$).

CRE results in altered recovery for many monocyte subtypes in both OPA and OPI groups

Within OPA, the recovery time point was lower than pre-exercise following CRE for intermediate and non-classical monocytes, as well as CCR5+ classical, CD11b+, CCR5+, CX3CR1+, and CCR2+ intermediate, and CD11b+, CX3CR1+, and CCR2+ non-classical monocytes ($p<0.05$). Within OPI, the same cells are significantly lower than pre-exercise ($p<0.05$), with the exception of non-classical monocytes and all of the non-classical monocyte subsets.

RE did not result in any significant cellular changes at the recovery time point for OPA, but in OPI, non-classical monocytes, as well as CCR5+ and CX3CR1+

non-classical monocytes were significantly higher compared to pre-exercise at the recovery time point ($p<0.05$).

Many monocyte subtypes remain elevated at the recovery time point following RE compared to CRE in OPI but not in OPA

Within the OPI group only, RE mobilized more cells post-exercise than CRE for total, classical, and intermediate monocytes, as well as for CD11b+, CX3CR1+, and CCR2+ classical monocytes, and CD11b+, CCR5+, and CX3CR1+ intermediate monocytes ($p<0.05$). Within the OPI group only, the number of cells at the recovery time point following RE was significantly higher compared to CRE for total, classical, intermediate, and non-classical monocytes, as well as CCR5+, CX3CR1+, and CCR2+ classical, CCR5+ and CX3CR1+ intermediate, and CX3CR1+ and CCR2+ non-classical monocytes ($p<0.05$). There were no significant differences between CRE and RE at post-exercise for the OPA group, and only intermediate, CD11b+ and CX3CR1+ intermediate monocytes were significantly higher following RE compared to CRE at the recovery time point ($p<0.05$).

MFI data does not reflect cellular changes

MFI data for the cell subtypes does not follow the same pattern as the cell count changes. This data is presented in Table 2.2. There was a pre-exercise to post-exercise increase in MFI for CD11b+ expression on classical for OPA after CRE ($p=0.02$, $z=-2.38$), and CCR5+ expression on non-classical monocytes for OPA after RE ($p=0.02$, $z=2.46$), and a decrease in MFI from pre-exercise to post-exercise

for CCR2+ expression on classical monocytes for OPA after CRE (p=0.01, z=2.70) and OPI after RE (p=0.03, z=2.29). At the recovery time point, MFI was lower than pre-exercise for CD11b+ expression on classical and intermediate monocytes in OPA after both CRE (classical: p<0.01, z=-4.08; intermediate: p<0.01, z=-3.51) and RE (classical: p=0.02, z=-2.36; intermediate: p=0.02, z=-2.31), but CD11b+ expression on classical monocytes was higher than pre-exercise in OPI after RE (p=0.04, z=-2.06). MFI for CX3CR1+ expression on intermediate monocytes was significantly higher at the recovery time point compared to pre-exercise in both OPA and OPI after RE (OPA: p<0.01, z=-3.08; OPI: p<0.01, z=-4.43) and on non-classical monocytes in OPI after RE (p=0.01, z=2.62). MFI for CCR2+ expression on classical monocytes at the recovery time point was lower compared to pre-exercise for OPA after RE (p=0.03, z=2.24).

Cortisol is not increased with exercise and responds the same in OPA and OPI for both CRE and RE

For both CRE and RE within both OPA and OPI, cortisol is highest at pre-exercise and there is no difference between OPA and OPI. Cortisol is lower than pre-exercise at both post-exercise (OPA: CRE: p=0.03, z=-2.26, RE: p=0.02, z=-2.34; OPI: CRE: p<0.01, z=-4.11, RE: p<0.01, z=-4.16) and recovery time points (OPA: CRE: p<0.01, z=-4.12, RE: p<0.01, z=-4.48; OPI: CRE: p<0.01, z=-6.62, RE: p<0.01, z=
-6.08). There were no differences between CRE and RE or between OPA and OPI at any time point (p>0.05). Cortisol data are presented in Figure 2.3.

LPS-stimulated IL-6 production

There were no baseline differences between OPA and OPI per CD14+ cell LPS-stimulated IL-6 production (Figure 2.4). There were also no significant pre-exercise to post-exercise changes within OPA or OPI groups for CRE or RE. At the recovery time point, physiological stimulation (1ng/mL LPS) IL-6 production was significantly higher than pre-exercise IL-6 within OPI RE only ($p=0.04$, $z=2.97$). There was a significant 3-way interaction between group (OPA vs OPI), modality (CRE vs. RE), and time (pre-exercise, post-exercise, and recovery) for 1ng/mL IL-6 production at the recovery time point only ($p<0.05$, $z=-2.00$).

LPS-stimulated TNF production

LPS-stimulated TNF production is shown in Figure 2.4 B. Per CD14+ cell LPS-stimulated TNF production was significantly higher in OPI for maximal (25 μg/mL) TNF production at baseline on the RE trial day only ($p=0.04$, $z=1.09$). There were significant pre-exercise to post-exercise increases with CRE within both OPA and OPI for physiological stimulation (1 ng/mL) TNF production (OPA: $p=0.04$, $z=2.12$; OPI: $p=0.04$, $z=2.04$), maximal (25 μg/mL) TNF production (OPA: $p<0.01$, $z=3.21$; OPI: $p<0.01$, $z=4.03$) and maximal + PMB (25 μg/mL + PMB) TNF production (OPA: $p<0.01$, $z=3.64$; OPI: $p<0.01$, $z=4.03$). TNF production at the recovery time point was higher than pre-exercise only in the RE condition in the OPI group for physiological stimulation (1 ng/mL) TNF production ($p=0.01$, $z=2.55$), maximal (25 μg/mL) TNF production ($p<0.01$, $z=2.99$) and maximal + PMB (25

μg/mL + PMB; p<0.01, z=3.27) TNF production and with CRE for maximal (25 μg/mL) TNF production (p=0.03, z=2.28) and maximal + PMB (25 μg/mL + PMB) TNF production (p<0.01, z=2.99). Within OPA, the unstimulated cells at the recovery time point following CRE produced less TNF compared to pre-exercise (p=0.03, z=2.22). Within OPA, CRE elicited a significantly larger TNF production compared to RE from pre-exercise to post-exercise for physiological (1 ng/mL) TNF production (p<0.01, z=-2.62), maximal (25 μg/mL) TNF production (p<0.01, z=-3.24) and maximal + PMB (25 μg/mL + PMB) TNF production (p<0.01, z=-4.87), and for OPI for maximal TNF production only (p=0.01, z=2.45).

Glucocorticoid assay for IL-6 production

Results from the glucocorticoid assay are shown in Figure 2.5 A. Unstimulated IL-6 production per CD14+ cell was significantly higher in OPA compared to OPI at baseline on the RE trial day only (p<0.01, z=2.87). There were significant pre-exercise to post-exercise differences for LPS-stimulation only within OPA RE (p=0.02, z=-2.49). No other time points within either OPA or OPI were significantly different from pre-exercise values. Nevertheless, within OPA only, significantly less IL-6 was produced at post-exercise following RE compared to CRE at the intermediate dose of dex (100 pg/mL LPS + 10^{-7} M dex; p=0.04, z=2.00), and IL-6 production was significantly lower following CRE compared to RE at the recovery time point for dex only (10^{-6} M dex; (p<0.01, z=2.91).

Glucocorticoid Assay for TNF production

Results from the glucocorticoid assay are shown in Figure 2.5 B. There were no baseline differences between OPA and OPI per CD14+ cell glucocorticoid-assay stimulated TNF production. There was a significant pre-exercise to post-exercise decrease in TNF production in unstimulated cells within OPA RE ($p=0.04$, $z=2.07$) and dex-stimulation only cells (10^{-6} M dex) within OPI RE ($p=0.04$, $z=2.01$), and a significant increase in TNF production from pre-exercise to post-exercise in the LPS-stimulation only cells (100 pg/mL LPS) within OPA CRE ($p=0.04$, $z=2.05$). Within OPA, there was a significant decrease in TNF production from pre-exercise to the recovery time point in unstimulated cells after RE ($p<0.01$, $z=3.93$), and a significant increase in TNF production at the recovery time point in the higher dose of dex (100 pg/mL LPS + 10^{-6} M dex) after CRE ($p=0.03$, $z=2.20$). Within OPI, there was a significant decrease in TNF between pre-exercise and recovery time points for the intermediate dose of dex (100 pg/mL LPS + 10^{-7} M dex) after CRE ($p<0.01$, $z=2.80$), and a significant increase after RE ($p=0.04$, $z=2.13$). In the LPS-stimulated only cells (100 pg/mL LPS), TNF production was significantly lower at post-exercise following RE compared to CRE for both OPA and OPI groups (OPA: $p<0.01$, $z=-2.87$; OPI: $p=0.02$, $z=2.38$). Significant 3-way interactions were found for unstimulated blood; $p<0.01$, $z=3.06$), the higher dose of dex (100 pg/mL LPS + 10^{-6} M dex; $p=0.01$, $z=2.55$), and the intermediate dose of dex (100 pg/mL LPS + 10^{-7} M dex; $p<0.01$, $z=-3.02$).

Discussion

This is the first study to compare the effects of acute cardiorespiratory (CRE) and resistance (RE) exercise on monocyte subset responses and functional

measures of monocytes in older adults in a randomized, complete crossover study design. We report that both CRE and RE mobilized monocytes and monocyte subtypes into the circulation in both OPA and OPI. However, at the recovery time point following RE, more monocyte subtypes remained elevated either compared to pre-exercise or compared to the CRE response in the OPI group only. Furthermore, we showed that with LPS stimulation, CRE resulted in an increase in pro-inflammatory TNF production post-exercise, whereas RE did not.

The most notable baseline difference between OPA and OPI was the lower MFI for CCR2+ expression on non-classical monocytes in OPA. CCR2 is the receptor for monocyte chemoattractant protein-1 (MCP-1, or CCL2). MCP-1 and its ability to rapidly recruit monocytes to areas of inflammation has been implicated in cardiovascular disease progression [98]. However, it remains to be determined whether increased CCR2 expression on monocytes is related to increases in monocyte recruitment to the endothelium in a way that might facilitate CVD progression or perpetuate low-grade inflammation. Nevertheless, this is the first study to report differences in CCR2 expression among older individuals of various physical activity levels and future research should aim to confirm these findings and assess potential implications.

OPA and OPI exhibited similar monocyte responses to exercise when looking at the immediately post-exercise time point, whereby monocytes and monocyte subtypes expressing surface markers CD11b, CCR5, CX3CR1, and CCR2 were mobilized into the circulation following an acute bout of exercise. CD11b, CCR2, and CX3CR1 are chemokine receptors found on monocytes, among other cells, that act

as adhesion molecules and are largely implicated in CVD [25], while CCR5 is a chemokine receptor for several chemokines including macrophage inflammatory protein (MIP) 1 alpha, and has been implicated in other inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease [99]. Thus, the monocyte receptors examined within this study are all related to the pathogenesis of a variety of pro-inflammatory conditions.

A significant mobilization from pre-exercise to post-exercise of monocytes expressing these receptors is in line with the emerging hypothesis that acute exercise preferentially mobilizes cells with high effector functions, migratory capacity, and inflammatory potential in order to enhance the body's ability to respond to a pathogenic challenge in the minutes and hours following exercise cessation [41, 79]. Such a response is thought to enhance immunity by priming the body to launch a robust immune response if encountered with a threat. The present study adds to the body of literature by supporting that both CRE and RE elicit a significant mobilization of monocytes with these properties (high effector functions, migratory capacity, and inflammatory potential) in older adults.

Contrary to our hypotheses, RE mobilized more monocyte subtypes from pre-exercise to post-exercise than CRE in both OPA and OPI individuals. One potential explanation for this is that resistance exercise utilized the glycolytic energy system to a larger extent than CRE. Although not measured in the present study, the difference in energy system utilization would likely result in increased lactate production with RE compared to CRE. Higher intensities of exercise have been shown to elicit greater CD16+ monocyte mobilization even when the duration of the

exercise performed is shorter [43]. While the present study had participants exercising at the same relative intensity (approximately 70% of maximum) for both CRE and RE for a similar amount of time (approximately 30 minutes), the demands of CRE compared to RE required using different energy systems such that the RE protocol most likely relied more on glycolysis compared to CRE. Nevertheless, the role of energy system utilization and blood lactate concentration on the mobilization of monocytes has not been extensively examined, as the movement of monocytes to and from the circulation in response to exercise has been largely attributed to increases in catecholamines and glucocorticoids [43].

Indeed, the OPI group exhibited a greater number of significant differences in monocyte subtype mobilization from pre-exercise to post-exercise between CRE and RE than OPA, which suggests that RE was more novel for these individuals and may have elicited a larger lactate or hormonal response. In support of this, researchers looking at the effects of training status on the exercise-induced mobilization of leukocytes have reported that untrained individuals exhibit more robust absolute responses to exercise compared to trained counterparts [100, 101], so differences with the OPI group in CRE compared to RE responses may be due to familiarity with the activity performed. In other words, the OPI group may have mounted a larger physiological response (i.e. increased lactate production, catecholamine secretion, and/or cardiovascular adaptations) to RE, which they were inexperienced with, compared to CRE, which consisted of walking and therefore was not an activity that OPI participants were unaccustomed to.

Additionally, β_2-adrenergic receptor (β_2-AR) signaling may play a role in the more robust monocyte subtype response to acute exercise in OPA and OPI groups. It is well known that leukocytes express varying density of β_2-AR's and therefore some leukocyte subtypes are more responsive to the catecholamine secretion that accompanies exercise than others [39, 41]. Intermediate and non-classical monocytes (i.e. CD16+ monocytes) exhibit significant mobilization into the circulation with acute exercise as a result of β_2-AR signaling [41, 43]. Upper body-based exercises elicit greater sympathetic nervous system (SNS) activity [102] and catecholamine secretion compared to lower body exercise [103]. The RE protocol used in the present study incorporated both upper and lower body exercises, whereas the CRE protocol was primarily lower body (walking). Thus, although not directly measured in the present study, it is possible that RE elicited a greater catecholamine response than CRE, which would help to explain the more robust cellular mobilization to RE.

At the recovery time point following CRE, most monocyte subtypes had returned to, or were significantly below, their pre-exercise numbers in both OPA and OPI, which is in line with the widely reported biphasic response of leukocytes to acute exercise [55, 88]. However, in the OPI group only at the recovery time point following RE, many monocyte subtypes remained elevated compared either to pre-exercise numbers, or to their response to CRE. In other words in OPI individuals, more monocytes remained in the circulation 1h following exercise cessation with RE and not CRE, suggesting a potential impairment in exercise recovery. According to the aforementioned hypothesis by Campbell and Turner (2018) whereby acute

exercise may enhance immunity, a prominent reason for immune enhancement is likely due to the redistribution of leukocytes with high effector functions and inflammatory potential to mucosal barriers and other systemic locations like the lymphatic system where they are most likely to encounter a potential threat [79]. However, if these monocyte subpopulations that express surface markers implicated in increased inflammation remain in the circulation, which they appear to be doing in OPI only at the recovery time point after RE, it is possible that they are not being adequately redistributed to the locations in which they might be needed to mount a robust immune or inflammatory response. The prolonged elevation in monocyte subtypes 1h following exercise cessation may additionally be attributed to differences in hormones known to impact leukocyte mobilization and egress (i.e. catecholamines and cortisol) that persisted into the recovery period in OPI, and should be examined in future work.

Cortisol plays a role in monocyte egress from the blood compartment following exercise [62], however changes in cortisol do not adequately explain these findings since cortisol did not differ between CRE and RE or between OPA and OPI. Specifically, neither CRE nor RE produced a significant increase in cortisol in OPA or OPI. Cortisol decreased following exercise, and again into the recovery period for both groups, likely a result of diurnal variations [104] since all exercise bouts in the present study were performed in the morning and both CRE and RE trials were completed at the same time of day for each participant.

Results from the LPS-stimulation assays showed an increase in IL-6 production at the recovery time point in OPI following RE. IL-6 is often viewed as a

pro-inflammatory cytokine, and increases in IL-6 production by monocytes has been implicated in aging and increased systemic inflammation [105]. Furthermore, although literature on LPS-stimulated cytokine response to acute exercise is lacking, longitudinal studies implementing regular exercise programs have reported reductions in LPS-stimulated IL-6 production [60]. Therefore, increased LPS-stimulated IL-6 production at the recovery time point in OPI after RE supports the notion of a potential impairment in the recovery from exercise and potentially increased inflammation.

In both OPA and OPI, LPS-stimulation elicited an increase in TNF production immediately following CRE but not RE, and the TNF response to LPS stimulation was significantly greater in CRE compared to RE. This was unexpected, particularly since RE resulted in a greater mobilization of CD16+ monocytes than CRE in both OPA and OPI groups. This means that the circulating numbers of intermediate and non-classical monocytes cannot fully explain the differences in the inflammatory response post-exercise. However, since TNF is produced as a result of LPS acting on the TLR4 signaling pathway of monocytes [29], it is possible that RE mobilized fewer TLR4-expressing monocytes compared to CRE. However, TLR4 was not measured in the present study, which serves as a limitation. The cellular data indicated that RE mobilized more monocyte subpopulations expressing surface receptors that are related to promoting inflammation than CRE; therefore, potential differences between RE and CRE in mobilizing TLR4-expressing monocytes appears to be an insufficient explanation. Future research should aim to identify

mechanistic insight as to why CRE and RE are causing differential responses with regard to LPS-stimulated TNF secretion.

In the glucocorticoid assays, LPS stimulation was effective in eliciting a TNF and IL-6 response and all concentrations of dex used in the present study greatly blunted the stimulated IL-6 and TNF response. The largest group and exercise differences were at the recovery time point. In OPA but not OPI, CRE caused a significant increase in TNF in the higher dose of dex (100 pg/mL LPS + 10^{-6} M dex), while RE did not. Conversely, in OPI but not OPA, RE caused a significant increase in TNF in the intermediate dose of dex (100 pg/mL LPS + 10^{-7} M dex), while CRE did not. The group differences between TNF production at the recovery time point appear paradoxical. Repeating the experiment with lower concentrations of dex could help delineate meaningful differences between active and inactive groups and CRE compared to RE.

The dex concentrations in the glucocorticoid assay were selected because the protocol was adapted from Bower et al. (2007), who performed the assay using the same concentrations of cortisol [106]. However, the results in the present study were difficult to compare, as Bower et al. (2007) reported their results using transformed data and did not delineate between cytokine production at the various concentrations of glucocorticoid [106]. Nevertheless, some differences between the present study and Bower et al. (2007) are that Bower et al. (2007) used cortisol rather than dex in their glucocorticoid assay, and that they examined a population of cancer survivors [106], who may exhibit increased systemic inflammation compared to our cohort of healthy older adults. However, additional research will be necessary

in order to discern whether physical activity status and/or exercise modality play a role in LPS and glucocorticoid-mediated TNF production.

When examining glucocorticoid assay-mediated IL-6 production at post-exercise, the intermediate dose of dex (100 pg/mL LPS + 10^{-7} M dex) stimulated a greater increase in IL-6 production per monocyte following CRE compared to RE within the OPA group only, with no significant changes with exercise within OPI. This reflects the general trend observed with the functional assays of the present study of blunted IL-6 production per monocyte at the post-exercise time point.

Taken together, the results from the present study provide novel insight into the potential influence of exercise modality and physical activity status on the cellular monocyte and pro-inflammatory responses of monocytes among older adults. We showed that monocytes that express surface receptors implicated in perpetuating pro-inflammatory conditions are mobilized into the circulation to a greater extent immediately following RE compared to CRE in both OPA and OPI, but OPI may exhibit impaired exercise recovery at 1h post-exercise following RE. The results from the functional assays suggest that the pro-inflammatory response in both OPA and OPI following RE may be lower than CRE, as evidenced by lower TNF production with LPS-stimulation, and that glucocorticoid receptor sensitivity did not differ between OPA and OPI, but that it may be influenced by acute exercise and exercise modality. In summary, moderate-intensity RE may operate via different physiological mechanisms compared to CRE to elicit cellular and functional monocyte responses, and physically inactive individuals may not recover from RE in the same way as their active counterparts.

Table 2.1: The effects of an acute bout of cardiorespiratory (CRE) or resistance (RE) exercise on circulating numbers of monocytes and monocyte subsets expressing surface markers CD11b, CCR5, and CX3CR1 in older physically active (OPA) and older physically inactive (OPI) participants. Blood samples were taken immediately prior to exercise (pre), immediately upon exercise cessation (post) and after 1h of rest following exercise cessation (recovery) during both CRE and RE trials.

		OPA		**OPI**	
		CRE (Mean ± SE)	**RE** (Mean ± SE)	**CRE** (Mean ± SE)	**RE** (Mean ± SE)
Total Monocytes (cells/uL)					
(CD14+/++)	Pre	242 ± 27	236 ± 27	224 ± 27	216 ± 27
	Post	299 ± 27*	310 ± 27*	261 ± 27*	309 ± 27*^
	Recovery	227 ± 27	230 ± 27	211 ± 27	245 ± 28^
CD11b+ Classical (cells/uL)					
(CD14++ CD16- CD11b+)	Pre	160 ± 22	162 ± 22	174 ± 22	166 ± 22
	Post	194 ± 22*	208 ± 22*	207 ± 22*	239 ± 22*^
	Recovery	146 ± 22	161 ± 22	167 ± 23	179 ± 23
CD11b+ Intermediate (cells/uL)					
(CD14++ CD16+ CD11b+)	Pre	23 ± 5	31 ± 5	17 ± 5	15 ± 5
	Post	24 ± 5	36 ± 5*	15 ± 5	21 ± 5^
	Recovery	16 ± 5*	25 ± 5*^	10 ± 5	17 ± 5
CD11b+ Non-classical (cells/uL)					
(CD14+ CD16+ CD11b+)	Pre	5 ± 2	7 ± 2	7 ± 2	6 ± 2
	Post	8 ± 2*	11 ± 2*	8 ± 2*	10 ± 2*
	Recovery	5 ± 2	5 ± 2	7 ± 2	8 ± 2
CCR5+ Classical (cells/uL)					
(CD14++ CD16- CCR5+)	Pre	22 ± 10	38 ± 10	53 ± 10	38 ± 10
	Post	24 ± 10	48 ± 10*	56 ± 10	50 ± 10*
	Recovery	17 ± 10*	34 ± 10	40 ± 11*	40 ± 11^
CCR5+ Intermediate (cells/uL)					
(CD14++ CD16+ CCR5+)	Pre	8 ± 2	15 ± 2	7 ± 2	6 ± 2
	Post	9 ± 2	18 ± 2*	7 ± 2	10 ± 2*^
	Recovery	5 ± 2*	11 ± 2	5 ± 3*	8 ± 3^
CCR5+ Non-classical (cells/uL)					
(CD14+ CD16+ CCR5+)	Pre	1 ± 1	1 ± 1	2 ± 1	2 ± 1
	Post	1 ± 1	3 ± 1*	3 ± 1*	3 ± 1*
	Recovery	1 ± 1	1 ± 1	2 ± 1	3 ± 1*
CX3CR1+ Classical (cells/uL)					
(CD14++ CD16- CX3CR1+)	Pre	180 ± 22	171 ± 22	184 ± 22	176 ± 22
	Post	216 ± 22*	219 ± 22*	214 ± 22*	251 ± 22*^
	Recovery	175 ± 22	173 ± 22	176 ± 22	198 ± 22^
CX3CR1+ Intermediate (cells/uL)					
(CD14++ CD16+ CX3CR1+)	Pre	24 ± 5	31 ± 5	17 ± 5	15 ± 5
	Post	25 ± 5	37 ± 5*	15 ± 5	21 ± 5*^
	Recovery	18 ± 5*	26 ± 5^	10 ± 5*	17 ± 5^
CX3CR1+ Non-classical (cells/uL)					
(CD14+ CD16+ CX3CR1+)	Pre	10 ± 3	13 ± 3	13 ± 3	12 ± 3

	Post	15 ± 3*	21 ± 3*	18 ± 3*	20 ± 3*
	Recovery	9 ± 3*	11 ± 3	12 ± 3	18 ± 3*

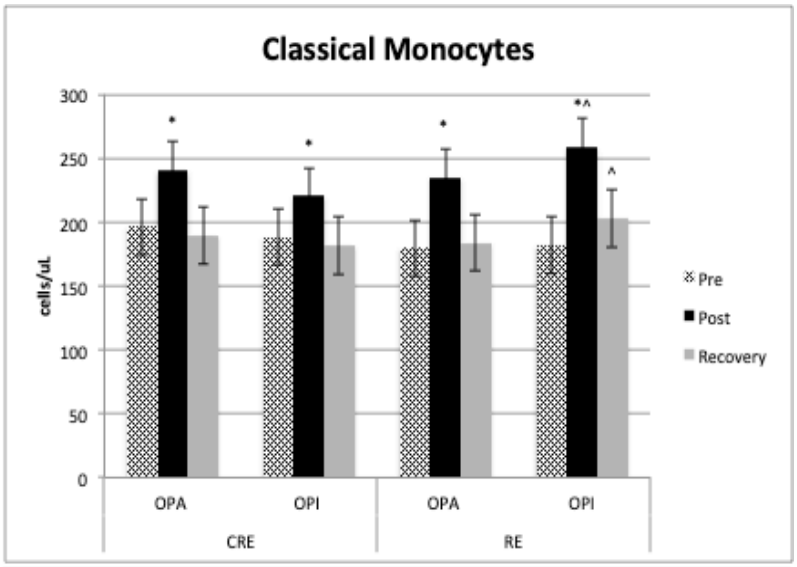
* denotes significant within-group difference compared to pre-exercise ($p<0.05$)
^ denotes significant within-group differences from the same time point in CRE compared to RE while controlling for pre-exercise values ($p<0.05$)
# denotes significant difference at pre-exercise between OPA and OPI groups ($p<0.05$)

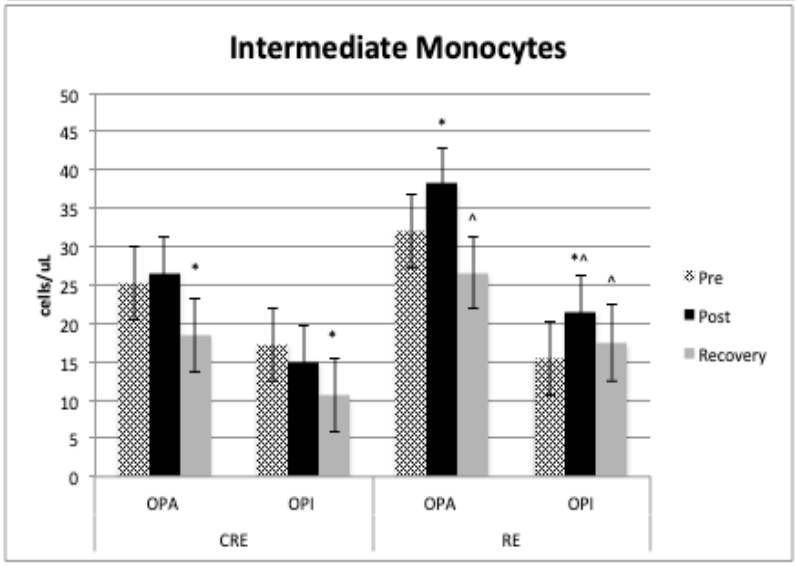
Table 2.2: The effects of an acute bout of cardiorespiratory (CRE) or resistance (RE) exercise on circulating monocyte subset median fluorescence intensity (MFI) for CD11b, CCR5, and CX3CR1 in older physically active (OPA) and older physically inactive (OPI) participants. Blood samples were taken immediately prior to exercise (pre), immediately upon exercise cessation (post) and after 1h of rest following exercise cessation (recovery) during both CRE and RE trials. Data are expressed in arbitrary units.

		OPA		OPI	
		CRE (Mean ± SE)	**RE** (Mean ± SE)	**CRE** (Mean ± SE)	**RE** (Mean ± SE)
CD 11b+ Classical					
(CD14++ CD16- CD11b+)	Pre	160 ± 22	162 ± 22	174 ± 22	166 ± 22
	Post	194 ± 22*	208 ± 22^	207 ± 22	239 ± 22
	Recovery	147 ± 22*	161 ± 22*	167 ± 23	179 ± 23*
CD11b+ Intermediate					
(CD14++ CD16+ CD11b+)	Pre	29 ± 4	33 ± 4	38 ± 4	38 ± 4
	Post	27 ± 4	34 ± 4	35 ± 4	37 ± 4
	Recovery	23 ± 4*	29 ± 4*	35 ± 4	36 ± 4
CD11b+ Non-classical					
(CD14+ CD16+ CD11b+)	Pre	12 ± 1	13 ± 1	14 ± 1	12 ± 1
	Post	12 ± 1	14 ± 1	13 ± 1	12 ± 1
	Recovery	13 ± 1	12 ± 1	13 ± 1	12 ± 1
CCR5+ Classical					
(CD14++ CD16- CCR5+)	Pre	7 ± 1	6 ± 1	7 ± 1	8 ± 1
	Post	8 ± 1	6 ± 1	7 ± 1	7 ± 1
	Recovery	7 ± 1	6 ± 1	8 ± 1	8 ± 1
CCR5+ Intermediate					
(CD14++ CD16+ CCR5+)	Pre	13 ± 3	16 ± 3	13 ± 3	13 ± 3
	Post	11 ± 3	12 ± 3	11 ± 3	12 ± 3
	Recovery	13 ± 3	15 ± 3	15 ± 3	12 ± 3
CCR5+ Non-classical					
(CD14+ CD16+ CCR5+)	Pre	12 ± 5	14 ± 5	14 ± 5	16 ± 5
	Post	15 ± 5	18 ± 5*	11 ± 5	16 ± 5
	Recovery	12 ± 5	20 ± 5^	13 ± 5	14 ± 5
CX3CR1+ Classical					
(CD14++ CD16- CX3CR1+)	Pre	11 ± 2	12 ± 2	16 ± 2	16 ± 2
	Post	10 ± 2	12 ± 2	15 ± 2	15 ± 2
	Recovery	11 ± 2	12 ± 2	17 ± 2	17 ± 2
CX3CR1+ Intermediate					
(CD14++ CD16+ CX3CR1+)	Pre	27 ± 7	27 ± 7	41 ± 7	40 ± 7
	Post	28 ± 7	30 ± 7	41 ± 7	44 ± 7
	Recovery	28 ± 7	31 ± 7*	45 ± 7	46 ± 7*^
CX3CR1+ Non-classical					
(CD14+ CD16+ CX3CR1+)	Pre	40 ± 4	43 ± 4	55 ± 4	52 ± 4
	Post	39 ± 4	41 ± 4	56 ± 4	53 ± 4
	Recovery	42 ± 4	49 ± 4	56 ± 4	61 ± 4*
CCR2+ Classical					
(CD14++ CD16- CCR2+)	Pre	12 ± 2	14 ± 2	16 ± 2	16 ± 2
	Post	11 ± 2*	15 ± 2^	15 ± 2	15 ± 2*
	Recovery	12 ± 2	12 ± 2*	16 ± 2	16 ± 2

CCR2+ Intermediate					
(CD14++ CD16+ CD11b+)	Pre	15 ± 3	19 ± 3	18 ± 3	18 ± 3
	Post	14 ± 3	18 ± 3	19 ± 3	16 ± 3
	Recovery	14 ± 3	18 ± 3	20 ± 3	18 ± 3
CCR2+ Non-classical					
(CD14+ CD16+ CD11b+)	Pre #	8 ± 4	7 ± 4	22 ± 4	19 ± 4
	Post	11 ± 4	14 ± 4	12 ± 4	20 ± 4^
	Recovery	8 ± 4	9 ± 4	18 ± 4	23 ± 4

* denotes significant within-group difference compared to pre-exercise ($p<0.05$)
^ denotes significant within-group differences from the same time point in CRE compared to RE while controlling for pre-exercise values ($p<0.05$)
# denotes significant difference at pre-exercise between OPA and OPI groups ($p<0.05$)





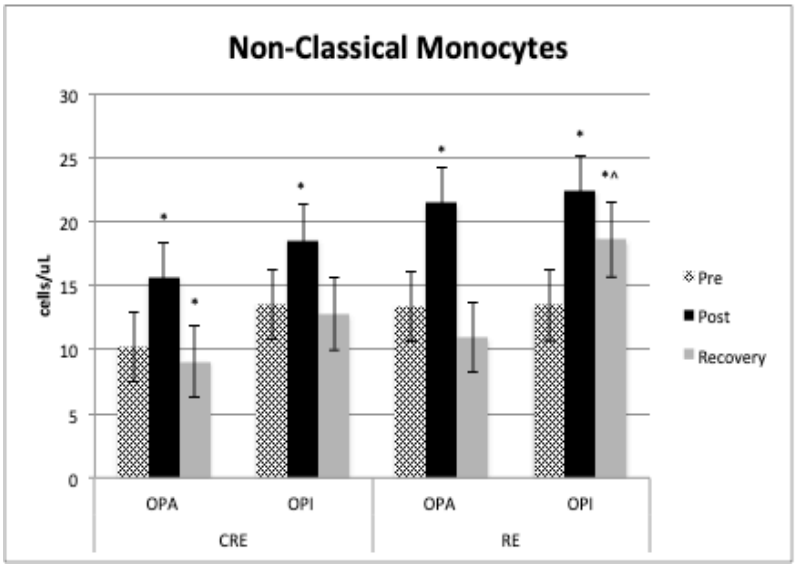


Figure 2.1: Classical, intermediate, and non-classical monocytes at pre-exercise, post-exercise, and recovery in response to cardiorespiratory training (CRE) and resistance training (RE) in older physically active (OPA) and older physically inactive (OPI) groups

* denotes significant difference compared to pre-exercise ($p<0.05$)
^ denotes significant within-group differences from the same time point in CRE while controlling for pre-

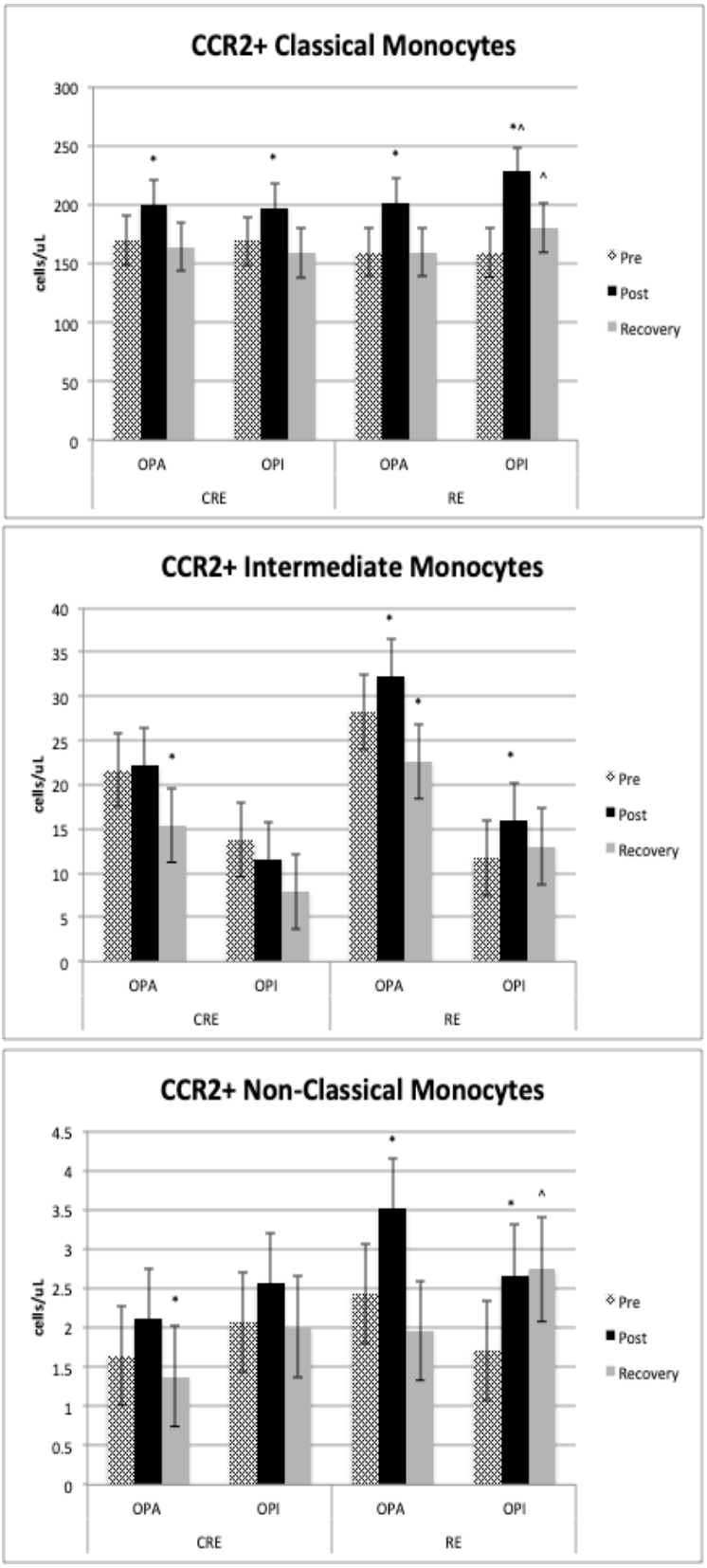


Figure 2.2: Circulating numbers of CCR2+ classical, intermediate, and non-classical monocytes at pre-exercise, post-exercise, and recovery in response to cardiorespiratory training (CRE) and resistance training (RE) in older physically active (OPA) and older physically inactive (OPI) groups

* denotes significant difference compared to pre-exercise ($p<0.05$)
^ denotes significant within-group differences from the same time point in CRE while controlling for pre-exercise values ($p <0.05$)

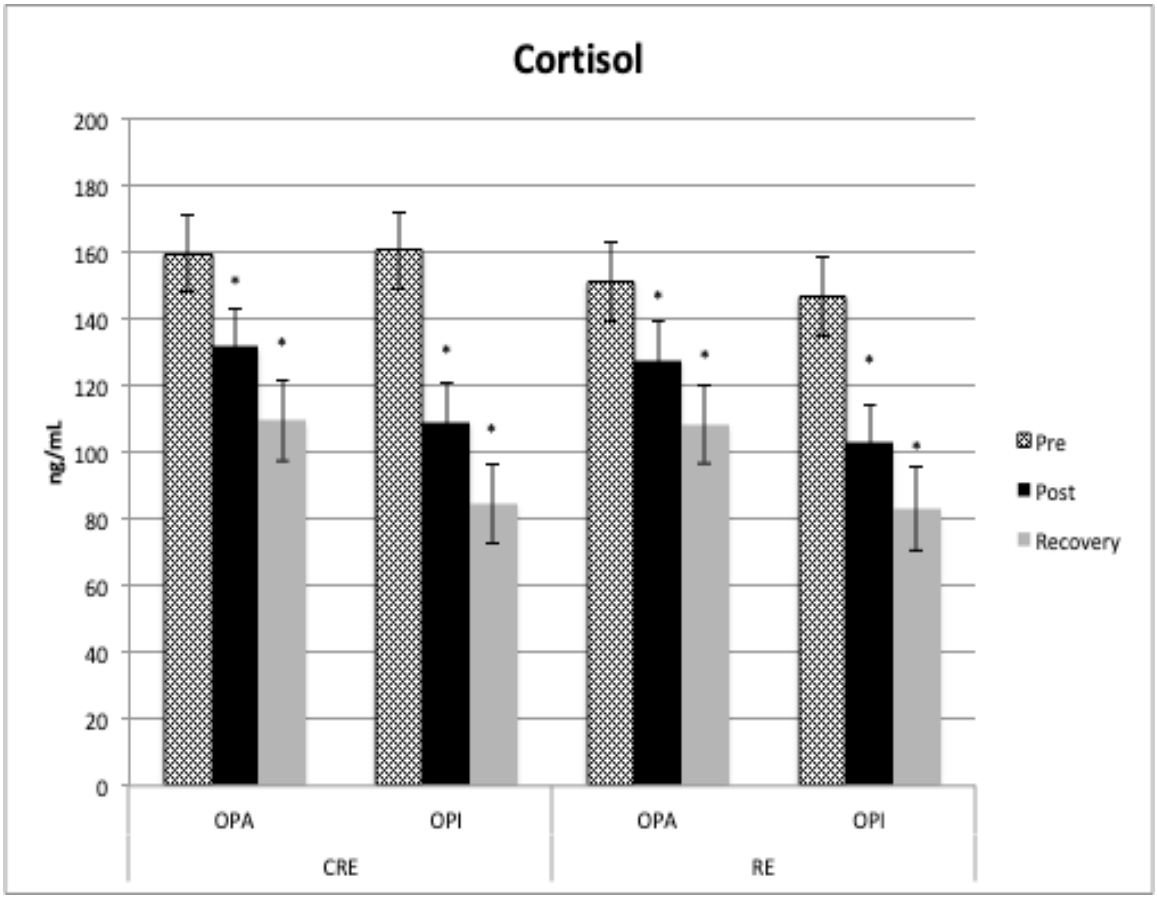


Figure 2.3: Serum cortisol at pre-exercise, post-exercise, and recovery in response to cardiorespiratory (CRE) and resistance (RE) exercise in older physically active (OPA) and older physically inactive (OPI) groups

* Indicates significant difference compared to pre values ($p < 0.05$)

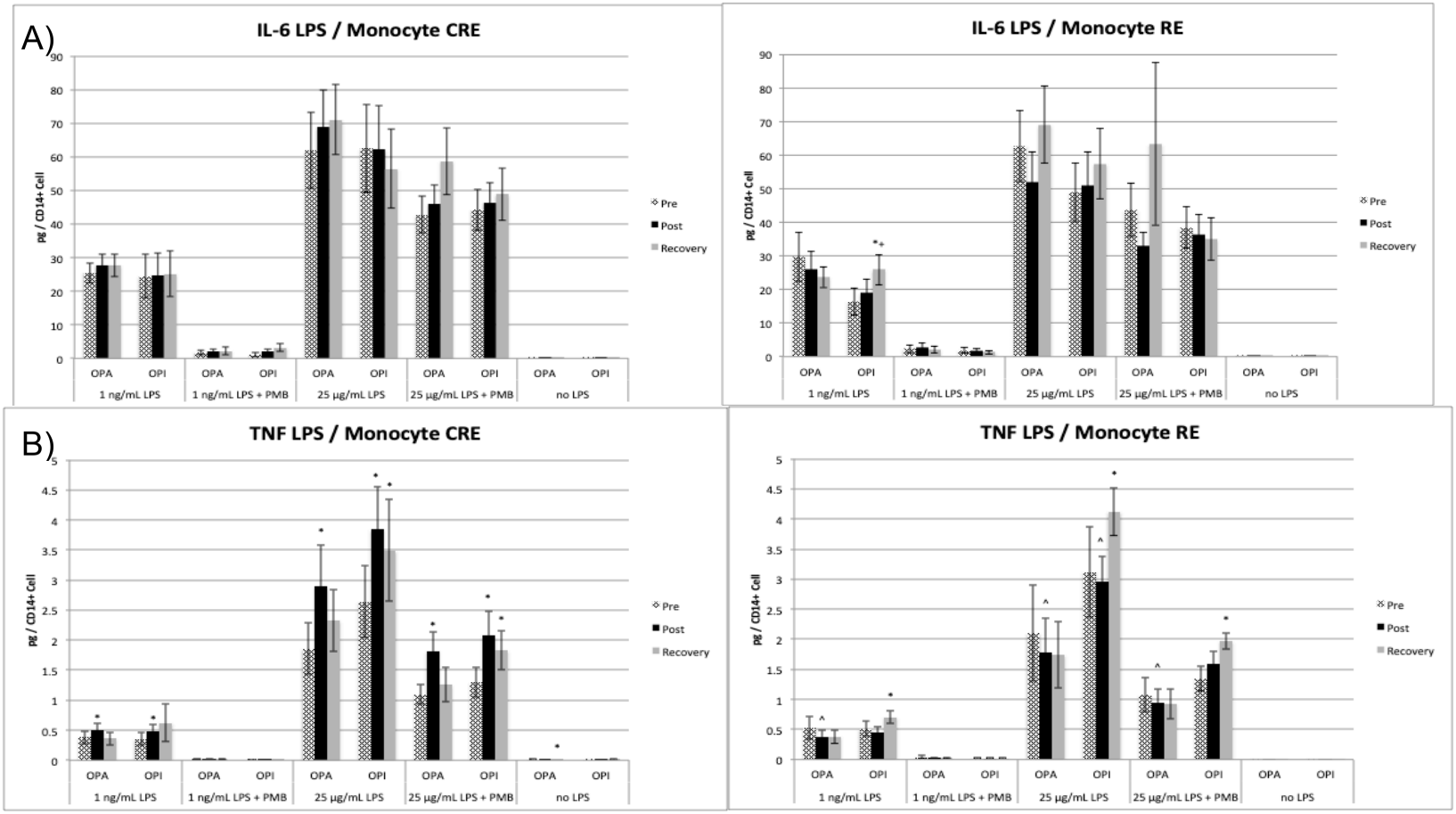


Figure 2.4: IL-6 and TNF production in response to whole blood lipopolysaccharide (LPS) stimulation with various concentrations of LPS and polymixin B (PMB) in older physically active (OPA) and older physically inactive (OPI) participants. Blood samples were taken immediately prior to exercise (pre), immediately upon exercise cessation (post) and after 1h of rest following exercise cessation (recovery) during both CRE and RE trials.

* denotes significant difference compared to pre-exercise ($p<0.05$)
^ denotes significant within-group differences from the same time point in CRE while controlling for pre-exercise values ($p <0.05$)
+ denotes significant 3-way interaction between group, exercise, and time

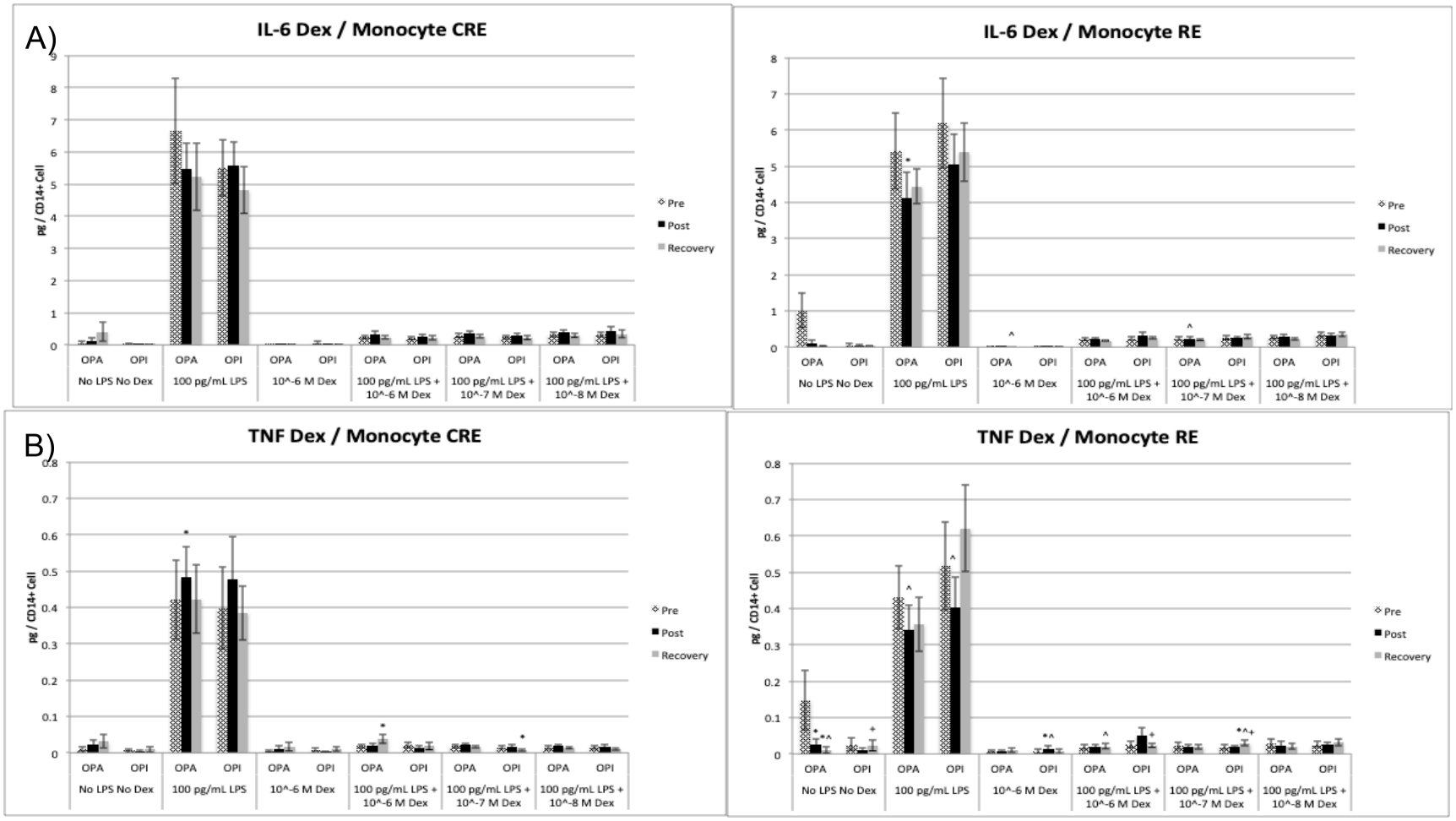


Figure 2.5: IL-6 and TNF production in response to whole blood glucocorticoid (dexamethasone; dex)stimulation with various concentrations of dex and 100pg/mL lipopolysaccharide (LPS) in older physically active (OPA) and older physically inactive (OPI) participants. Blood samples were taken immediately prior to exercise (pre), immediately upon exercise cessation (post) and after 1h of rest following exercise cessation (recovery) during both CRE and RE trials.

* denotes significant difference compared to pre-exercise ($p<0.05$)
^ denotes significant within-group differences from the same time point in CRE while controlling for pre-exercise values ($p <0.05$)
+ denotes significant 3-way interaction between group, exercise, and time

CHAPTER FIVE

Discussion

Physical inactivity is associated with increased systemic inflammation and a variety of pro-inflammatory diseases such as CVD, diabetes, arthritis, and obesity. Aging is similarly associated with these conditions, although increased inflammation is likely more related to age-associated increases in sedentary behavior and its ramifications than to the biological effects of aging itself [2]. Thus, it is recommended to maintain a physically active lifestyle to mitigate the deleterious effects of physical inactivity and aging on immunological properties and functioning. Remaining physically active through the aging process therefore serves as a multifaceted means of facilitating longer, healthier lives among older adults [5]. The present study examined resting (pre-exercise) T-cell, monocyte, and inflammatory characteristics of older adults who were either physically active (OPA) or physically inactive (OPI). Additionally, the present study examined how each of these variables responded to acute cardiorespiratory (CRE) compared to resistance (RE) exercise both immediately following exercise cessation (post-exercise) and 1h after exercise (recovery).

Aging is associated with a variety of unfavorable immunological changes that alter the characteristics and the functional abilities of the immune system [5]. While it has been proposed that many of these negative characteristics are exacerbated by physical inactivity [2], immunosenescence is a canopy term that is often used to describe specifically an age-related decline in immunity [5]. Indeed, both innate and adaptive arms of the immune system are subject to age-related changes that

contribute to the development of chronic disease, increased susceptibility to infection, and reduced vaccine efficacy [5].

Subject Characteristics and Exercise Testing Measures

All participants in both OPA and OPI groups completed both CRE and RE protocols and participant characteristics and exercise data are displayed in Tables 1.1 and 1.2. Participants in OPA and OPI groups did not differ among age, body weight, BMI, or resting BP. Significant differences in physical characteristics between OPA and OPI were found for relative body fat and resting heart rate, whereby OPA was lower than OPI. Despite no significant differences in BMI between the groups, the significant differences in relative body fat may be due to the number of females in each group (OPI had more females), as the range of healthy body fat percentages for females are higher than that of males [81]. Additional group differences were reported in measures of skeletal muscle strength (predicted 1 RM for leg press, chest press, leg curl, lat pull down, weighted calf raise, triceps extension, and seated row), as well as for the volume of exercise performed on the RE testing day. It was surprising that OPA and OPI did not differ in strength on the leg extension or in predicted VO_{2max}, although the OPA group averaged ~10 mL/kg/min higher than OPI, which may be clinically meaningful despite lack of statistical significance.

Cellular Characteristics and Responses

As previously discussed (Chapter 2), T-cells, which are part of the adaptive immune system, have many important roles such as recognizing novel pathogens and mounting an immune response, as well as cytotoxic capabilities, which enable

them to wipe out invading antigens [19]. The success of T-cells in eradicating infection and maintaining host immunity is dependent, in part, on the diversity of the T-cell repertoire, which is also known to undergo age-related decline [17]. The innate immune system also undergoes changes that fall under the umbrella of immunosenescence. Monocytes are one cell type that have been shown to undergo age-related changes, namely among the proportions of its subsets (classical, intermediate, and non-classical; [27]). Specifically, aging is associated with an increase in the resting proportions of non-classical and intermediate monocytes, which express the CD16 surface marker and are heavily implicated in a variety of pro-inflammatory properties, and a reduction in classical monocytes, which have more anti-inflammatory capabilities than monocytes that express CD16 [27, 107].

There were several significant differences at rest (pre-exercise) between OPA and OPI groups. Namely, OPA had lower cell numbers of circulating CD57+ EMRA CD4+ T-cells, and lower CCR2 expression (MFI) on non-classical monocytes compared to OPI. Both CD57+ EMRA CD4+ T-cells and CCR2+ non-classical monocytes are known to express properties of elevated effector and inflammatory functions. This is significant, as lower circulating numbers of these particular cells may be indicative of better immune functioning and lower systemic inflammation [23, 99, 107, 108]. Furthermore, aging is associated with increased serum MCP-1 [107], the ligand for CCR2, and thus, if non-classical monocytes express more CCR2 as they did in OPI, it is possible that they are more heavily involved in extravasating the circulation and engaging in pro-inflammatory activity at the sites of MCP-1 signaling. As non-classical monocytes are implicated in CVD development and progression

[25], this data supports the idea that one way in which regular physical activity may prevent CVD is via attenuating the increases in CCR2 expression on non-classical monocytes that is observed in OPI compared to OPA.

In response to acute exercise, T-cell and monocyte dynamics were largely similar between OPA and OPI groups. Both CRE and RE exercise mobilized highly differentiated T-cells and monocytes with greater inflammatory capabilities into the circulation immediately post-exercise. This response has been consistently demonstrated throughout the exercise immunology literature [33, 41-43]. Specifically, both CRE and RE elicited a significant mobilization of EMRA CD8+ T-cells and CD57+ EMRA CD8+ T-cells, non-classical monocytes, and monocyte sub-populations expressing cell surface receptors CD11b, CCR5, CX3CR1, and CCR2, which are all implicated in conditions of impaired immunity and increased inflammation [17, 25, 99]. Furthermore, these cell types did not remain elevated in the circulation at the recovery time point, indicating that most of them egressed out of the circulation [55].

In an effort to debunk the long-standing open window theory, researchers have begun to accept the opinion that this preferential mobilization and subsequent egress of immune cells that express specific properties including increased effector functions, migratory capacity, and inflammatory potential with exercise contributes to heightened immunity rather than impaired immunity [79]. This emerging hypothesis postulates that there is a physiological advantage to mobilizing immune cells with these specific characteristics into the circulation with exercise, as it primes the body to be able to mount a robust and effective response to any potential threat. Physical

exercise, which represents a situation of acute stress on the body, initiates the primal fight or flight response, in which the body prepares to either encounter a trauma, or to flee [33, 109]. Thus, using the analogy presented by Dhabhar (2009), the initiation of an acute stressor (such as exercise) stimulates the “soldiers” (leukocytes with discrete properties) to leave their “barracks” (sites where leukocytes typically reside such as the lungs, spleen, or other marginal pools), and enter the “boulevards” (blood vessels), where they can be quickly and effectively delivered to any site of antigen invasion or trauma that would require an immune response [109].

According to this hypothesis of enhanced immunity following acute exercise, the preferential response of T-cells and monocytes with discrete phenotypes and properties with exercise provides the body with the opportunity to redistribute these cells to locations that are most likely to encounter a potential threat, such as the lungs and gut, as well as to the bone marrow where senescent cells can undergo apoptosis and stimulate the production of new immune cells [79]. Thus, even when leukocyte numbers decrease below baseline in the minutes and hours following exercise cessation, it is likely that the individual remains at a heightened state of immune competency [79]. While there are a plethora of published works that suggest an “open window”, or a period of increased susceptibility to opportunistic infection following acute exercise [61, 88], there are a variety of methodological concerns regarding these reports, and there is mounting evidence to suggest that acute exercise is more likely to enhance immunity in the recovery period [79]. For example, re-examining the body of previous literature that reported increased incidences of URTI following acute vigorous exercise has led researchers to suggest

that most URTI symptoms reported in these studies were unlikely to be indicative of infection, and those that were, were more likely a result of an individual's wellbeing independent of the exercise, or the environment in which they performed the exercise (i.e. sporting event that involves travel and/or large crowds) [79]. This means that the engaging in physical exercise may have acute in addition to chronic benefits on immunity.

While OPA and OPI had largely similar responses to exercise, there were a few notable differences between CRE and RE. Namely, at the recovery time point following RE, several T-cell and monocyte subtypes remained elevated, particularly within the OPI group either compared to pre-exercise (EM CD4+ T-cells and CM CD8+ T-cells, non-classical, CCR5+ non-classical, and CX3CR1+ non-classical monocytes) or compared to their response to CRE (CD57+ EMRA CD8+ T-cells, total monocytes, classical, intermediate, and non-classical monocytes, CCR5+ classical and intermediate monocytes, CX3CR1+ classical and intermediate, and CCR2+ classical and non-classical monocytes). This may suggest an impaired recovery from the exercise bout in OPI. Tying these findings into the hypothesis of enhanced immunity in the window of recovery following acute exercise, it appears that in OPI, these cell types are not being redistributed during exercise recovery as efficiently as in OPA. This would mean that in OPI, fewer immune cells that possess elevated effector functions are re-distributed to the mucosal barriers following exercise, which may impair the effectiveness of the immune response to a potential antigenic threat. Future research should aim to examine the mechanisms of cellular egress following exercise and why this may be different in physically inactive

individuals, particularly when the mobilization of these cells was not significantly different from their active counterparts.

Additionally, RE was responsible for mobilizing more T-cell and monocyte subtypes into the circulation post-exercise than CRE. For T-cells, this was true only for the OPI group. However, for monocytes, this was true for both groups. The greater mobilization of T-cells and monocytes with RE was somewhat surprising, particularly among the OPA group, as we hypothesized that the magnitude of cell mobilization would be greater following CRE. The reason for this hypothesis was because the primary mechanisms responsible for exercise-induced leukocyte mobilization are increases in hemodynamic shear stress and catecholamine secretion [38]. Generally speaking, the discontinuous nature of resistance training is thought to elevate shear stress to a lesser degree than continuous moderate-intensity cardiovascular exercise [110], and we expected catecholamine response to be similar or lower with RE compared to CRE especially in OPA, who are accustomed to RE. Nevertheless, the results indicate that RE mobilized more cells than CRE in both groups. Aside from measuring catecholamine levels, a potential explanation for this includes insufficient training experience among the OPA group with the specific stacked weight machines used in the RE protocol, thereby resulting in OPA participants reacting to the RE protocol as if it were a novel type of training. Nevertheless, this does not explain why similar group differences were not observed among T-cell populations. The difference in T-cell mobilization between OPA and OPI participants with RE may be due to differences in catecholamine secretion. Although not directly measured in the present study, it is likely that OPI participants

secreted more catecholamines than OPA in response to RE, thereby eliciting the mobilization of more T-cell subsets.

Exercise Did Not Increase Cortisol in Older Adults

Serum cortisol was measured at all time points for both CRE and RE in both OPA and OPI. With both CRE and RE in both OPA and OPI groups, serum cortisol significantly decreased from pre-exercise to post-exercise, indicating that the exercise stimulus was not sufficient to stimulate cortisol secretion in a way that was detectable independent of diurnal variation. This is not entirely unexpected, as cortisol secretion is typically directly related to the intensity and duration of the exercise bout performed, with greater cortisol secretion at higher intensities and/or longer durations of exercise [111]. Indeed, Hill et al. (2008) examined the cortisol response to 30 minutes of exercise at various intensities and reported that moderate and high intensity (60% and 80% of maximal oxygen uptake (VO_{2max})) exercise resulted in significant increases in serum cortisol, but that low intensity exercise (40% VO_{2max}) resulted in a significant decrease in serum cortisol in a cohort of young adults [111]. The body of literature examining the effects of acute exercise intensity and duration on serum cortisol among older adults is lacking [112]. However, the present findings are in line with those of Heaney et al. (2013), who also reported a significant decrease in cortisol immediately post-exercise and 1h post-exercise in a cohort of older adults who were both trained and untrained [97]. It is therefore possible that the intensity thresholds may shift in older adulthood or that the intensities calculated in the present study (60-70% HRR for CRE and 70% estimated 1RM) under-predicted a true moderate to vigorous exercise intensity.

Inflammatory Responses

Chronic systemic inflammation serves as a hallmark feature of a variety of chronic diseases and is also often associated with aging [113]. Cytokines are key players in chronic inflammation and have many roles in its perpetuation and maintenance [113]. IL-6 and TNF are two such cytokines that are known to have potent inflammatory properties and are also released from monocytes, among other sources [29, 105]. TNF is strictly a pro-inflammatory cytokine while IL-6 is known to have both pro- and anti-inflammatory roles - particularly in regards to exercise [114, 115]. Specifically, chronic elevations in resting IL-6 are typically associated with increased systemic inflammation, whereas muscle-derived IL-6, which is released as a result of muscle contraction, is often associated with more anti-inflammatory effects [115]. It is unknown as to whether IL-6 that is released from monocytes following acute exercise is associated with more pro- or anti-inflammatory properties.

Responses to LPS Stimulation

LPS stimulation did not elicit many changes in IL-6 production per CD14+ monocyte in either OPA or OPI with CRE or RE. However, the IL-6 at the physiological dose of LPS was elevated at the recovery time point in OPI after RE, which represented a significant deviation from the OPA response. Although data on LPS-stimulated cytokine response to acute exercise is lacking, chronic exercise training is associated with reduced LPS-stimulated IL-6 production per monocyte [60]; therefore elevated IL-6 in the recovery from exercise may be considered unfavorable. However, more research is needed in order to derive any firm conclusions from these results. It can be speculated that RE provided more of a pro-

inflammatory stimulus to OPI compared to OPI due to the increased T-cell and monocyte mobilization in conjunction with increased LPS-stimulated IL-6 production.

Much more prominent differences were observed between CRE and RE with LPS-stimulated TNF secretion per CD14+ monocyte. Specifically, among both OPA and OPI, CRE resulted in an increase in TNF production, while RE demonstrated a decrease. Similar to IL-6, reductions in LPS-stimulated TNF production with chronic exercise training would likely be seen as favorable but have yet to be shown longitudinally [9, 60]. In a cohort of fatigued compared to non-fatigued breast cancer survivors, LPS-stimulated cytokine response to an acute psychological stressor increased in fatigued survivors and decreased in non-fatigued survivors [106], but the implications of this remain unknown. More research regarding the mechanisms behind the differential response to CRE compared to RE must be performed. Nevertheless, it appears that monocytes mobilized into the circulation immediately post-exercise with RE may exhibit fewer inflammatory properties than those mobilized with CRE. As there is much evidence to suggest that exercise exerts anti-inflammatory effects [63], the mechanisms behind the anti-inflammatory effects of exercise are not fully understood. These findings suggest that RE may have additional anti-inflammatory effects compared to CRE.

Responses to LPS + Dex Stimulation (Glucocorticoid Assays)

No differences between glucocorticoid stimulated IL-6 or TNF production per monocytes were found between OPA and OPI at pre-exercise. Such findings indicate that there were not differences in glucocorticoid sensitivity between OPA and OPI groups.

Similar to the LPS stimulation results for IL-6, the glucocorticoid assay also did not elicit many changes in IL-6 production per monocyte. Within OPA only, the intermediate dose of dex (100 pg/mL LPS + 10^{-7} M dex) at post-exercise was significantly lower after RE compared to CRE. Since this difference in IL-6 production with LPS and dex stimulation is present only at the post-exercise time point, the implications of these results remain to be determined. If, however, IL-6 production from monocytes following acute exercise is seen as unfavorable due to the chronic inflammatory potential of IL-6, then it may be that RE results in more anti-inflammatory effects than CRE. This finding, however, was only observed among OPA.

Regarding TNF production, the glucocorticoid assay confirmed the findings from the LPS stimulation, as evidenced by the LPS only results, which noted a significant difference between CRE and RE in both OPA and OPI groups, whereby TNF production was blunted post-exercise with RE. The major differences in glucocorticoid-stimulated TNF production were found at the recovery time point. At the recovery time point and 100 pg/mL LPS+ 10^{-6} M dex, TNF production increased in OPA only after CRE but not RE. However, at the same time point with 100 pg/mL LPS + 10^{-7} M dex, TNF increased in OPI only with RE but not CRE. Activity status and exercise modality appear to be important in influencing the glucocorticoid-stimulated TNF production per monocytes at both concentrations of dex (10^{-6} M and 10^{-7} M) at the recovery time point, as evidenced by the significant 3-way interaction effect. Nevertheless, the results appear opposite between the two concentrations. In order to confirm these findings and draw potential conclusions, the experiment

should be repeated, perhaps with lower concentrations of dex, as the difference between LPS-stimulated TNF production and TNF production with LPS and any amount of dex was quite large.

Findings from the functional assays are novel and informative, as this is the first study that functional assays were performed in a cohort of physically active and physically inactive older adults in response to acute exercise of different modalities. Nevertheless, previous research in older adults has reported that while aging was related to phenotypic shifts in monocytes, resting functional characteristics examined using various methodologies did not change [107]. It is possible, however, that even if resting functional properties of monocytes are better maintained throughout the aging process, functional differences following a stressor (i.e. exercise), may provide a more sensitive indication of age-related changes. However, insignificant findings were reported examining glucocorticoid sensitivity in a cohort of fatigued compared to non-fatigued breast cancer survivors following an acute psychological stressor [106]. In the referenced breast cancer survivors protocol, however, the researchers compared the pre-stress response to 30-minutes post-stress response, which is different from the sampling timeline used in the present study. It is possible, then, that changes in glucocorticoid sensitivity may have occurred in the cells present in the circulation immediately following the stressor, but were no longer present 30 minutes following the stressor due to monocyte extravasation from the circulation or adhesion to the endothelium. Taken together, it appears that there are no major differences in the glucocorticoid sensitivity of monocytes in OPA compared to OPI,

as their responses to prolonged (20h) elevations in glucocorticoid were largely the same.

Strengths and Limitations

The present study has a variety of strengths that arise as a result of its methodology. The complete crossover design whereby each participant completed both CRE and RE protocols in a randomized order makes this study the first of its kind in examining differences in exercise modality and the acute immune response in a cohort of older adults. Using the same participants for CRE and RE allows for direct comparison of the exercise modalities and adjustment for individual variation. The CRE and RE protocols themselves were matched for relative intensity (approximately 70%), and were designed according to ACSM recommendations to mimic standard exercise workouts typically performed by older adults. Furthermore, the strict inclusion / exclusion criteria, which eliminated those with comorbidities or medications that may confound the results, ensured that it was a relatively homogenous sample of older adults whose most prominent difference was activity level (physically active vs. physically inactive).

Nevertheless, the present study also carries several limitations. Because of the strict inclusion/exclusion criteria, both OPA and OPI groups were quite healthy, which might explain why we did not find many major group differences. For example, participants were carefully screened in order to exclude those who had chronic diseases often associated with physical inactivity, such as type 2 diabetes and obesity. Also as a consequence, the results are only generalizable to very healthy active and inactive older adults, thus excluding the majority of older adults who do

possess various comorbidities (i.e. diabetes, CVD, those on beta blockers or statins for high BP or high cholesterol, etc.). Additionally, latent CMV infection was not measured or controlled for in the present study. Latent CMV infection is known to influence the number of senescent T-cells, particularly CD8+ T-cells, without known effects on number or function of monocytes [116, 117]. Nevertheless, individuals both with and without CMV demonstrate a similar T-cell mobilization to exercise, the magnitude is often much larger in those who are CMV seropositive [38]. This was partially controlled for in the present study by using each participants' pre-exercise cell counts as a covariate when examining exercise responses. Furthermore, there was not an even distribution of males and females between the groups, which may serve as a confounding variable [118]. The sample sizes of 12 per group remain too small to examine sex differences at this time. There was also a large range in inclusion criteria for what qualified as physically active. We used ACSM recommendations for physical activity [81], which set a minimum amount of cardiorespiratory and resistance exercise that must be performed. However, some of the active participants focused more on one type of training than the other, and it remains to be determined whether there is a training specificity (i.e. strength athletes vs. endurance athletes) or a training threshold (physically active vs. very physically active) that may also influence the immune responses of older adults.

Directions for Future Research

Future work should aim to confirm the findings of the present study by employing a similar crossover study design. Controlling more tightly for physical activity level among the active individuals may add additional benefit, as it would be

interesting to see if there was a larger difference in resting and exercise immune and inflammatory characteristics among those who are highly physically active (as opposed to just meeting ACSM recommendations) compared to those who are physically inactive. Once immune and inflammatory responses to acute exercise have been confirmed, future work should aim to examine repeat exercise bouts, and ultimately the effects of chronic exercise training, particularly among OPI to determine whether implanting exercise training in older adults can yield positive immunological changes. Ultimately, the effects of CRE and RE on older adults with common co-morbidities (i.e. type 2 diabetes, obesity, CVD, high blood pressure, etc.) should also be examined to delineate additional mechanisms through which exercise may be able to offset or reverse these conditions.

Results from the present study suggest that while there may not be major differences in the cellular T-cell and monocyte response to acute exercise, there may be differences in the functional response to CRE compared to RE. Future work should aim to identify potential mechanisms for the differences in inflammatory properties found among the difference exercise modalities. Doing so will help to fill in essential gaps in the literature regarding the effects of resistance training on immune and inflammatory responses of older adults and help to inform future exercise prescription for this population.

Conclusion

Physical exercise is proposed as a means of countering the negative immunological and inflammatory changes that typically accompany aging and physical inactivity [2]. The results from the present study indicate that there are

indeed potentially meaningful differences in resting immune characteristics among OPA and OPI individuals, as evidenced by the findings of significant pre-exercise differences in CD57+ EMRA CD4+ T-cells and CCR2+ non-classical monocytes.

Furthermore, the cellular responses to exercise remained largely similar between OPA compared to OPI, and reflected previously reported information characterized by the significant mobilization of immune cells with high effector functions, migratory capacity, and inflammatory potential [41]. Although major differences between OPA and OPI were not observed, potential differences between CRE and RE were. Specifically, RE elicited a more robust cellular response than CRE, which is the first time this has been demonstrated in a crossover study design.

Similar findings were noted among the functional assays, whereby OPA and OPI groups demonstrated similar responses to each other, but responses to CRE compared to RE were different. TNF response to LPS-stimulation increased after exercise with CRE and decreased with RE.

Taken together, findings from the present study show that the cellular and inflammatory responses are very similar among healthy OPA and OPI individuals. Larger differences may exist between CRE compared to RE. These findings should be examined further with future research and may be used to help inform exercise prescription for the older adult by providing insight into the functional and inflammatory nature of the immune response to exercise. It is our hope that the findings from the present study contribute to the body of literature aimed at developing exercise prescriptions designed to maximize immunity and reduce chronic inflammation in older adults.

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APPENDICES

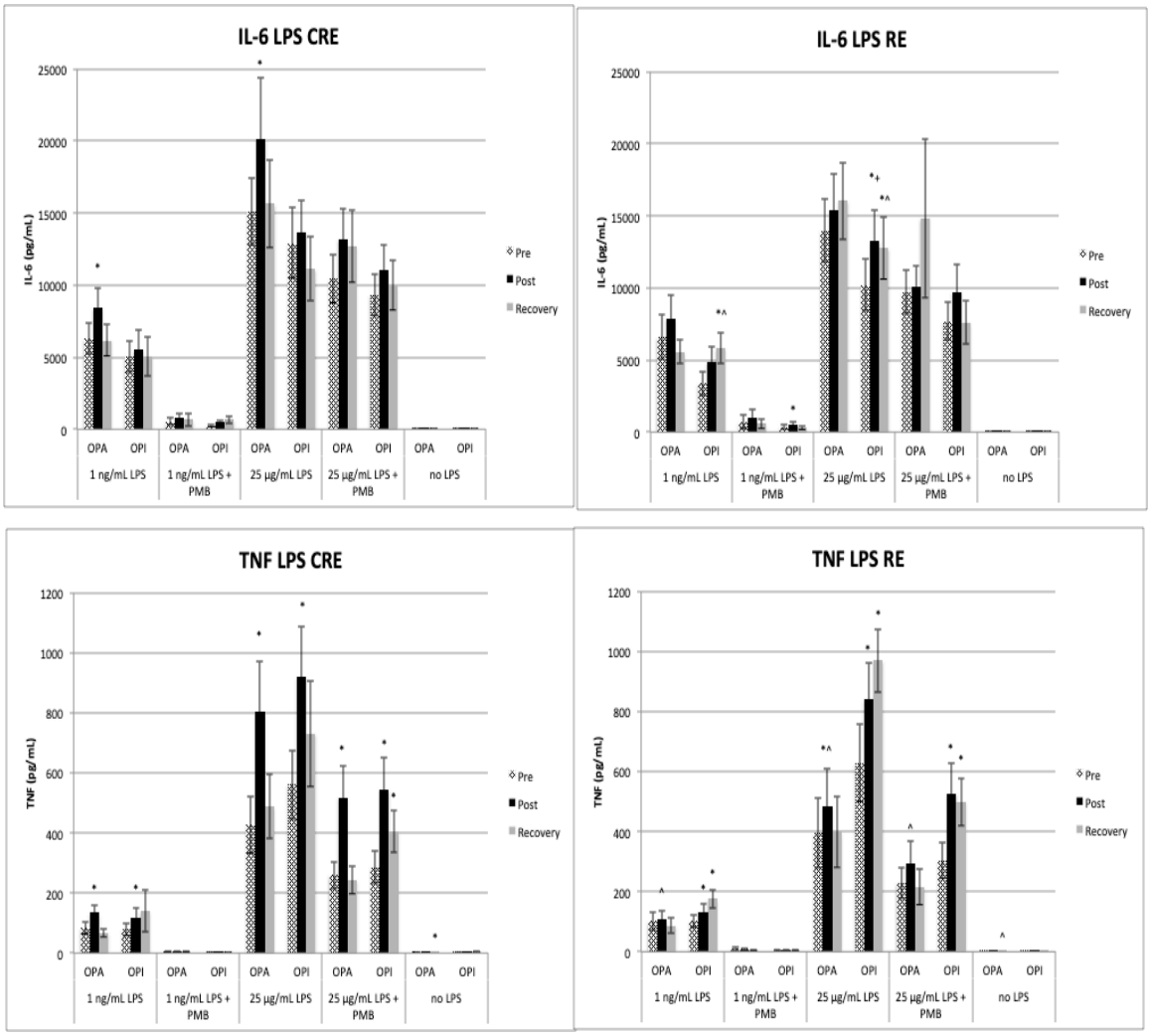

Figure S1: IL-6 and TNF production in response to whole blood lipopolysaccharide (LPS) stimulation with various concentrations of LPS and polymixin B (PMB) in older physically active (OPA) and older physically inactive (OPI) participants. Blood samples were taken immediately prior to exercise (pre), immediately upon exercise cessation (post) and after 1h of rest following exercise cessation (recovery) during both CRE and RE trials. The above data is not adjusted per CD14+ monocyte

* denotes significant difference compared to pre-exercise ($p<0.05$)
^ denotes significant within-group differences from the same time point in CRE while controlling for pre-exercise values ($p <0.05$)
+ denotes significant 3-way interaction between group, exercise, and time

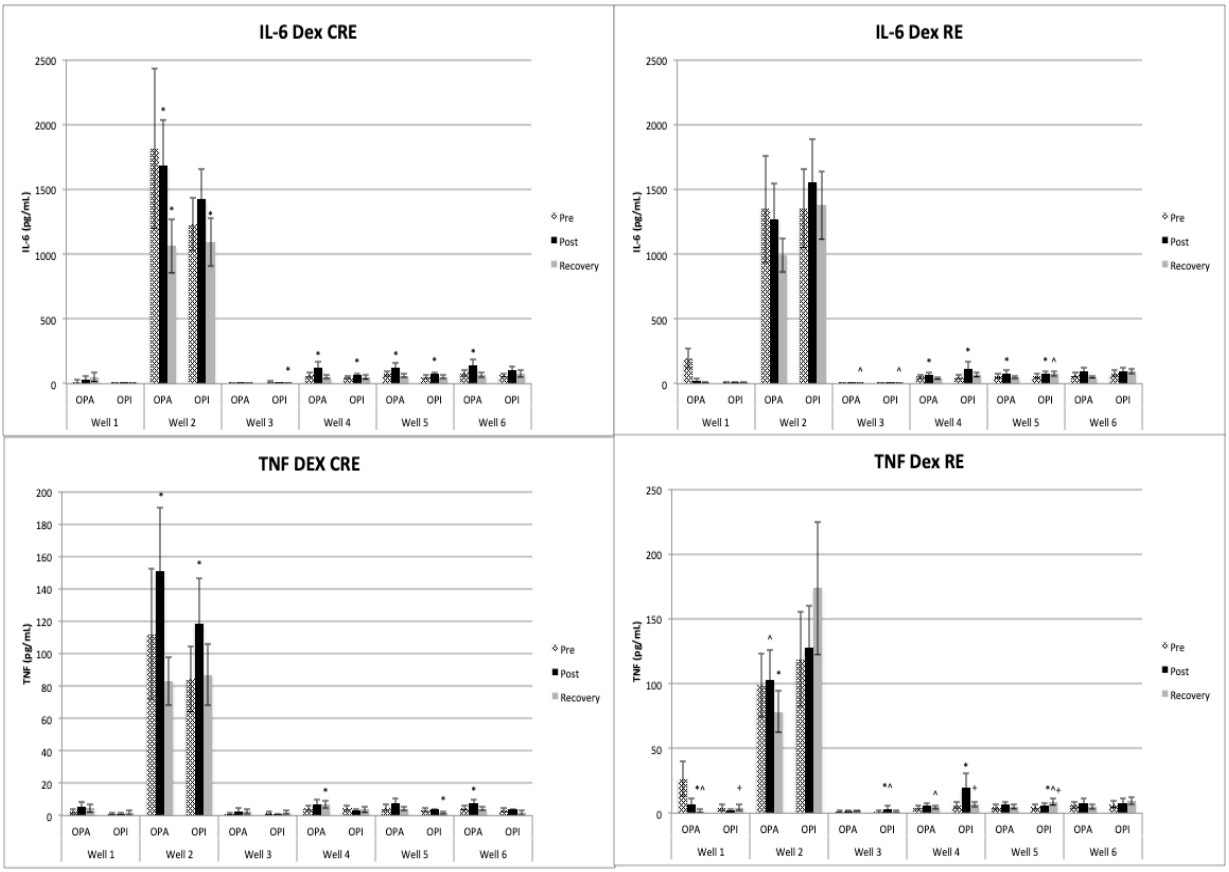

Figure S2: IL-6 and TNF production in response to whole blood glucocorticoid (dexamethasone; dex) stimulation with various concentrations of dex and 100pg/mL lipopolysaccharide (LPS) in older physically active (OPA) and older physically inactive (OPI) participants. Blood samples were taken immediately prior to exercise (pre), immediately upon exercise cessation (post) and after 1h of rest following exercise cessation (recovery) during both CRE and RE trials. The above data is not adjusted per CD14+ monocyte

* denotes significant difference compared to pre-exercise ($p<0.05$)
^ denotes significant within-group differences from the same time point in CRE while controlling for pre-exercise values ($p <0.05$)

Table S1: IL-6 Dex / Monocyte Data

Table S1: IL-6 Dex / Monocyte

	Well 1 (No LPS No Dex)		Well 2 (100 pg/mL LPS)		Well 3 (10^-6 M Dex)		Well 4 (100 pg/mL LPS + 10^-6 M Dex)		Well 5 (100 pg/mL LPS + 10^-7 M Dex)		Well 6 (100 pg/mL LPS + 10^-8 M Dex)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
OPA CRE Pre	0.06	0.06	6.65	1.63	0.00	0.00	0.25	0.06	0.29	0.06	0.31	0.07
OPA CRE Post	0.12	0.10	5.48	0.80	0.00	0.00	0.34	0.09	0.36	0.07	0.39	0.08
OPA CRE Recovery	0.41	0.29	5.22	1.04	0.00	0.00	0.24	0.06	0.27	0.05	0.29	0.06
OPA RE Pre	1.01	0.47	5.42	1.05	0.01	0.00	0.21	0.03	0.24	0.04	0.28	0.05
OPA RE Post	0.11	0.08	4.11	0.74 *	0.01	0.00	0.22	0.04	0.24	0.05 ^	0.28	0.07
OPA RE Recovery	0.03	0.01	4.44	0.49	0.01	0.00 ^^	0.18	0.02	0.21	0.03	0.22	0.03
OPI CRE Pre	0.02	0.02	5.50	0.87	0.06	0.04	0.21	0.05	0.24	0.06	0.31	0.07
OPI CRE Post	0.01	0.01	5.58	0.72	0.01	0.00	0.26	0.05	0.30	0.06	0.43	0.13
OPI CRE Recovery	0.00	0.00	4.81	0.72	0.00	0.00	0.21	0.06	0.22	0.06	0.34	0.12
OPI RE Pre	0.05	0.04	6.19	1.23	0.00	0.00	0.24	0.04	0.26	0.05	0.35	0.07
OPI RE Post	0.03	0.02	5.04	0.85	0.01	0.00	0.31	0.09	0.26	0.04	0.32	0.06
OPI RE Recovery	0.03	0.02	5.38	0.81	0.01	0.00	0.26	0.04	0.29	0.05	0.35	0.06

* denotes significant difference compared to pre-exercise (p<0.05). **(p<0.01)
^ denotes significant within-group differences from the same time point in CRE while controlling for pre-exercise values (p <0.05). ^^ (p<0.01)
+ denotes significant 3-way interaction between group, exercise, and time (p<0.05). ++ (p<0.01)

Table S2: TNF Dex / Monocyte Data

Table S2: TNF Dex / Monocyte

	Well 1 (No LPS No Dex)			Well 2 (100 pg/mL LPS)			Well 3 (10^-6 M Dex)			Well 4 (100 pg/mL LPS + 10^-6 M Dex)			Well 5 (100 pg/mL LPS + 10^-7 M Dex)			Well 6 (100 pg/mL LPS + 10^-8 M Dex)		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
OPA CRE Pre	0.01	0.01		0.42	0.11		0.01	0.00		0.02	0.01		0.02	0.01		0.01	0.00	
OPA CRE Post	0.02	0.01		0.48	0.08	*	0.01	0.01		0.02	0.01		0.02	0.01		0.02	0.00	
OPA CRE Recovery	0.03	0.02		0.42	0.09		0.02	0.01		0.04	0.01	*	0.02	0.00		0.01	0.00	
OPA RE Pre	0.15	0.08		0.43	0.09		0.01	0.00		0.02	0.01		0.02	0.01		0.03	0.01	
OPA RE Post	0.03	0.02	*	0.34	0.07	^^	0.01	0.00		0.02	0.01		0.02	0.01		0.02	0.01	
OPA RE Recovery	0.01	0.01	**^^	0.36	0.07		0.01	0.01		0.02	0.01	^	0.02	0.01		0.02	0.01	
OPI CRE Pre	0.01	0.00		0.40	0.11		0.01	0.00		0.02	0.01		0.01	0.00		0.02	0.00	
OPI CRE Post	0.00	0.00		0.48	0.12		0.00	0.00		0.01	0.00		0.02	0.01		0.02	0.00	
OPI CRE Recovery	0.01	0.01		0.38	0.07		0.01	0.01		0.02	0.01		0.01	0.00	**	0.01	0.00	
OPI RE Pre	0.03	0.02		0.52	0.12		0.01	0.01		0.03	0.01		0.02	0.01		0.03	0.01	
OPI RE Post	0.01	0.01		0.40	0.08	^	0.01	0.01	*^	0.05	0.02		0.02	0.01		0.02	0.01	
OPI RE Recovery	0.02	0.02	++	0.62	0.12		0.01	0.01		0.02	0.01	+	0.03	0.01	*^^++	0.03	0.01	

* denotes significant difference compared to pre-exercise (p<0.05). **(p<0.01)

^ denotes significant within-group differences from the same time point in CRE while controlling for pre-exercise values (p <0.05). ^^ (p<0.01)

+ denotes significant 3-way interaction between group, exercise, and time (p<0.05). ++ (p<0.01)

Table S3: IL-6 LPS / Monocyte Data

Table S3: IL-6 LPS / Monocyte

	Well 1 (1 ng/mL LPS)			Well 2 (1 ng/mL LPS + PMB)		Well 3 (25 µg/mL LPS)		Well 4 (25 µg/mL LPS + PMB)		Well 5 (no LPS)	
	Mean	SE		Mean	SE	Mean	SE	Mean	SE	Mean	SE
OPA CRE Pre	25.52	3.52		1.79	0.68	61.87	6.10	42.76	5.10	0.01	0.00
OPA CRE Post	27.86	3.01		2.02	0.72	68.80	11.24	46.07	5.55	0.01	0.01
OPA CRE Recovery	27.68	3.43		2.24	1.22	71.04	10.36	58.71	9.96	0.01	0.00
OPA RE Pre	29.66	7.22		2.31	1.18	62.72	10.64	43.73	7.91	0.01	0.00
OPA RE Post	26.05	5.31		2.84	1.39	52.12	8.75	32.91	4.03	0.01	0.00
OPA RE Recovery	23.69	3.16		2.01	0.95	68.98	11.51	63.36	24.26	0.01	0.00
OPI CRE Pre	24.49	5.19		1.19	0.43	62.51	11.18	44.20	6.30	0.07	0.05
OPI CRE Post	24.79	6.50		1.93	0.69	62.24	13.04	46.35	6.09	0.07	0.04
OPI CRE Recovery	25.18	6.77		3.21	1.25	56.45	11.70	48.84	7.74	0.07	0.05
OPI RE Pre	16.36	4.05		1.91	0.88	48.81	8.68	38.42	6.16	0.09	0.06
OPI RE Post	18.95	4.20		1.77	0.63	50.90	10.11	36.20	6.15	0.07	0.04
OPI RE Recovery	25.93	4.56	**+	1.32	0.57	57.38	10.46	34.97	6.26	0.07	0.05

* denotes significant difference compared to pre-exercise (p<0.05). **(p<0.01)

^ denotes significant within-group differences from the same time point in CRE while controlling for pre-exercise values (p <0.05). ^^ (p<0.01)

+ denotes significant 3-way interaction between group, exercise, and time (p<0.05). ++ (p<0.01)

Table S4: TNF LPS / Monocyte Data

Table S4: TNF LPS / Monocyte

	Well 1 (1 ng/mL LPS)			Well 2 (1 ng/mL LPS + PMB)			Well 3 (25 µg/mL LPS)			Well 4 (25 µg/mL LPS + PMB)			Well 5 (no LPS)		
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
OPA CRE Pre	0.38	0.11		0.02	0.01		1.86	0.43		1.10	0.16		0.01	0.01	
OPA CRE Post	0.50	0.11	*	0.02	0.01		2.89	0.69	**	1.82	0.31	**	0.01	0.00	
OPA CRE Recovery	0.36	0.10		0.02	0.01		2.33	0.52		1.26	0.29		0.00	0.00	*
OPA RE Pre	0.53	0.19		0.04	0.02		2.10	0.80		1.07	0.28		0.01	0.00	
OPA RE Post	0.37	0.11	^^	0.02	0.01		1.78	0.57	^^	0.94	0.23	^^	0.01	0.00	
OPA RE Recovery	0.38	0.11		0.02	0.01		1.74	0.55		0.92	0.25		0.01	0.00	
OPI CRE Pre	0.36	0.11		0.01	0.00		2.64	0.59		1.30	0.25		0.00	0.00	
OPI CRE Post	0.48	0.11	*	0.01	0.00		3.84	0.72	**	2.08	0.39	**	0.00	0.00	
OPI CRE Recovery	0.62	0.31		0.01	0.00		3.49	0.85	*	1.83	0.33	**	0.01	0.01	
OPI RE Pre	0.51	0.13		0.02	0.01		3.12	0.75		1.35	0.21		0.00	0.00	
OPI RE Post	0.46	0.09		0.02	0.01		2.97	0.41	^	1.59	0.20		0.01	0.00	
OPI RE Recovery	0.70	0.10	*	0.02	0.01		4.12	0.40	**	1.97	0.14	**	0.00	0.00	

* denotes significant difference compared to pre-exercise (p<0.05). **(p<0.01)
^ denotes significant within-group differences from the same time point in CRE while controlling for pre-exercise values (p <0.05). ^^ (p<0.01)
+ denotes significant 3-way interaction between group, exercise, and time (p<0.05). ++ (p<0.01)