T-HELPER 17 RESPONSE TO AN ACUTE EXERCISE BOUT

AND TO 12-WEEKS EXERCISE TRAINING

by

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T-HELPER 17 RESPONSE TO AN ACUTE EXERCISE BOUT

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CHAPTER 1. INTRODUCTION

Background

Over the last decade, the prevalence of obesity has increased exponentially worldwide. In the United States, one-third (~33%) of the adult population is considered obese (body mass index ≥ 30 kg•m^{-2}), representing a major public health and social concern [3,5]. Obesity promotes the pathogenesis of pro-inflammatory diseases and inactivity-related conditions, including type-2 diabetes mellitus (T2D) and cardiovascular disease (CVD) [5]. Hypertension (HT) is the leading chronic cardiovascular disease (CVD), where approximately 70-90 percent of hypertensive cases are classified as "essential" HT, meaning the specific cause of elevated blood pressure (BP) is unknown [43]. Obesity is associated with chronic low-grade systemic inflammation and vascular dysfunction, which play a key role in the development and maintenance of CVD and HT [5,26]. The specific cellular or molecular mechanisms that trigger vascular inflammation and blood pressure disruptions, however, remain elusive. Recently, several authors reported a causative link between chronic low-grade inflammation and the adaptive immune system, with specific focus on the production and activation of T-lymphocytes, in the incidence of obesity-associated diseases [17, 24, 25, 39].

T-lymphocytes, a type of circulating leukocyte (white blood cell), are part of the adaptive immune system. T-lymphocytes can be further subdivided into T-helper cells (Th) and cytotoxic T cells [50]. Functionally distinct CD4$^+$ T-helper cells play a key role in host defense immunity against invading pathogens. Th17 lymphocytes are key cells of the adaptive immune system, as they respond to extracellular bacteria and fungi, as well as recruit neutrophils to infection sites by regulating the secretion of interleukin 17 (IL-17) and granulocyte-colony-stimulating factor

(GM-CSF). Th17 lymphocytes through the release of IL-17 into circulation propagate the inflammatory response via the regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), a protein complex that regulates the expression of pro-inflammatory cytokines [50]. Circulating IL-17 binds to its corresponding receptor on epithelial cells and promotes further pro-inflammatory cytokine and chemokine production, along with recruitment of T-cells and neutrophils to the site of inflammation. Although Th17 lymphocytes are critical in the adaptive immune system response to invading pathogens, Th17 cells are also linked to the induction and maintenance of multiple chronic inflammatory and autoimmune diseases [17, 24, 25, 39, 58]. Several authors report that patients with multiple sclerosis, acute myocardial infarction, unstable angina, rheumatoid arthritis and cancer all have elevated circulating Th17 lymphocytes and associated cytokines, as well as an elevated Th17/Treg ratio [23, 38, 44].

A growing body of literature also supports the role of Th17 cells and their main cytokine (IL-17) in obesity, hypertension and various forms of CVD [25, 26, 38, 65]. Kim et al. (2015) reported a greater circulating Th17 lymphocyte concentration (700%) in hypertensive patients when compared to normotensive individuals ($p<0.01$). Similarly, in a rodent model, Madhur et al. (2010) determined that infusion of angiotensin-II, a potent vasoconstrictor [21], for two weeks up-regulated the production of IL-17 by >5-fold and the expression of Th17 cells by 2 to 3-fold in hypertensive mice. Moreover, elevated levels of circulating Th17 cells and Th17-associated cytokines are linked with development of atherosclerosis and severity of carotid artery plaques [44]. T-cell activation and proliferation, particularly that of Th17 cells, may thus orchestrate the chronic inflammatory state prevalent in various forms of CVD/hypertension.

Based on aforementioned results, a general model for the involvement of T-helper lymphocytes and adaptive immunity in hypertension has emerged. Harrison (2014) proposed that

HT results from an antigen-specific T-lymphocyte-dependent autoimmune response [59]. Consistent exposure to hypertensive stimuli and physiological stressors, such as cortisol, angiotensin-II and high salt intake, cause initial systolic BP elevations (130 to 140 mmHg), leading to a pre-hypertensive state [25]. Even low to moderate elevations in BP promote chronic low-grade inflammation in the vasculature [25]. The inflammatory state is propagated through continuous mechanical and oxidative stress (elevated stress hormones and inflammatory cytokines) which causes protein damage and/or dysregulation of protein production, leading to "neoantigen" formation [25]. Neoantigens are endogenous proteins ("self-molecules") that have been modified or excessively upregulated due to mechanical or oxidative stress and are no longer recognized as "self" by the immune system. Recognition of neoantigens by our immune system elicits an inflammatory response through T-lymphocyte activation and proliferation in the vasculature influencing organs and systems that regulate blood pressure, thus promoting HT. T-lymphocyte-dependent autoimmunity toward endogenous neoantigens is highly involved in the chronic low-grade inflammatory state present in CVD in animal and human models [25, 26, 38, 65].

Obesity-associated, chronic low-grade inflammation is recognized as a primary risk factor for T2D and CVD. Recently, the importance of the interaction between the immune system and the pathogenesis of obesity-associated diseases has been examined [20,58]. Several authors reported elevated concentrations of circulating Th17 lymphocytes and IL-17 in obese human and mice models when compared to lean controls [26, 57, 62]. After inducing obesity with a high fat diet, Winer et al. (2009) reported that Th17 differentiation and IL-17 production were greater in the obese mice when compared to the regular diet group. In a human clinical observation study, Luczynski et al. (2015) reported that Th17 cell concentration was greater in children with central obesity as compared to lean children. Similarly, Sumarac-Dumanovic

(2009) demonstrated that obese middle-aged women possess greater circulating Th17-related (IL-17 and IL-23) cytokine concentrations, when compared to lean individuals of the same sex and age (16 ± 6 pg/ml vs. 10 ± 5 pg/ml, IL-17 concentration, respectively; $p<0.01$). Several factors may be responsible for the enhanced Th17 differentiation and elevated circulating IL-17 levels in obesity, including elevated production of IL-6, elevated serum amyloid A proteins, high fatty acid metabolism and greater differentiation of pro-inflammatory macrophages.

Exercise training is a non-pharmacological, generally inexpensive means to reduce risk for inactivity-related diseases, as it decreases low-level systemic inflammation that promotes disease processes. Consistent exercise decreases key inflammatory modulators such as C-reactive protein and IL-6, which are associated with the development and maintenance of inactivity-dependent diseases [42, 55]. Moving forward we must further examine "how" exercise exerts its anti-inflammatory effects. The question remains whether exercise training reduces risk of developing HT, CVD and type-II diabetes mellitus by attenuating the up-regulated Th17 profile present in obese individuals. Golzari et al. (2010), observed a reduction in plasma IL-17 after eight weeks of moderate intensity exercise training among multiple sclerosis patients. No previous research has been conducted to examine the Th17 phenotype and Th17-dependent cytokine responses to an acute exercise bout in an at-risk CVD population, such as obese postmenopausal women. Perry et al. (2012) reported that a moderately intense aerobic exercise bout increased the Th17/Treg ratio in peripheral blood, both in healthy males and in patients with chronic lymphocytic leukemia [53]. Similar results were reported in 2013, where intense exercise (marathon)-induced increases in TGF-β and IL-6 promoted Th17 differentiation [52]. The exercise-induced increase in Th17 lymphocytes and the reciprocal decrease in Tregs may influence the delicate balance of immune tolerance and autoimmune phenomena. No one has examined the

influence of an acute exercise bout on the Th17 cell distribution or the Th17 cell-dependent cytokine responses. Because some chronic training-dependent changes in Th17 variables may potentially be residual effects from the previous exercise day, we will examine both the acute and chronic model. Chronic exercise training, without changes in body weight, has the potential to exert anti-inflammatory effects and stimulate systemic hormonal or cellular mechanisms to reduce cardiovascular and inactivity-related disease risk. Various authors previously reported that twelve weeks of combined endurance and resistance training or resistance training alone reduces pro-inflammatory cytokine production and modulates monocyte surface expression of TLR4 in elderly women [54,55]. Further investigation is required to determine the effects of both acute and chronic exercise on Th17 cell number and IL-17 concentration in obese individuals to help improve our understanding of the various mechanisms through which exercise reduces systemic inflammation and the risk of inactivity-related diseases.

Purpose and Hypotheses

The purpose of this study will be fourfold:

- To determine the influence of adiposity and metabolic markers of obesity (BMI, percent fat, hemoglobin A1c) on circulating levels of Th17 lymphocytes and Th17-dependent cytokines.
- To examine the effects of a single resistance and aerobic exercise bout on circulating Th17 lymphocytes in obese post-menopausal women.
- To examine the effects of a 12-week combined (resistance and aerobic) exercise training program on resting circulating Th17 lymphocytes and their related cytokines in overweight/obese post-menopausal women.

- To examine the effects of 12-weeks of exercise training on the exercise-dependent response of circulating Th17 lymphocytes and associated cytokines in obese trained post-menopausal women.

We hypothesize:

- Th17 lymphocyte number and percent, as well as circulating IL-17 and Il-23 concentration, will be greater in obese post-menopausal women when compared to lean controls. Additionally, we predict that circulating number of Th17 lymphocytes and Th17-dependent cytokines' concentration will be associated with anthropometrics (BMI, %body fat), metabolic markers of obesity and blood glucose control (HbA1c).
- Due to known acute exercise-induced elevations in TGF-β and IL-6, we hypothesize that the acute exercise bout will increase Th17 number and percent, as well as IL-17 and IL-23 concentration, for up to 2H post-exercise when compared to non-exercise education participants.
- Twelve weeks of exercise training will decrease Th17 lymphocyte number and percent, as well as Th17-dependent cytokines and HbA1c when compared to non-exercise education participants.
- The acute exercise response of Th17 lymphocytes and related cytokines will be blunted after 12-weeks of exercise training in trained obese post-menopausal women.

Significance

The results from this study could assist in understanding the mechanisms by which exercise decreases the risk of inactivity and inflammatory-related diseases. Additionally, the

results from this study could shed light on the influence of exercise on the proposed autoimmune response coordinated by the inflammatory Th17 cells in a population at risk for CVD and Type 2 diabetes mellitus. To our knowledge, we would be the first to examine the influence of a training intervention on the Th17 cell and IL-17 response in a CVD at-risk group, obese post-menopausal women. Examining the physiological processes involved in disease and their responses to both acute and chronic exercise provides insight to disease causes and regulation, as well as ideas for potential treatment. Consistent exercise is a non-pharmacological, generally inexpensive means to reduce risk for inactivity-related diseases as it decreases low-level, systemic inflammation that promotes disease processes. Moreover, data from the proposed project may help to support the "Exercise is Medicine" campaign promoted by the American College of Sports Medicine (ACSM) and promote physician's awareness of the benefits of exercise prescription as a therapeutic treatment that targets reduction of Th17 cells to help regulate blood pressure and systemic inflammation in at risk CVD population. There is currently no published work on auto-immune-driven hypertension and CVD in the exercise science literature. We would be the first to examine the influence of a single exercise bout of moderate-strenuous intensity, as well as 12-weeks of supervised consistent exercise training on circulating Th17 lymphocytes and Th17 phenotype-dependent production of cytokines in an at-risk group for CVD. It is probable that this project will lead to additional investigation into the subtleties of autoimmune triggers involved in CVD and the mechanisms by which exercise mitigates these responses.

CHAPTER 2. REVIEW OF LITERATURE

Background

CVD is the leading cause of death in developed countries, contributing to high rates of morbidity and mortality worldwide [5] and its high prevalence is typically attributed to behavioral factors such as sedentary lifestyle and poor dietary habits [3, 5, 49]. Hypertension (HT) is the one of the most important modifiable risk factors for stroke and CVD [5]. Low-grade systemic inflammation and vascular dysfunction are known to play a key role in the development and maintenance of hypertension, yet the mechanisms that trigger vascular inflammation and BP elevations remain elusive [59]. Several authors report that the adaptive immune system, specifically Th17 cells, may be a causative component of vascular dysfunction and HT [17, 25, 38]. Physical activity is recognized by experts as one of the best methods to reduce obesity, as well as lower the likelihood of developing CVD and HT. Despite this knowledge, one third of the American population is considered obese and 60% of the population is considered sedentary [5,49]. High percent of body fat and an excessive accumulation of adipose tissue is correlated with high levels of low-grade systemic inflammation and pro-inflammatory cytokine production, further promoting the T-lymphocyte-dependent hypertensive response [20,49]. The obesity-induced polarization of T-cell autoimmunity may be one of the reasons why obese individuals are at a greater risk for developing HT and CVD.

The Immune system

The primary function of the immune system is to defend the body from disease-causing organisms known as pathogens. In order to fight infections (pathogens) successfully, immunity is subdivided into two interconnected classifications: innate and adaptive immune systems [50]. Innate immunity is a genetically pre-programmed set of responses that can be immediately

mobilized to fight infection and create a state of inflammation in the infected tissue [50]. Innate immunity is composed of predominantly antigen presenting cells (APCs), the toll-like-receptor (TLRs) pattern recognition system and complement activation (C3-C9). APCs, like monocytes and dendritic cells, phagocytize infecting pathogens and present fragments on the cell surface to adaptive immune cells with the purpose of mounting a specific adaptive immune response [50]. APCs are able to recognize infected or damaged cells/tissue from healthy cells due to their signaling cell-surface receptors that identify structural features that distinguish microbial carbohydrates, lipids, proteins and nucleic acids [50]. This discrimination allows the innate immune system response to be directed to the invading organism and only damaged human cells. The main type of signaling receptors are the family of toll-like receptors (TLRs) that recognize pathogens and instruct APCs to recruit additional immune cells to the infected tissue. TLR's are transmembrane proteins that are located intracellularly (TLR-7 and TLR-9) or on the cell surface of APCs (TLR-2 and TLR-4) and contain leucine-rich repeats (LRR) that recognize foreign pathogen associated molecular patterns (PAMP) or host-derived danger signals known as damage associated molecular patterns (DAMP) [50]. Through signaling pathways, TLR's contribute to the release of pro-inflammatory cytokines, chemokines and cell adhesion molecules from monocytes, macrophages, neutrophils and dendritic cells. TLR4, for example, specifically recognizes bacterial lipolysaccharide (LPS), found on the surface of gram negative bacterial species (*salmonella and E. coli*), along with several other components of pathogens (lipid A) and damaged endogenous molecules. After LPS-dependent activation, TLR4 induces the initiation of intracellular chain reactions that lead to the synthesis of pro-inflammatory cytokines (IL-6, IL-1B, TNF-α and IL-12) and chemokines via the regulation of NF-kB [19]. Cytokines are small soluble proteins acting in a paracrine, autocrine and endocrine fashion. When cytokines bind to

their specific intra- or extracellular receptors on immune cells, they cause functional changes such as induce the expression of surface receptors, which may change the responsiveness to target cells [3, 25, 50]. Consequently, through the recognition of PAMPs or DAMPs, APCs (dendritic cells and macrophages) induce adaptive immune system activation, pro-inflammatory cytokine production and promote tissue accumulation of inflammatory cells. Molecules such as nitric oxide (NO), superoxide radicals and cytokines can also regulate the expression of vascular adhesion molecules and chemokines that promote T-cell infiltration to the tissues [50]. The responses of the innate and adaptive immune systems are, therefore, integrated and mediated by the milieu of circulating cytokines and chemokines.

The Adaptive Immune System

The adaptive immune system is highly specific and is able to adapt to the nuances of the infecting pathogen. Immunological memory, defined by augmented response upon repeated exposure to a previously encountered pathogen, is a key feature of adaptive immunity and is the basis for vaccination against infection [25, 44, 50]. The key cells that play a role in the adaptive immune response include, T-lymphocytes and B-lymphocytes [50]. These cells require prior sensitization to foreign antigen for their most effective and potent response. T-cells and B-cells recognize different types of antigen. B-lymphocytes have the main function to recognize foreign antigen epitopes, which are specific regions of the antigen that are recognized by means of cell-surface immunoglobulins on B-lymphocytes. When B-cells recognize the antigen, they differentiate into plasma cells that secrete highly specific antibodies that bind to the target pathogen and initiate its neutralization or destruction [50]. T-lymphocytes, in contrast, are activated through T-cell receptor recognition of antigen from APC's that present the pathogen on their cell surface in a major histocompatibility complex (MHC) [24, 50]. The MHC binds and presents antigen

fragments on APCs' cell surface, along with a co-stimulatory signal, to T-lymphocytes. There are two types of MHC's: MHC class-I and MHC class-II. Cytotoxic T cells ($CD8^+$) recognize intracellular antigens presented by MHC I on APCs, while T-helper cells ($CD4^+$) recognize extracellular pathogens presented by MHC II on APCs [50]. The ligand for a T-cell receptor is, therefore, not simply a peptide antigen (pathogen) but the specific combination of peptide bound to MHC presented on the surface APCs and the presence of a co-stimulatory signal.

All hematopoietic cells are continuously produced in the bone marrow. Naïve T-cells migrate towards the thymus, where each cell undergoes DNA rearrangement to generate a unique and antigen-specific T-cell receptor [50]. During thymic maturation, the developing T-lymphocyte lineage is tested against every protein in our body (negative selection) to determine whether the T-cell recognizes "self" vs. "non-self", with the purpose of eliminating by apoptosis T-cells that recognize "self" with high affinity. Naïve T-cells that survive negative selection become activated and undergo maturation along with proliferation in the thymus into distinct lineages under the influence of specific cytokines. Cytokines are circulating modulators of inflammation, participating in acute and chronic inflammation via complex interactions with circulating leukocytes and play an important role in naïve T-cell differentiation. The Th17 lymphocyte lineage is dependent on the cooperative interaction of transforming growth factor beta (TGF-β), interleukin one beta (IL-1β) and interleukin six (IL-6) signaling pathways [50]. Peripheral T-lymphocytes require 2 signals for activation. The first signal involves the interaction of the T-cell receptor with an antigen-peptide presented in the MHC on APCs. The second signal, refers to co-stimulatory interactions of receptors in proximity to the T-cell receptor with specific ligands on APCs [1]. The complex immune defense mechanism, therefore, relies on the simplistic idea of innate and adaptive cells discerning "self" from "non-self".

Effector Th17 lymphocytes play a critical role in host defense against extracellular bacteria and fungi, as well as immune protection against virus-associated pathologies [50]. Th17 lymphocytes are best known for their high concentration and important role in mucosal and fungal immunity [50]. The key role of Th17 lymphocytes in mucosal immunity is highlighted by the increased susceptibility to recurrent bacterial and fungal infections in individuals with hyper-IgE syndrome (HIES) [50]. Individuals with HIES have mutations in signal transducer and activator of transcription 3 (STAT3), which prevents Th17 lineage differentiation due to an inability to express RORyT, the differentiating transcription factor for Th17 lymphocytes [50]. After development and differentiation of the Th17 phenotype, interleukin 23 (IL-23) induces expansion and stabilization of Th17 lymphocytes in the periphery [10, 50]. Cosmi et al. (2008) reported that the Th17 lineage differentiates in the absence of exogenous TGF-ß and in the presence of both IL-1ß and IL-23. Santarlasci et al. (2009) determined that TGF-ß indirectly contributes to Th17 lineage expansion through inhibition of Th1 cells. Regulatory T-cells (Tregs) are another subset of T-helper lymphocytes that play an important role in the maintenance of self-tolerance, prevention of autoimmunity and modulation of immune responses against infectious pathogens [1, 50]. Th17 cells and Tregs pay opposite roles in the immune response. Differentiation pathways of Th17 cells and Tregs are reciprocally regulated, that is an increased production of Th17 lymphocytes suppresses Treg differentiation [10] and the balance of development can influence the outcome of immune responses, particularly in autoimmune or inflammatory diseases. Bettelli et al. (2014), reported that TGF-β and IL-6- dependent activation of T-lymphocytes induced a predominant differentiation of Th17 cells and fewer Tregs, such that the unbalanced differentiation led to exacerbated autoimmune disease development and progression. As previously mentioned, upon antigen recognition, T-cell activation and

proliferation requires signal from the T-cell receptor with MHC and CD28 co-stimulatory signal from the APC [26]. The small fraction of T-lymphocytes bearing the highly specific receptor that binds to the pathogen will be stimulated to divide and differentiate into "active" cells. Activation of cells from the adaptive immune system, particularly T-cells, in response to "self" proteins are connected to the development of chronic inflammatory diseases, CVD and hypertension.

Inflammation, Immunity and Hypertension

In recent years, various authors presented data linking hypertension to an autoimmune response [24, 25, 48, 59, 60]. Guzik et al. (2007), studied recombination-activating gene (RAG) deficient ($RAG^{-/-}$) mice, which do not generate T or B lymphocytes due to absence of the main gene that is essential for their T- or B-cell receptor development and function. The authors demonstrated that $RAG^{-/-}$ rodents had a 50% attenuated response (160 mmHg vs. 130 mmHg measure of systolic pressure) to the development of hypertension after the infusion of a potent vasopressor, angiotensin-II. The authors confirmed that the blunted hypertensive response in $RAG^{-/-}$ mice was not caused by differences in angiotensin-II receptor amount or signaling. To do so, the authors measured the levels of the key angiotensin receptors. The expression of angiotensin-1-receptor (AT1R) (0.58 vs. 0.61 band density, respectively) and angiotensin-2-receptor (AT2R) (0.62 vs. 0.55 band density, respectively) were not different in RAG-/- mice when compared to C57BL/6 (wild type) mice [24]. Interestingly, vascular oxygen production and loss of endothelium-dependent vasodilation that generally accompany angiotensin-II infusion was also blunted in $RAG^{-/-}$ rodents. Additionally, the adoptive transfer of T cells, but not B cells, led to hypertension (150 mmHg vs. 120 mmHg respectively, as measured by the tail cuff method; $p<0.01$), vascular superoxide production and impaired endothelium-dependent vasodilation [24]. Crowley et al. (2010) reported a similar response in immunodeficient mice,

where the animals experienced reduced left ventricular hypertrophy, cardiac fibrosis and albuminuria compared to non-immunodeficient mice following angiotensin-II infusion. Moreover, Mattson et al. (2013) deleted the RAG1 gene in Dahl salt-sensitive mice and demonstrated an attenuation of hypertension and kidney damage in the genetically transformed mice. Therefore, in addition to angiotensin-II induced hypertension, authors report that T-lymphocytes are essential for development of acetate-salt, high fat diet, DOCA salts, salt-sensing kinase and norepinephrine dependent hypertension, thus emphasizing that many forms of CVD/hypertension may be dependent on a T-cell response.

For hypertension to be considered a T-cell dependent autoimmune disease, T-cells must receive both activating signals (T-cell receptor antigen recognition and CD28 co-stimulation) in response to neoantigens or hypertensive stimuli. Vinh et al. (2010) reported that the elimination of the T-cell co-stimulatory signal, thus preventing T-cell activation, blunted the hypertensive response and vascular superoxide production. The authors, along with the work of Guzik et al. (2010) demonstrated that inhibition of the adaptive immune response improves high blood pressure in a murine model, confirming the involvement of the T-cell response in hypertension [26,59]. Based on available evidence, a proposed autoimmune mechanism for inflammation and hypertension has surfaced. Chronic elevations in circulating hypertensive stimuli, including excess salt intake and angiotensin II, induces initial elevations in blood pressure. If the chronic hypertensive stimuli are not removed, the inflammatory state is propagated through continuous mechanical and oxidative stress (elevated stress hormones and pro-inflammatory cytokines), which cause protein damage and/or dysregulation of protein production, leading to “neoantigen” formation. A neoantigen is an endogenous molecule that is modified and, therefore, no longer recognized as “self” by the immune system [25]. In hypertensive individuals, T-helper lymphocytes

appear to recognize and mount an inflammatory response to various neoantigens. The vicious T-cell activating cycle has been supported by the discovery of several “neoantigens”, including excessive concentration of heat shock proteins (HSPs), oxidized low-density lipoprotein (LDL) and degradation products of hyaluron (vessel damage) that are associated with chronic inflammation [11, 33, 61, 64]. Xu et al. (1995) investigated heat shock protein (HSP) 70 as the neoantigen involved in the inducement of hypertension in rodents. Mechanical stress, initial elevations of blood pressure, or hemodynamic alterations lead to the induction of abnormal levels of HSP70 in mice. Elevated levels of HSP70 or oxidized LDLs are recognized as “non-self” and initiate T cell proliferation and infiltration to the vasculature and organs to promote inflammation and the chemotactic recruitment of other inflammatory cells [25]. The authors reported that T-lymphocyte proliferation in response to elevated levels of HSP70 is critical to sustain low grade inflammation in hypertensive kidneys [33]. In addition, various hypertensive stimuli increase reactive oxygen species (ROS) that lead to lipid oxidation and formation of isoketal-modified proteins, which are also considered as circulating neoantigens [61]. Modified isoketal or lipid formation induces the polarization of T-lymphocytes to the Th17 lineage and the production of pro-inflammatory cytokines associated with the Th17 phenotype, including Il-6, IL-17 and IL-23. Authors have also reported that oxidized LDL and the production of reactive oxygen species associated with atherosclerosis induce Th17 cell differentiation, contributing to the IL-17-dependent pathway of vascular inflammation [61]. Overall, the involvement of self-antigens and the activation of the adaptive immune system suggests that loss of tolerance, increased hypersensitivity and autoimmunity are an underlying factor in the pathogenesis of hypertension.

Centrally Mediated Hypertension

The central nervous system (CNS) is also involved in the immune-cell dependent development of hypertension. Lymph nodes and the spleen are innervated with sympathetic nerves (SNS) which, through release of norepinephrine, may stimulate T-cell activation and proliferation [37, 39]. In order to determine the influence of CNS input in the autoimmune-dependent hypertensive response, Marvar et al. (2010) examined the effects of electrolytic lesions at the anteroventral third ventricle (AV3V)/CVO region in RAG deficient MHC class II-restricted transgenic mice. These regions of the brain surround the ventricular system and are key senders and receivers of input coming from the cardiovascular control centers of the brain stem. Because the AV3V/CVO lacks a blood brain barrier, it can be influenced by vasopressors, such as angiotensin-II. The lesions in the AV3V region caused a reduction of sympathetic outflow, led to a decreased recruitment of $CD4^+$ T-cells and blunted the hypertensive effects of a high-dose (490 ng/kg/min) of angiotensin II in the rodents [39]. The authors argued that central actions are required for the activation of T-lymphocytes that promote vascular infiltration and pro-inflammatory cytokine production. The hypothesis proposed by Harrison and colleagues also demonstrates the involvement of the sympathetic drive in the autoimmune essence of hypertension [25]. The current hypothesis suggests that the CNS can, therefore, serve as a key mediator of inflammation through its communication with the immune system and its ability to orchestrate the T cell response leading to chronic inflammation and hypertension.

Th17 Lymphocytes Mediate the Hypertensive Autoimmune Response

The activation of T-lymphocytes evidently plays a crucial role in the development of hypertension, however, the specific lineage subsets involved are still unknown. In 2005, a new T-helper subset was described, designated Th17, which produces IL-17 and other cytokines such

as IL-23, IL-6, IL-22 and IFN-γ . The neutrophil chemotactic role of IL-17, along with the IL-23-dependent production of IL-22 is crucial for epithelial induction of antibacterial responses and host defense [50]. Th17 cells, are activated as effector cells by transcription of RORyT, the master regulator and secrete the cytokine IL-17 [50]. Augmented Th17 lineage expansion promotes tissue damage during inflammation, as the Il-17/IL-23 cytokine axis appear to be involved in the development and maintenance of chronic inflammatory diseases. Th17 lymphocytes are known as key players in the pathogenesis of multiple autoimmune and inflammatory diseases, such as rheumatoid arthritis, multiple sclerosis, asthma, acute myocardial infarction and unstable angina [26, 38, 59, 62]. Elevated circulating levels of IL-17 are associated with the development and maintenance of autoimmune diseases, such as lupus erythematosus, multiple sclerosis and pre-eclampsia, which are all commonly associated with chronic inflammation [23]. Recent evidence also supports the role of Th17 cells and their main cytokine (IL-17) in hypertension and various CVD [36]. Winer et al. (2009), reported increased IL-17 production in response to angiotensin II infusion in aortic and mesenteric vessels. Moreover, the authors demonstrated that angiotensin-induced hypertension is not sustained in IL-17 knockout mice [62]. IL-17 knockout mice also displayed enhanced vascular function, decreased superoxide production and reduced T-cell aortic infiltration when compared to wild type mice. Similarly, treatment with anti-IL-17 antibodies significantly reduced blood pressure, as well as circulating pro-inflammatory mediators in the heart and kidney of DOCA-salt rats [4]. IL-17 modulates target genes with the purpose of propagating the pro-inflammatory response through the release of secondary cytokines and chemokines. Madhur et al. (2010), determined that angiotensin-II infusion for two weeks up-regulated the production of IL-17 by >5 fold and the expression of Th17 cells by 2 to 3-fold in mice. In addition, the authors studied the inducement of hypertension in IL-17 deficient mice.

The investigators reported that IL-17$^{-/-}$ mice did not experience hypertension and determined that IL-17 contributes directly and indirectly to the vascular dysfunction prevalent in hypertensive individuals [38]. Moreover, the authors showed that serum IL-17 concentration is a key player in human hypertension, specifically in type 2 diabetic patients (7.1 ± 1.6 pg/mL hypertensive individuals vs. 2.2 ± 1.4 pg/mL control, p=0.005) [38]. In addition, IL-17 can indirectly alter vascular reactivity by promoting chemotaxis of inflammatory cells and elevated superoxide production. IL-17 elevates blood pressure when infused into mice and induces phosphorylation of the endothelial N synthase (eNOS) on threonine 495, an inhibitory site, leading to impaired endothelium-dependent vasodilation [48]. The pro-inflammatory role Th17 lymphocytes may exacerbate tissue damage in hypertensive pathology, and blunting Th17 phenotype signaling may blunt the chronic inflammation associated with HT.

The Th17 lineage is highly dependent on circulating TGF-β and IL-6 and is reciprocal to the Treg cell lineage [10]. Hypertensive stimuli increase the local IL-6 concentrations, which stimulates a differentiation imbalance towards the Th17 phenotype [10]. Elevated circulating IL-6 concentration appears to force Th17 differentiation, while reducing the percent of effector Treg cells in the periphery by blocking FoxP3 mRNA expression. Tregs are cells responsible for the control of peripheral immunity, tolerance to “self” and immune homeostasis [50]. The diverted balance of Treg cells lead to decreased immune tolerance and high levels of hyper-sensitization. In a model of Tacrolimus-induced hypertension, BP elevation and endothelial dysfunction was linked to an increase in Th17 cell differentiation with a decrease in the Treg lineage [15]. The literature suggests that elevated Th17 lymphocyte number promotes further Th17/Treg ratio imbalance, resulting in elevated IL-17 concentrations, which promote endothelial dysfunction and may be one of the mechanisms by which Th17 cells contribute to the development of HT.

Obesity Induces Th17 Activation

The importance of the interaction between the immune system and the pathogenesis of obesity- associated diseases has been recently examined. Medical professionals recognize the augmented production of pro-inflammatory cytokines as a marker for obesity and metabolic changes [3, 20, 62]. Obese individuals have greater circulating IL-6 concentration when compared to lean controls [3]. Elevated IL-6 concentration, as well as excessive salt and fat consumption, can dysregulate the balance of Th17 to Treg ratio [14], promoting Th17 cell development and production of IL-17 [10]. Furthermore, IL-17 production is regulated via a positive feedback loop as it can increase IL-6 production by stimulation of the NF-kB pathway, resulting in a cytokine-inducing-cytokine vicious cycle [10, 22]. Luczynski et al. (2015) examined Th17 cell number and percent in children with central obesity. The authors reported that Th17 cell concentration was greater in children with central obesity as compared to lean children of the same age [12, 36]. In addition, the authors positively correlated total plasma cholesterol with Th17/Treg ratio, such that a child with elevated cholesterol would have a greater proportion of peripheral Th17 cells [36]. Similarly, Sumarac-Dumanovic (2009) demonstrated in a clinical study that obese middle-aged women possess a greater concentration of circulating Th17-related cytokines (IL-17 and IL-23) when compared to lean individuals of the same sex and age (17 ± 10 pg/ml vs. 10 ± 5 pg/ml IL-17 concentration, respectively $p>0.01$). Interestingly, the authors did not report significant correlations between Th17-dependent cytokines (IL-17 and IL-23) and anthropometric or metabolic markers of obesity (leptin). Sumarac-Dumanovic suggested that the Th17 phenotype may not differentiate at the same rate as accumulation of adipose tissue. In disagreement with the work of Summarac-Dumanovic, Howard et al. (1999) suggest that high concentration of circulating leptin in obese individuals might play a role in Th17 activation and IL-17 production

[27]. Leptin is an adipocyte-derived hormone whose plasma concentrations are proportional to percent body fat [20, 27]. There is increasing evidence that leptin is a key regulator of naïve T cell differentiation. Howard et al (1999). reported that T cell development in the thymus was deficient in leptin-deficient mice. The leptin receptor signals through signal transducer activator of transcription-3 (STAT3) inducing pro-inflammatory responses in different cells. STAT's are proteins that translocate into the nucleus and change the pattern of gene expression in the target cell [50]. Consistently, Yu et al. (2013) reported that $CD4^+$ T cells from leptin deficient mice had a reduced in vitro Th17 differentiation. IL-17 production and Th17 differentiation may thus be associated with greater percent body fat and obesity.

Elevated circulating concentrations of the pro-inflammatory cytokine IL-17 may be an important clinical marker of obesity. Cheng et al. (2014) reported elevated levels of pro-inflammatory Th17 cells in adipose tissue of obese individuals when compared to lean controls. The results also indicate that when co-cultured with adipose tissue dendritic cells (ATDC), the concentration of IL-17 is significantly elevated when compared to control cells (0.45 pg/ml vs 0.21 pg/ml, respectively) [14]. Dendritic cells are primary APCs from the innate immune system, thus the augmented IL-17 production could be dependent on increased Th17 activation in response to "neoantigens".

Obese individuals also have elevated levels of serum amyloid A and an elevated rate of fatty acid metabolism compared to lean controls. Serum amyloid A proteins are adipokines that contribute to augmented LDL oxidation and directly mediate obesity-dependent inflammation [7]. Serum amyloid A in obesity increases production of IL-23 from Th17 lymphocytes and other T-helper cells, which acts as an inducer of the Th17 cell lineage by up-regulating the transcription factor retinoic acid-related orphan receptor γ*t* (RORγ*t*), which controls Th17 cell development [7].

High-fat-diet-induced obesity augments the expression of enzymes associated with fatty acid metabolism. A high rate of fatty acid metabolism increases acetyl-coA carboxylase 1 (ACC1) activity, an enzyme involved in fatty acid biosynthesis [20]. The elevated rate of ACC1 activity in obese individuals increases monounsaturated fatty acid synthesis, which act as endogenous ligands for RORγt and Th17 cell differentiation [20]. Pharmacological inhibition or genetic deletion of ACC1 impaired the development and expansion of Th17 lymphocytes [20]. ACC1-induced fatty acid biosynthesis is required for the ligand-dependent RORγt activation during Th17 differentiation. IL-17A was shown to be capable of inhibiting adipogenesis and triggering inflammation in vitro, suggesting that local effects of Th17 cells in adipose tissue promote adipocyte stress and block the formation of new adipocytes in the face of high caloric load.

Finally, obese individuals have greater numbers of inflammatory resident macrophages when compared to lean counterparts. Metabolic health and appropriate adipogenesis rely on macrophage content and level of activation of adipose tissue macrophages [41]. Obesity induces a switch from an anti-inflammatory macrophage profile (M2) towards “activated” inflammatory resident macrophages (M1) that secrete pro-inflammatory cytokines (IL-6, IL-1β, IL-23) and polarize T-cell development towards a Th17 phenotype [32]. The literature suggests that obesity-associated diseases involve the contribution of various immune cells, among which Th17 lymphocytes play a crucial role. As mentioned, the differentiation balance towards a Th17 phenotype is induced by a high concentration of circulating pro-inflammatory cytokines, hypercaloric/ high fat diets and high adiposity. The connection between Th17 cells and inflammatory-related diseases (CVD, T2D, asthma) elucidates reasons why obesity is a major risk factor in these diseases.

Overall, the literature suggests that Th17 lymphocyte concentration may be an important marker for obesity in routine clinical assessments. Enhanced Th17 differentiation and production of circulating IL-17 may lead to inflammation and alterations in the vasculature that could cause chronic inflammatory diseases (HT, CVD, cancer) among the obese population.

Exercise as a Non-pharmacological Anti-inflammatory Mediator

Exercise helps to reduce the prevalence of obesity [41, 46]. Continuous exercise training has also been associated with significant reductions in plasma inflammatory mediators, such as CRP, IL-6 and TNF-B [3, 41, 46, 55]. Phillips et al. (2012), demonstrated that resistance training reduced circulating CRP (-33%), leptin (-18%) and TNF-alpha (-29%) independent of changes in body composition in obese post-menopausal women. The question remains whether exercise can serve as means to reduce the up-regulated Th17/IL-17 pro-inflammatory profile in obese individuals. Golzari et al. (2010), observed a significant decrease in plasma IL-17 in an 8-week exercise training program in multiple sclerosis (MS) patients. The authors reported reduced expression of IL-17 in plasma in individuals with MS, an inflammatory disease, after the intervention [23]. No one has yet examined the influence of consistent exercise training on the proposed hypertensive-related autoimmune response coordinated by the inflammatory Th17 cells in a population at risk for CVD and Type 2 diabetes mellitus.

In order to establish the benefits of continuous exercise training on the Th17 phenotype, the changes that occur with a single acute bout of exercise must be examined. Perry et al. (2012) reported that a moderate bout of aerobic running increased percent Th17 lymphocytes 1-hour after the exercise bout in patients with chronic lymphocytic leukemia (2.53 ± 1.8% pre-exercise vs. 3.96 ± 3% 1H post exercise). As a baseline control, the authors examined the acute exercise response to the same aerobic run in healthy young male athletes. Exercise-induced IL-6 and

TGF-B increases induced an increase in percent Th17 cells 1-hour after the exercise bout (1.8 ± 1.4% pre-exercise vs. 2.6 ± 1.9% 1-hour after the exercise bout). The authors suggested that the cytokine profile at the end of the exercise bout (elevated IL-6 and TGF-B) induced proliferation of Th17 cells during recovery, while suppressing the Treg phenotype [53]. Perry et al. (2013), reported similar results in a high intensity endurance exercise bout on the Th17/Treg peripheral balance. The marathon exertion induced a significant increase in Th17 levels while significantly reducing Tregs blood concentration (12 cells/ml vs. 2.7 cells/ml respectively) [52]. The exercise-induced IL-6 production induced a transient increase in Th17 cell proliferation with a reciprocal decrease in Tregs [52]. Limited research has been conducted examining the role of exercise on Th17 cell expression. Further investigation is required to determine the effects of exercise training on Th17 cells and the pathogenic role of IL-17 in obese individuals.

Th17 Lymphocyte Surface Receptors

T-cell subsets can be phenotypically defined by the combinations of cell surface markers and intracellular transcription factors. The CD4 extracellular marker is expressed on the surface of all T-helper lymphocytes and differentiates T-helper cells from cytotoxic T-cells ($CD8^+$). The CD196 extracellular marker is present in all IL-17 producing cells and plays an important role in the migration to inflamed tissues. Additionally, it serves as the CCL20, an inflammatory chemokine, binding receptor on the surface of Th17 cells [34]. Authors have previously reported that CD196 is involved in the recruitment of pathogenic Th17 cells in rheumatoid arthritis (RA), allergic pulmonary inflammation and psoriasis [16, 26, 29]. The CD196 agonist, CCL20 has been shown to be upregulated in an autocrine fashion by IL-17 production, promoting a vicious positive loop. The literature suggests that Th17 cells possess a high level of plasticity (similarity) with Th1 cells. The extracellular surface marker on IL-17 producing cells, CD161, is the co-

activating C-leptin receptor, which promotes antigen-dependent T-cell proliferation and induces the production of IL-17 upon substrate binding [8, 30]. Additionally, CD161-expressing T-lymphocytes respond to IL-23 to become fully activated Th17 lymphocytes and respond to target-organ inflammation. Dipeptidyl peptidase IV (CD26) is involved in T-cell activation and cell infiltration through cell adhesion and invasion to inflammatory sites. Human Th17 lymphocytes are characterized by a high expression of CD26 in peripheral blood and are thus labeled as $CD26^{bright}$ [8]. Interestingly, CD26 is also an enzymatically active molecule, as it can degrade numerous substrates including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [47]. Membrane bound CD26 on Th17 lymphocytes may be an important regulator to pathogenesis of metabolic diseases, such as CVD, obesity and diabetes [8, 31, 47]. T-lymphocytes may assume different phenotypes in response to antigen stimulation. The fate of a T-cell phenotype is dependent on factors, including cytokines, immunomodulatory products and antigen specificity. These signals may influence the surface profile of the T-cell, leading to developmental and functional changes. Thus, by using flow cytometry and different functional T-cell markers, we can gain a better understanding of how Th17 lymphocytes impact disease development and progression.

Summary

Obesity is a major public health concern, as the obesity rates continue to increase in the United States. Research suggests that obese and sedentary individuals display a cytokine profile associated with chronic inflammatory diseases. A large body of evidence has accumulated to support that Th17 cells and IL-17 production are key mediators of obesity-associated disease and provide a new perspective on the pathways of inflammation associated with the metabolic syndrome. Future research should focus on exercise as a non-pharmacological therapeutic model

with the potential to reduce excessive Th17 differentiation and IL-17 production in populations at risk of developing CVD.

CHAPTER 3. METHODOLOGY

Participants

Forty-four sedentary, overweight-obese (BMI= 26-47kg•m^{-2}) and 14 moderately active lean (BMI = 18-24 kg•m^{-2}) post-menopausal women between the ages of 55- 75 years participated in this study. Post-menopausal, for the purpose of the investigation, was defined as not experiencing a menstrual cycle for two or more years due to natural or surgical menopause. "Sedentary" was defined as not having participated in regular (more than one mild-moderate exercise bout per week) exercise for the previous six months. Participants represented a variety of ethnicities, including seven Mexican American/Latino, two Native American/Asian and 35 Caucasians. Exclusionary criteria included chronic inflammatory diseases or autoimmune disorders, such as Addison's disease, Graves'disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis and psoriaticarthritis), HIV/AIDS, acute or chronic infection, previous myocardial infarction, peripheral artery disease (PAD), Type 1 or 2 diabetes mellitus, chronic respiratory condition, blood disorders, oral steroid or statins use and those unable to exercise due to an injury or long-term illness.

Experimental Protocol

Human subjects' approval by the TCU Institutional Review Board (IRB) (IRB # 1512-104-1601) was obtained prior to subject recruitment and data collection. Participants were recruited from the university campus and surrounding community via newspaper adds, flyers, electronic postings and word-of-mouth. Participants were asked to carefully review and sign the informed consent form and complete a medical history form and a physical activity questionnaire before participating in the study. Participants were also asked to obtain approval to participate in the study from their personal physician and then undergo a medical screening by our study

physician (Jay Haynes, M.D.) which included a review of their medical history and a physical examination to identify musculoskeletal or flexibility limitations and indicate any contraindications to exercise.

Experimental Design

In order to examine the influence of adiposity on circulating Th17 cell number and percentage, a one-factor, cross-sectional design was employed. All (n=47) overweight-obese participants (OO) were compared to a lean control group (LN) at rest (PRE), prior to exercise or education intervention (BT). All overweight-obese participants (OO) were then randomly assigned to either the exercise-training group (EX) or the education group (ED). Random assignment was stratified for age, body composition and medications, such that prior to training (BT) both groups were not significantly different from each other among age and anthropometric measurements. A repeated measures ANOVA was then utilized to determine the influence of a single exercise bout up to 2-hours after the exercise bout before the 12-week exercise and education intervention (BT). To examine the influence of 12-weeks of exercise training, a three-factor design was employed. The first factor was group, comprised of two levels: exercise (EX) and education control (ED) groups. The second factor was intervention time point with two levels: before training (BT) and after training (AT). The third factor was experimental trial time point with four levels: pre-exercise (PR), post-exercise (PO), one-hour post-exercise (1HR) and two-hours post-exercise (2HR). Finally, we employed a one-factor, cross sectional design at rest with a between group comparison of the obese exercise trained women (EX AT), obese education control (ED AT) and the lean group (LN).

Preliminary Testing. Participants first reported the Texas Christian University Exercise Physiology Lab for anthropometrics measurements. Subjects' height was measured, without

shoes, using a wall-mounted stadiometer to the nearest 0.1 centimeter. Body weight was measured in light clothing and without shoes, to the nearest 0.1kg on an electronic scale. Body mass index (BMI) was calculated for each participant using height and weight ($kg \cdot m^{-2}$). Percent body fat was assessed by dual energy x-ray absorptiometry (DEXA). Waist circumference was assessed 2 cm superior to the umbilicus, while hip circumference was obtained at the largest region of the buttocks using a Gullick measuring tape. Waist to hip ratio was calculated to the nearest centimeter (cm). Participants (EX, ED) underwent a submaximal (85% heart rate reserve (HRR)) treadmill exercise test to estimate maximal oxygen consumption (VO_2 max). VO_2 max was predicted using the linear extrapolation method, where the obtained submaximal VO_2 and heart rate were extrapolated to age-predicted maximum heart rate (208- (0.7*age)).

Acclimation protocol. ED and EX groups completed 1-week (MWF) acclimation to resistance exercises. Eight exercises were completed for the resistance portion of the training: leg extension, leg flexion, leg press, hip adduction, hip abduction, chest press, seated row and ‘‘lat’’pull-down. On acclimation day 1, participants were taught proper lifting techniques and every subject’s 8RM was assessed for each of the resistance exercises. On acclimation day 2, participants completed three sets of each exercise at 50% of their estimated 1RM, where the first two sets consisted of 8-repetitions and the last set was to muscular failure or the equivalent of 15 repetitions. On acclimation day 3, participant’s 8RM for all exercises was reassessed. Acclimation and testing was repeated for the ED group after the education intervention.

12-Week Intervention. EX reported for combined exercise training (endurance and resistance) three times per week on non-consecutive days (M,W,F). Each session included a 5-min walking warm-up, resistance training, aerobic training and a cool-down period. Participants first completed resistance exercises at 75–100% of measured 8RM, consisting of an initial set of

eight repetitions followed by a second set performed to ‘‘momentary muscular failure’’. The participants’ number of repetitions were reevaluated on a biweekly basis and the resistance (lbs) of each resistant exercise was adjusted. If participants were capable of performing 12 or more repetitions on 3 consecutive workouts, exercise load was increased by 10 lbs in lower body exercises and 5 lbs in upper body exercises. Eight-RM strength was reassessed at the end of the 12-week training period. Aerobic training consisted of treadmill walking for 25 min at 70–80% of heart rate reserve. ED met twice per week to attend talks on health and diet, crafts and games and personal safety to control for diurnal and seasonal variations, as well as social interaction. Acclimation week was repeated for non-exercise ED to re-familiarize participants to all resistance exercises and ensure accurate AT 8-RM assessment.

Experimental Testing. Both before and after the 12-week intervention period, EX and ED groups completed an experimental trial. Both groups reported to the Exercise Physiology Lab after a 10-hour overnight fast and assumed a supine position for 15-min prior to a resting heart rate, supine/standing resting blood pressure (BP) measurements and a blood sample (PR) via a single stick method. EX completed a combined (resistance and aerobic) exercise session, as described above. Immediately after exercise, within 0-6 minutes, a catheter was inserted into an arm vein for post-exercise blood samples at time points: PO, 1HR and 2HR. The ED group sat quietly in the lab during the experimental trial having blood samples taken at the same time points as EX, as shown in Table 1.

Table 1. Experimental Protocol

Preliminary Testing	**Acclimation**	**Experimental Trial**
Informed Consent Medical History Resting supine and standing BP DEXA Anthropometrics Physical Exam Submaximal TM test 8RM	**3-DAYS (MWF)** 8 Resistance Exercises: leg extension, leg flexion, leg press, hip adduction, hip abduction, chest press, seated row and "lat" pull-down	10-hour fast Resting HR, supine and standing BP **Resting Blood sample (PR)** **EXERCISE BOUT**: 2 sets of each RE, where the first set will be @ 80% of 1RM and the second set to failure and 25-min TM walking at 70-80% HRR **BLOOD SAMPLES** • **PO EX** • **1HR** • **2HR**

Lean Preliminary and Experimental Testing. LN first reported the Texas Christian University Exercise Physiology Lab for preliminary paperwork and anthropometrics measurements, as previously described. Participants also underwent a submaximal (85% heart rate reserve (HRR)) treadmill exercise test.VO_2 max was predicted using the linear extrapolation method, as described above. For experimental testing, LN arrived at the Exercise Physiology Lab after a 10-hour overnight fast and assumed a supine position for 15-minutes prior to a resting heart rate, blood pressure and a single blood sample (PR) via standard venipuncture techniques.

Blood Analysis. Blood was collected into chilled ethylenediaminetetraacetic acid (EDTA) tubes and was used to assess cytokines, chemokines and adhesion markers using ELISA or milliplex assays following manufacturer's instructions (Invitrogen, Carlsbad, CA). Blood collected into room temperature EDTA tubes was utilized for a complete blood count and 5-part differential analysis using an AcTDiff 5 hematolgy analyzer (Beckman Coulter, Brea, CA) and for flow cytometry analysis of Th17 cell number and percent using a BD FACS Celeste cytometer (BD Biosciences, San Jose, CA). Leukocytes were stained in whole blood using extracellular fluorochrome conjugated antibodies specific for human CD4, CD161, CD196, CD194 and CD26 for the Th17 assay. Th17 cells were defined as $CD4^+$, $CD26^{bright}$, $CD196^+$, $CD161^+$, $CD194^+$, as previously described in the literature (2, 8).

Flow Cytometry

Whole blood was stained and analyzed to identify Th17 lymphocytes using flow cytometry with the following extracellular cell surface markers: CD4, CD196, CD161, CD194 and CD26. After staining, all tubes were gently vortexed and incubated in the dark at room temperature for 20 minutes. After incubation, 2mL of 1X NH_4Cl red blood cell lysing buffer (see appendix A) were added to all tubes, samples were gently inverted, then incubated in the dark for 15 minutes. After lysing, tubes were centrifuged at 200xg for 6 minutes and the supernatant was decanted while leaving the pellet undisturbed. The pellet was resuspended and washed with 2.5mL 0.1% NaN_3 FACS buffer (phosphate-buffered saline (PBS), 5% fetal bovine serum (FBS), 1% NaN_3 and 1 mM EDTA). A second wash was performed as above. After the second wash, the supernatant was again removed and the remaining cells in the pellet were resuspended in

350µL of FACS buffer with 1% paraformaldehyde and analyzed on a FACSCelesta flow cytometer and FACSDiva™ software (BD Biosciences, San Jose, CA).

T-lymphocytes were first gated based using size and density properties on forward/side scatter. Prior to the start of the study, cells only, isotype controls, single stain controls and fluorescence minus one (FMO) controls were used to assess autofluorescence and determine gating parameters. Th17 cells were defined as $CD4^{+}$ $CD196^{+}$ $CD161^{+}$ $CD26^{bright}$.

Statistical Analyses

In order to assess the influence of adiposity on Th17 lymphocytes and Th17-dependent cytokines, an independent t-test was conducted on all anthropometrics and blood variables to determine differences between groups (OO vs. LN) at rest (PR), prior to intervention (BT).

A repeated measures analysis of variance (ANOVA) was employed to determine differences among groups (EX and ED groups) and experimental time points (PR, PO, 1HR and 2HR), prior to 12-weeks of intervention (BT).

A three-factor analysis of variance (ANOVA) with repeated measures on the within factor was employed to determine differences among groups (EX and ED groups), training (BT and AT) and experimental time points (PR, PO, 1HR and 2HR).

A one-way ANOVA was conducted to determine differences between EX AT, ED AT and LN. A Mauchlys test of sphericity was employed for analysis of variance. If the sphericity test confirmed that the covariance assumption was not satisfied the Huynh-Feldt adjustment was used to correct the degrees of freedom. If the ANOVA detected significant main effects or interactions, pairwise comparisons using Bonferroni adjustments was used to determine where the differences were located. Partial Eta squared effect size estimates was reported for all

analyzed blood variables to determine practical significance and generalizability of the reported results.

Finally, correlational analyses and stepwise linear regression predictions were conducted to determine relationships between relevant dependent variables. Descriptive variables were expressed as mean ± standard deviation. All other data was expressed as mean ± standard error. Statistical significance was accepted at $p < 0.05$.

CHAPTER 4. RESULTS

Cross Sectional Analyses of Obese vs. Lean

Anthropometrics, Blood Pressure and Oxygen Consumption

Anthropometrics, blood pressure and descriptive blood variables for LN and OO at rest (PR), before the 12-week intervention (BT) are listed below (Table 2). The mean age, height, bone mineral density (BMD) and resting heart rate (RHR), were not significantly different between LN and OO. OO had a significantly greater body mass, BMI, waist-to-hip ratio, percent body fat ($p = 0.0001$), bone mineral density (BMD) ($p = 0.026$) and resting systolic blood pressure (SBP) ($p = 0.039$) when compared to LN. The resting diastolic blood pressure (DBP) and mean arterial pressure, however, were not different between groups ($p=0.259$, $p = 0.055$, respectively). Percent lean mass and estimated maximum relative oxygen consumption (VO_2) were lower ($p = 0.0001$, $p = 0.008$, respectively) in OO compared to LN.

	Lean (LN) (Mean ± SD)	**Overweight-Obese (OO)** (Mean ± SD)
Group Size	n = 8	n = 48
Age (years)	64.0 ± 6.4	64.1 ± 5.3
Height (cm)	163.2 ± 8.3	162.7 ± 6.0
BMD ($gm \cdot cm^{-2}$)	1.03 ± 0.11	1.13 ± 0.12*****
RHR (bpm)	62.8 ± 8.4	67.2 ± 8.3
Body Mass (kg)	54.0 ± 5.1	87.8 ± 13.5*****
BMI ($kg \cdot m^{-2}$)	20.3 ± 1.14	33.1 ± 4.5*****
Body Fat %	29.7 ± 5.2	47.4 ± 3.9*****
Waist-to-Hip ratio	0.76 ± 0.06	0.90 ± 0.07*****
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	29.6 ± 5.0	20.8 ± 3.7*****
SBP (mmHg)	112.6 ± 10.3	123.8 ± 10.8*****
MAP (mmHg)	85.6 ± 7.3	91.6 ± 8.1
Lean Mass (%)	66.0 ± 4.8	49.4 ± 3.8*****

Table 2. Anthropometrics of lean and overweight-obese post-menopausal women. The * indicates significantly different from LN

Blood and Flow Cytometry Variables

Leukocyte, lymphocyte and neutrophil number were not different between LN and OO. Similarly, neutrophil to lymphocyte ratio (NLR) and hemoglobin A1c (HbA1c) were not different among groups (p=0.103, p=0.396, respectively). The number and percent of all T-helper lymphocytes ($CD4^+$ cells) were significantly greater (p=0.031, p=0.020, respectively) in OO when compared to LN (Table 3). Th17 cells are expressed as a percent of total $CD4^+$ T-lymphocytes (percent of all T-helper cells). Neither percent Th17 lymphocytes nor the density of Th17 lymphocytes extracellular receptors (CD161, CD196, CD194 and CD26) differed between groups (Tables 3, 4). Th17 lymphocyte number (10^3 cells·mL^{-1}) in whole blood were greater (p=0.0001) in OO when compared to lean controls (Fig. 1, Table 3).

	Lean (LN) (Mean ± SE)	**Overweight-Obese (OO)** (Mean ± SE)	***p*-value**
Leukocyte (10^6 cells·mL^{-1})	4.8 ± 0.31	5.43 ± 0.20	0.203
Lymphocyte (10^6 cells·mL^{-1})	1.55 ± 0.13	1.68 ± 0.03	0.432
Neutrophil (10^6 cells·mL^{-1})	2.49 ± 0.22	3.00 ± 0.14	0.157
NLR	1.68 ± 0.19	1.86 ± 0.08	0.396
HbA1c (%)	5.43 ± 0.07	5.62 ± 0.05	0.103
$CD4^+$ Lymphocytes (%)	29.48 ± 4.0	39.06 ± 1.49 *****	0.020
Th17 Lymphocyte (%)	2.29 ± 0.38	3.01 ± 0.23	0.229

Table 3. Leukocyte and blood variables. The * indicates significantly different from LN. Values are expressed as mean ± SE.

	Lean (LN) (Mean ± SE)	**Overweight-Obese (OO)** (Mean ± SE)	***p*-value**
CD161 (MedFI)	3004 ± 527	3243 ± 127	0.524
CD196 (MedFI)	2642 ± 159	3017± 93.3	0.122
CD194 (MedFI)	1410 ± 123	1425 ± 50.7	0.913
CD26 (MedFI)	7704 ± 246	7493 ± 80.3	0.342

Table 4. Median fluorescent intensity (density) of characteristic Th17 phenotype extracellular receptors. No differences between LN and OO were reported ($p > 0.05$). Values are expressed as mean ± SE.

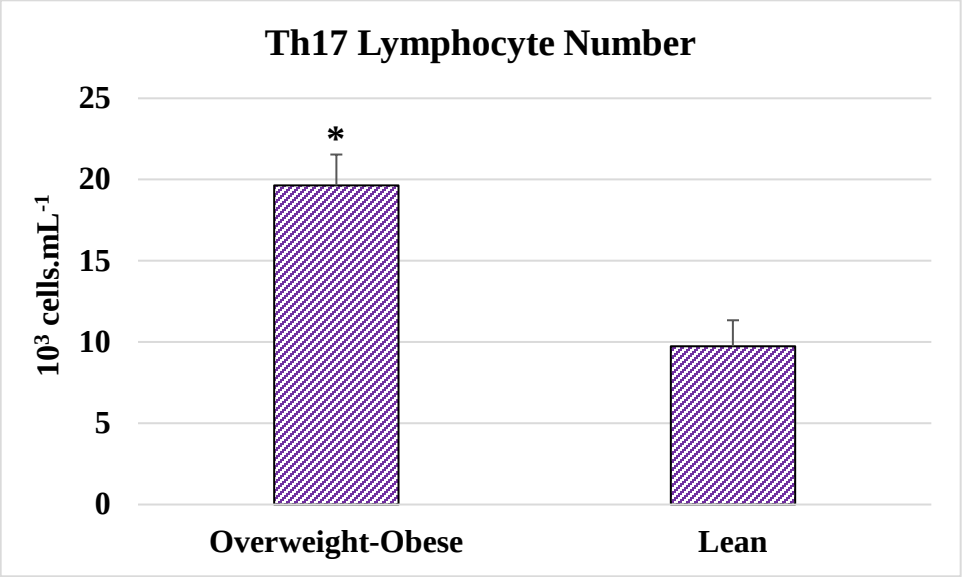

Figure 1. Resting, circulating Th17 lymphocytes in LN and OO. Th17 lymphocyte number in whole blood in overweight-obese and lean post-menopausal women. The * indicates that OO was significantly greater than the LN (p=0.0001). Values are expressed as mean ± SE.

Blood Variable Responses to the Exercise Bout (Before Intervention)

Leukocytes

We observed a time point by group interaction ($p < 0.0001$) for total leukocyte number. A single bout of aerobic and resistance exercise increased leukocyte number in EX at PO and 2H when compared to resting ED. Leukocyte number increased at PO and remained elevated at 2H

in EX, whereas we observed a diurnal effect in ED, as leukocyte number increased over time (PRE < PO, 1H and 2H; Table 5).

A time point by group interaction ($p < 0.0001$) for neutrophil number was observed. A single bout of exercise increased neutrophil number at PO and remained elevated up to 2-hours, highlighting significant differences between EX and ED at PO, 1H and 2H (Table 5). The immediate neutrophil increase from PRE to PO in EX was 40.8% compared to 17.1% in ED. Similar to leukocyte number, neutrophil number in ED significantly increased over time, from PRE to 2H (Table 5).

A time point by group interaction ($p < 0.0001$) in lymphocyte number was observed. The exercise bout induced lymphocytosis at PO, followed by lymphocytopenia at 1H in EX. Lymphocyte number returned to PRE values by the 2H time point in EX. Differences in lymphocyte number between EX and ED were present at PO and 1H (Figure 2). Diurnally, lymphocyte number increased significantly at 1H and 2H when compared to PRE in ED (Figure 2). Additionally, a time by group interaction ($p < 0.0001$) in percent lymphocyte indicated significant differences between groups at 1H and 2H. The exercise bout did not induce immediate changes in percent lymphocyte, however, 1 and 2 hours of recovery elicited a significant percent decrease from PRE in EX (Table 5). No diurnal changes were observed in ED.

A time point by group interaction ($p < 0.0001$) in NLR revealed that the exercise bout induced an increase in NLR at 1H and 2H in EX. NLR was greater in EX at 1H and 2H when compared to ED (Figure 7). No diurnal changes were observed in ED.

A time point by group interaction ($p=0.003$) was found for $CD4^+$ lymphocyte number. The exercise bout induced a transient increase at PO in $CD4^+$ lymphocyte number, followed by a

significant decrease at the 1H time point in EX, with differences between groups at 1H (Table 5). No diurnal variations were reported in ED.

The ANOVA detected a main effect of time point for both Th17 lymphocyte number and percent ($p = 0.002$ and $p < 0.0001$, respectively). Th17 number increased at PO and remained elevated through the 2H time point, independent of the exercise bout (Figure 3). There was a tendency for a group by time point interaction ($p = 0.099$), driven primarily by a non-significant decrease in Th17 number at 1H in EX (Figure 3). Similarly, percent Th17 lymphocyte increased at 1H and remained elevated up to 2H, independent of the exercise bout. Th17% increased by 18% from PR to 1H in EX and by 22% from PR to 1H in ED (Table 5).

Leukocytes (10^6 cells·mL^{-1})	**PRE**	**PO**	**1H**	**2H**
Leukocyte	5.44 ± 1.45 *****	7.64 ± 1.88 †	6.81 ± 1.58 *****	8.13 ± 1.68 †
	5.42 ± 1.22 *****	6.05 ± 1.52	6.2 ± 1.4	6.4 0 ± 1.45
Neutrophil	3.03 ± 1.01 *****	4.27 ± 1.27 †	4.58 ± 1.30 †	5.60 ± 1.47 ***** †
	2.96 ± 0.92 *****	3.47 ± 1.03	3.51 ± 0.99	3.60 ± 0.98
Lymphocyte	1.69 ± 0.43	2.44 ± 0.61 ***** †	1.51 ± 0.48 * †	1.74 ± 0.50
	1.67 ± 0.47 **a b**	1.84 ± 0.52	1.92 ± 0.52 **a**	2.02 ± 0.60 **b**
NLR	1.86 ± 0.54	1.81 ± 0.52	3.26 ± 1.17 * †	3.46 ± 1.28 ***** †
	1.87 ± 0.63	1.92 ± 0.55	1.87 ± 0.52	1.85 ± 0.58
CD4+ Lymphocyte Number	0.64 ± 0.24 **a**	0.77 ± 0.33 **a b**	0.58 ± 0.25 **b** †	0.65 ± 0.27
	0.67 ± 0.24	0.71 ± 0.28	0.78 ± 0.31	0.71 ± 0.36
% Th17 Lymphocyte	3.13 ± 0.31	3.13 ± 0.33	3.70 ± 0.43	4.07 ± 0.40
	2.87 ± 0.35	3.14 ± 0.38	3.50 ± 0.50	3.64 ± 0.46

Table 5. Leukocytes and T-lymphocyte variables in response to a single exercise bout. EX (n=27) means are represented as the top value and ED (n=21) means as the bottom values. The * indicates time point is different than all other time points within group. The † indicates differences between groups at the specified time point. Same letters indicate differences between time points within group. Values are expressed as mean ± SE.

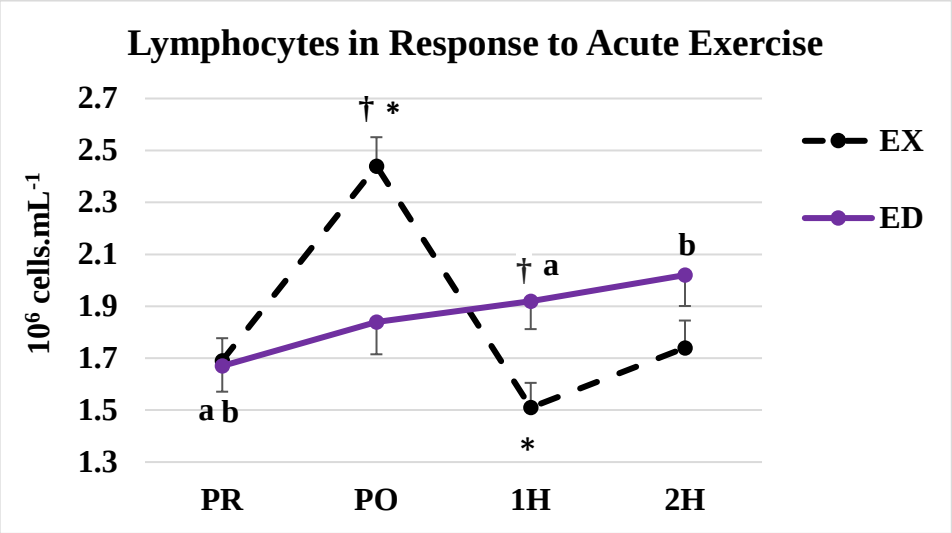

Figure 2. Lymphocyte number in response to an acute exercise (aerobic and resistance) bout in overweight-obese post-menopausal women. The * indicates time point is different than all other time points within groups. The † indicates differences between groups at the specified time point. Same letters indicate differences between time points within groups. Values are expressed as mean ± SE.

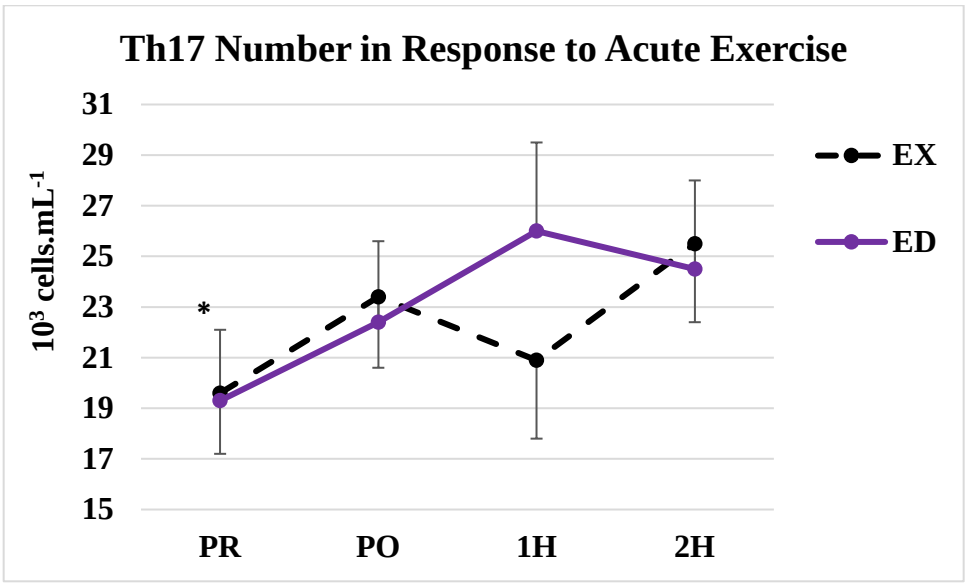

Figure 3. Th17 lymphocyte number in response an acute exercise (aerobic and resistance) bout. * indicates time point is different than all other time points, independent of group. EX n=27 and ED n=21. Values are expressed as mean ± SE.

Th17 Lymphocyte Extracellular Receptors

A main effect of time point was reported for CD196, CD26 and CD161 expression ($p<0.0001$, $p=0.003$, $p<0.0001$, respectively). CD196 expression on Th17 lymphocytes was transiently upregulated at PO, independent of group. The observed main effect of time point appears to be primarily driven by EX (Figure 4). CD196 expression (MedFI) percent change in EX and ED was calculated from PRE to all subsequent time points (PO, 1H and 2H). EX induced a 9.4 percent increase ($p=0.014$) in CD196 expression when compared to 2.4 percent in non-exercising ED. CD26 expression was downregulated at 1H and 2H, independent of the exercise bout (Table 6). CD161 expression was downregulated at 2H independent of the exercise bout, such that PRE and PO receptor expression were significantly greater than 2H (Table 6). No diurnal or exercise induced variations were reported for CD194 receptor expression (MedFI) (Table 6).

	PR	**PO**	**1H**	**2H**
CD196 (MedFI)	3009 ± 126 [a] 3026 ± 143	3336 ± 129 [a] 3099 ± 147	3008 ± 114 3006 ± 130	2966 ± 112 2856 ± 127
CD26 (MedFI)	7550 ± 107 [a b] 7419 ± 122	7476 ± 124 7322 ± 141	7388 ± 110 [a] 7166 ± 125	7410 ± 98 [b] 7189 ± 111
CD161 (MedFI)	3335 ± 170 [a] 3125 ± 193	3273 ± 193 [b] 3091 ± 219	3102 ± 194 2951 ± 220	2987 ± 200 [a b] 2826 ± 227
CD194 (MedFI)	1391 ± 68 1468 ± 77	1495 ± 70 1498 ± 80	1420 ± 62 1511 ± 71	1420 ± 67 1462 ± 76

Table 6. Th17 associated extracellular receptors in response to an acute exercise bout.
EX n=27, ED n=21. Same letters indicate differences between time points, independent of group. Top values are EX and bottom values are ED. Values are expressed as mean ± SE.

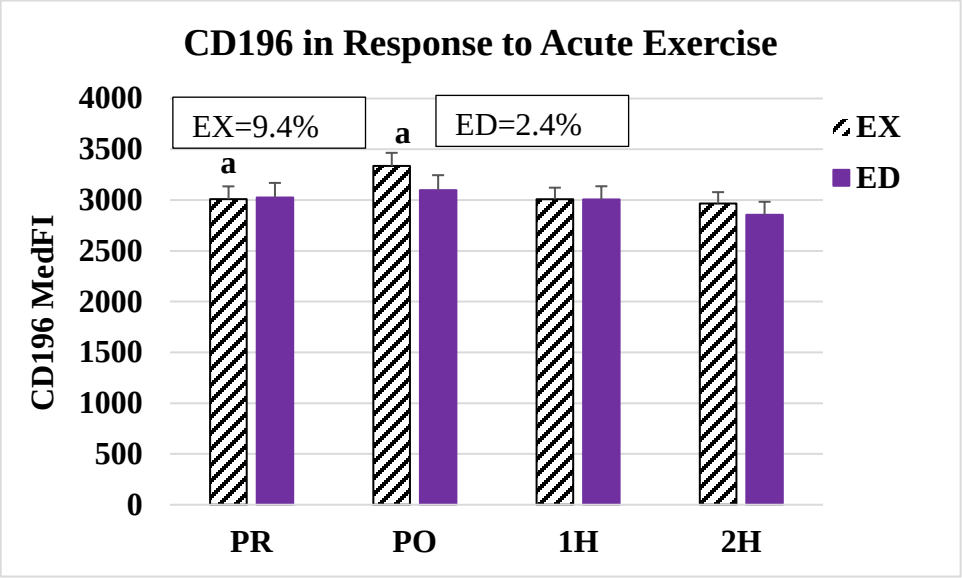

Figure 4. CD196 in response to an acute exercise bout. * indicates main effect of time point from PR. EX n=27 and ED n=21. Written percent indicate percent change from PR to PO in EX and ED (p=0.014). Values are expressed as mean ± SE.

Twelve-week Intervention

Anthropometrics, Oxygen Consumption and Strength Measurements

Anthropometrics for EX (n=12) and ED (n=12) before and after 12-weeks of exercise or education intervention are illustrated in Table 7. The average height, body mass, BMI, waist-to-hip-ratio, percent android fat and BMD did not significantly change from BT to AT in either group. A main effect of intervention for percent body fat and percent gynoid fat (p=0.043 and p=0.001, respectively), indicated that the 12-week intervention decreased both region and gynoid percent fat when compared to BT. The observed training effect is primarily driven by EX, where region percent body fat decreased by 2.8% in EX and 0.97% in ED. Similarly, percent gynoid fat decreased by 4.3% in EX and 1.8% in ED (Table 7). Moreover, a main effect of intervention in percent lean mass (p=0.018) indicated an increase AT when compared to BT, independent of group (Table 7).

	EX (Mean ± SD)	**ED** (Mean ± SD)	**p-value**
Height (cm)	163.7 ± 4.6 163.8 ± 4.6	160.8 ± 7.9 160.9 ± 8.0	0.97
BMD (gm·cm^{-2})	1.20 ± 0.16 1.20 ± 0.16	1.08 ± 1.0 1.09 ± 0.08	0.759
Body Mass (kg)	90.6 ± 18.2 89.9 ± 16.7	82.6 ± 9.5 83.6 ± 10.2	0.83
BMI (kg·m^{-2})	33.8 ± 6.0 33.5 ± 5.5	31.9 ± 2.4 32.3 ± 2.8	0.86
Waist-to-Hip Ratio	0.90 ± 0.06 0.90 ± 0.06	0.89 ± 0.08 0.90 ± 0.07	0.44
Body Fat (%)	46.6 ± 6.0 ‡ 45.3 ± 4.2	46.2 ± 4.7 ‡ 45.8 ± 4.2	0.043
Android Fat (%)	53.8 ± 9.1 52.5 ± 7.3	53.5 ± 5.9 53.1 ± 6.4	0.192
Gynoid Fat (%)	51.2 ± 4.8 ‡ 49.0 ± 3.4	50.5 ± 2.9 ‡ 49.6 ± 3.8	0.001
Lean Mass (%)	50.2 ± 5.8 ‡ 51.7 ± 4.6	50.5 ± 3.1 ‡ 51.0 ± 4.1	0.018

Table 7. Anthropometrics before and after 12-weeks of intervention in EX (n=12) and ED (n=12). Top values are BT and bottom values are AT. The ‡ indicates a main effect of intervention, independent of group (p <0.05). Values are expressed as mean ± SD.

A training by group interaction in estimated maximum oxygen consumption (VO_2 max) and resting heart rate (p=0.024 and p=0.003, respectively), revealed no differences between groups before or after the 12-week intervention period. EX improved their VO_2 max from BT to AT, while no significant intervention changes were observed in ED (Table 8). Resting heart rate tended to be lower in EX when compared to ED after the training intervention (p=0.108). Exercise training decreased resting heart rate when compared to BT in EX, whereas no change was observed in ED (Table 8). Additionally, a main effect of intervention revealed that HbA1c and SBP were significantly decreased (p=0.0001 and p=0.037, respectively) AT when compared to BT, independent of group. The average mean arterial pressure (MAP) did not significantly change from BT to AT in either group (Table 8).

	EX (Mean ± SD)	**ED** (Mean ± SD)
Group Size	n = 12	n = 12
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	21.4 ± 1.1 **ª** 24.3 ± 1.2 **ª**	22.9 ± 1.1 22.8 ± 1.3
RHR (bpm)	66.1 ± 9.4 **ª** 59.2 ± 5.4 **ª**	62.1 ± 7.5 64.0 ± 8.5
HbA1c (%)	5.6 ± 0.3 ‡ 5.4 ± 0.4	5.7 ± 0.3 ‡ 5.5 ± 0.3
SBP (mmHg)	129.4 ± 9.0 ‡ 125.7 ± 9.1	122.8 ± 16.2 ‡ 121.9 ± 14.8
MAP (mmHg)	95.3 ± 7.9 93.3 ± 6.3	87.8 ± 11.0 89.7 ± 10.4

Table 8. Maximal oxygen consumption, resting heart rate, HbA1c and blood pressure responses to 12-weeks of exercise or education intervention. BT means are represented as the top value and AT means as the bottom values. The ‡ indicates a training main effect, independent of group. Same letters indicate differences between time points, within groups. Values are expressed as mean ± SE.

The influence of 12-weeks of exercise training on upper and lower body strength, as represented by 8-RM (lbs) of chest press and leg press, respectively, is illustrated in Figure 5. Training by group interactions were found for strength measurements in all 8 resistance exercises (leg press, chest press, leg flexion, leg extension, seated row, lat pulldown, leg adduction and leg abduction; p=0.0001). EX had greater 8RM strength measures for all eight resistance exercises after the training intervention, as compared to BT (Figure 5).

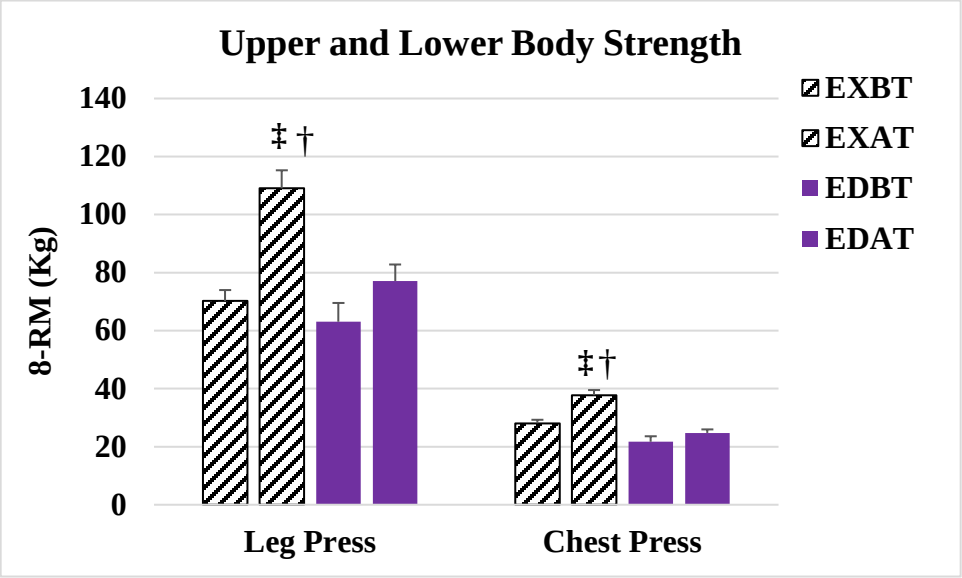

Figure 5. Upper and lower body strength as represented by chest press and leg press 8RM. The † indicates differences between groups (EX> ED). The ‡ indicates training differences within condition (AT>BT). Values are expressed as mean ± SE.

Leukocytes before and after 12-weeks of intervention

Twelve-weeks of exercise training did not alter the leukocyte response (10^6 cells·mL^{-1}) to an acute exercise bout in 12 participants (EX n=12) (Table 9, Figures 6-7). Similar to BT, we observed a time point by group interaction (p<0.0001) in leukocyte number. Leukocyte number increased acutely with exercise and remained elevated up to 2-hours after the exercise bout, independent of the 12-week intervention (Table 9). A significant diurnal increase in leukocyte number was reported at 1H and 2H when compared to PRE in ED. Additionally, a tendency (p=0.101) for a 3-way interaction in leukocyte number was observed. The tendency appears to be driven by an accentuated decrease in leukocyte number at 1H and 2H in EX (Table 9).

The ANOVA revealed a time point group interaction in both lymphocyte number and percent (p<0.0001). The exercise (resistance and aerobic) bout induced a significant transient increase in lymphocyte number, followed by lymphocytopenia, independent of training intervention (Figure 6). A strong diurnal increase in lymphocyte number was also observed at 1H

and 2H in ED (Figure 6). Percent lymphocyte of total leukocytes was significantly lower in EX when compared to ED at 1H and 2H, independent of training intervention. Percent lymphocyte was significantly decreased at 1H and 2H in EX when compared to PRE and PO, while no changes were observed in ED.

We also found a time point by group interaction ($p<0.0001$) in neutrophil number, where the exercise bout induced an immediate increase in neutrophil number and it remained elevated up to two hours post-exercise, independent of training intervention. A diurnal increase in neutrophil number was observed in ED at PO and 2H (Table 9). Neutrophil number, however, was not different between EX and ED at any time point, suggesting that diurnal variations were not disturbed by the acute exercise bout BT or AT. We observed a tendency ($p=0.063$) for neutrophil number to be greater in EX when compared to ED at 2H, independent of training intervention. Moreover, a tendency ($p=0.056$) for a training by time point interaction in neutrophil number, was driven by an intervention-dependent attenuation in neutrophil number at 1H and 2H, independent of group (Table 9).

No training effect was found for neutrophil to lymphocyte ratio (NLR), which serves as a measure of oxidative stress and subclinical inflammation. A time point by group interaction in NLR ($p<0.0001$), revealed an increase at 1H and 2H in EX when compared to ED (Figure 7). No diurnal variations were observed in ED.

Leukocytes (10^6 cells·mL^{-1})	PRE	PO	1H	2H
Leukocyte Number	4.40 ± 0.28 † * 5.51 ± 0.32 a b	6.43 ± 0.38 6.17 ± 0.44	5.52 ± 0.38 * 6.44 ± 0.43 a	6.69 ± 0.36 6.64 ± 0.41 b
Percent (%) Lymphocyte	32.7 ± 1.80 32.3 ± 2.08	34.1 ± 1.68 32.1 ± 1.93	23.8 ± 1.70 † * 33.2 ± 1.96	22.8 ± 1.72 † * 33.3 ± 2.00
Neutrophil Number	2.40 ± 0.22 * 2.98 ± 0.26 a b	3.48 ± 0.26 3.48 ± 0.30 a	3.67 ± 0.29 3.59 ± 0.34	4.56 ± 0.29 * 3.68 ± 0.34 b
CD4$^+$ Number	0.60 ± 0.07 0.67 ± 0.08 a	0.70 ± 0.07 a 0.78 ± 0.07	0.55 ± 0.08 a † 0.86 ± 0.07 a	0.58 ± 0.06 † 0.80 ± 0.07
Percent CD4$^+$ Lymphocytes	41.2 ± 2.77 a b 39.7 ± 3.04	32.0 ± 2.61 a † 41.1 ± 2.86	42.6 ± 1.74 b 43.5 ± 1.91	38.9 ± 2.57 39.1 ± 2.81

Table 9. Leukocytes in response to an acute exercise bout before and after 12-weeks of intervention. EX means are represented as the top value and ED means as the bottom values. The * indicates time point is different than all other time points within groups. The † indicates differences between groups at the specified time point. Same letters indicate differences between time points within groups. Values are expressed as mean ± SE.

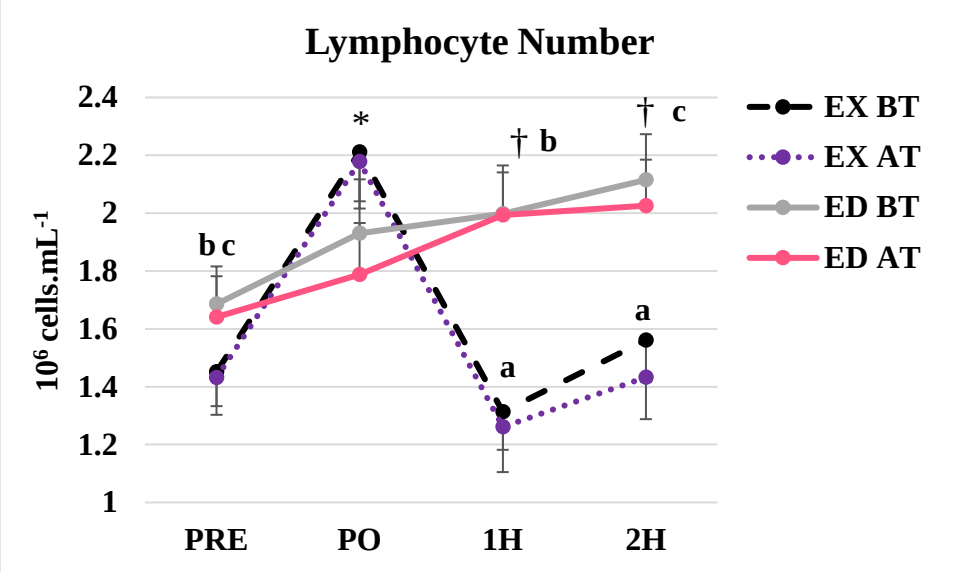

Figure 6. Lymphocyte number in response to an acute exercise bout, before and after 12-weeks of intervention. EX n=12, ED n=12. † indicates differences between groups at specified time point, independent of training. * indicates time point is different than all other time points, within groups. Same letters indicate difference between time points, within groups. Values are expressed as mean ± SE.

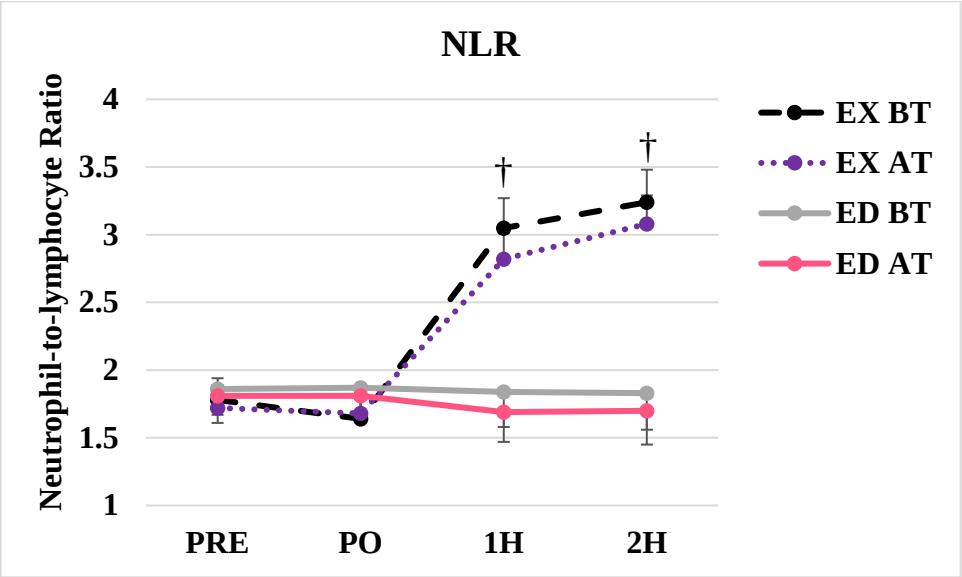

Figure 7. NLR in response to an acute exercise bout, before and after 12-weeks of intervention. EX n=12, ED n=12. † indicates differences between groups at specified time point, independent of training. Values are expressed as mean ± SE.

Flow cytometry variables before and after 12-weeks of exercise training

Twelve weeks of exercise training did not alter $CD4^+$ lymphocyte or Th17 lymphocyte number or percent in response to a single exercise bout. A time by group interaction in $CD4^+$ lymphocyte number (p=0.002), revealed increased $CD4^+$ cell number in ED at 1H and 2H when compared to EX, independent of training. The single exercise bout induced a significant decrease in $CD4^+$ lymphocytes at 1H when compared to PRE in EX (Table 9). We also observed a time point by group interaction in percent $CD4^+$ lymphocytes (p=0.013), where EX had a decrease in percent T-helper cells at PO when compared to ED (Table 9). Percent T-helper lymphocytes returned to resting levels at 1H.

The exercise training intervention did not induce changes in resting Th17 lymphocyte number or percent (Figure 9). A main effect of time point (p=0.002) in percent Th17 lymphocytes revealed an increase at 2H when compared to PO, independent of group or

intervention. In addition, we observed a tendency (p=0.079) for main effect of training in percent Th17 lymphocytes. Independent of group, percent Th17 lymphocytes appear to increase after 12-weeks of intervention. A main effect of time point (p=0.012) in Th17 number revealed no significant differences between experimental time points (Figure 8). A tendency was found for Th17 lymphocyte number to be greater at the 2H time point when compared to PRE, independent of group or training intervention (p=0.088, Figure 8). In addition, a tendency for a time point by group interaction (p=0.085) was driven by a diurnal increase in Th17 lymphocyte number in ED over time, independent of intervention (Figure 8-9).

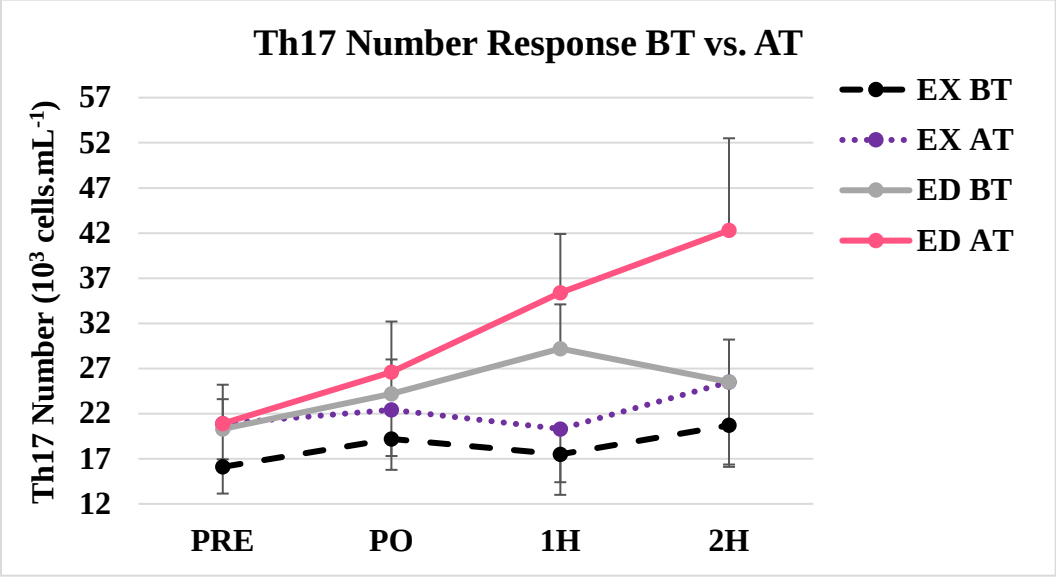

Figure 8. Th17 number in response to an acute exercise bout, before and after 12-weeks of intervention. EX n=12, ED n=12. Values are expressed as mean ± SE.

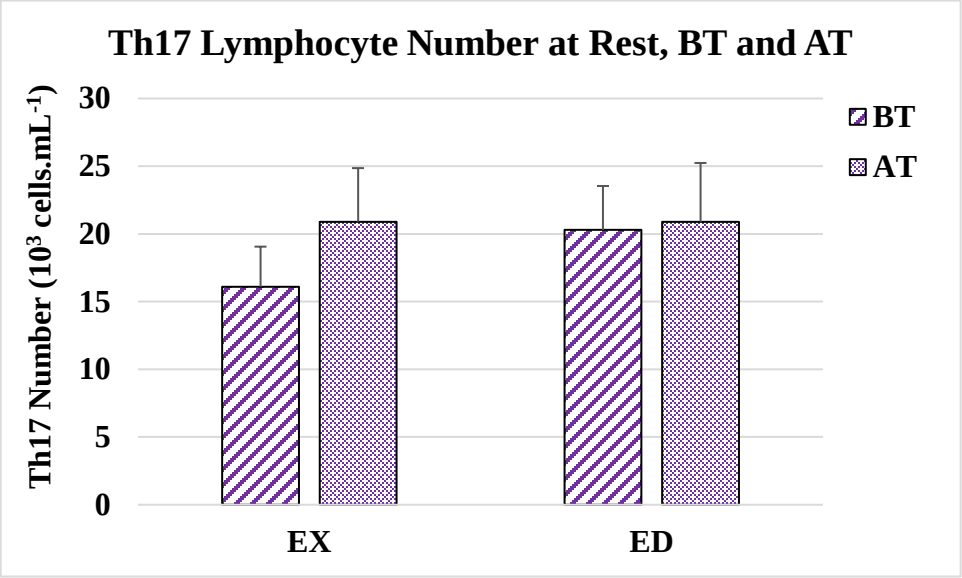

Figure 9. The influence of 12-weeks of exercise training on resting Th17 lymphocyte number. EX n=12, ED n=12. Values are expressed as mean ± SE.

Th17 Lymphocyte Extracellular Receptors

Extracellular Th17 lymphocyte receptor densities were influenced by acute exercise, independent of the 12-week intervention. A main effect of time point (p<0.0001) in CD161 MedFI, revealed a diurnal variation, where CD161 receptor density decreased significantly at 1H and 2H when compared to PRE and PO, independent of group. A tendency for a main effect of training (p=0.052) was also observed, where CD161 receptor density trends to be downregulated with exercise training.

A main effect of training (p=0.016) in CD196 MedFI revealed that the receptor density was significantly downregulated after 12-weeks of intervention at rest (PRE), regardless of group. A time point main effect (p=0.0001) in CD196 MedFI showed that PO was greater than both 1H and 2H, independent of group and intervention (Figure 10).

A three-way interaction (group * training * time point) for CD194 MedFI was found (p=0.047). CD194 MedFI was downregulated at PO in ED after 12-weeks of intervention (AT)

when compared to BT, while no training-induced changes were found in EX. An acute exercise bout induced an upregulation of CD194 receptor density, such that PO MedFI was significantly greater than both 1H and 2H after training (AT) in EX. No significant exercise-induced changes in CD194 receptor density were found BT.

A training by group interaction (p=0.013) was found for CD26 MedFI, where ED had an upregulation of the CD26 receptor AT when compared to BT (Figure 11). No significant training-associated changes were reported for EX. In addition, a time point main effect (p=0.0001) was found for CD26 MedFI where resting (PRE) CD26 receptor density was greater than both 1H and 2H, independent of group (Figure 12).

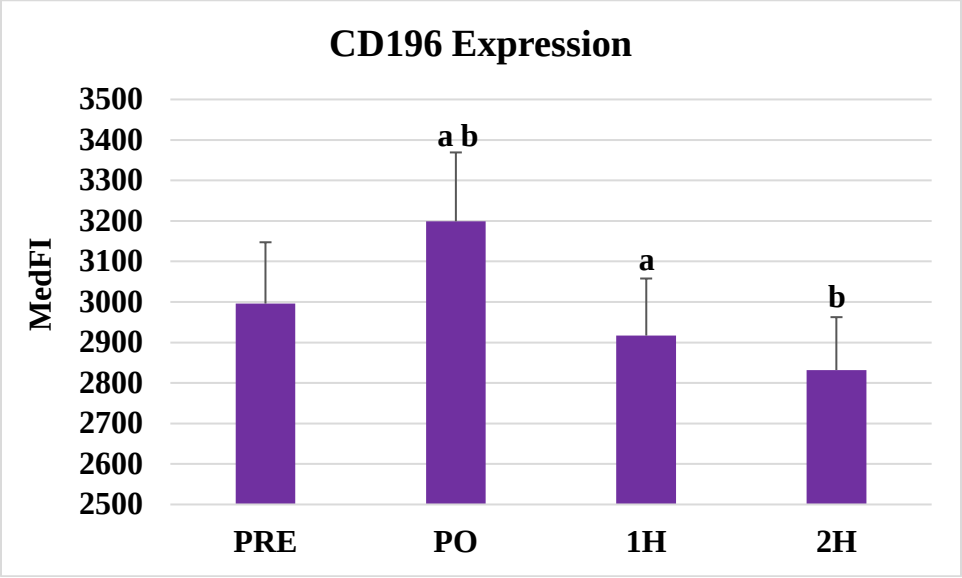

Figure 10. CD196 receptor expression (MedFI) with EX and ED collapsed across intervention. EX n=12, ED n=12. Same letters indicate differences between time points, independent of group or intervention. Values are expressed as mean ± SE.

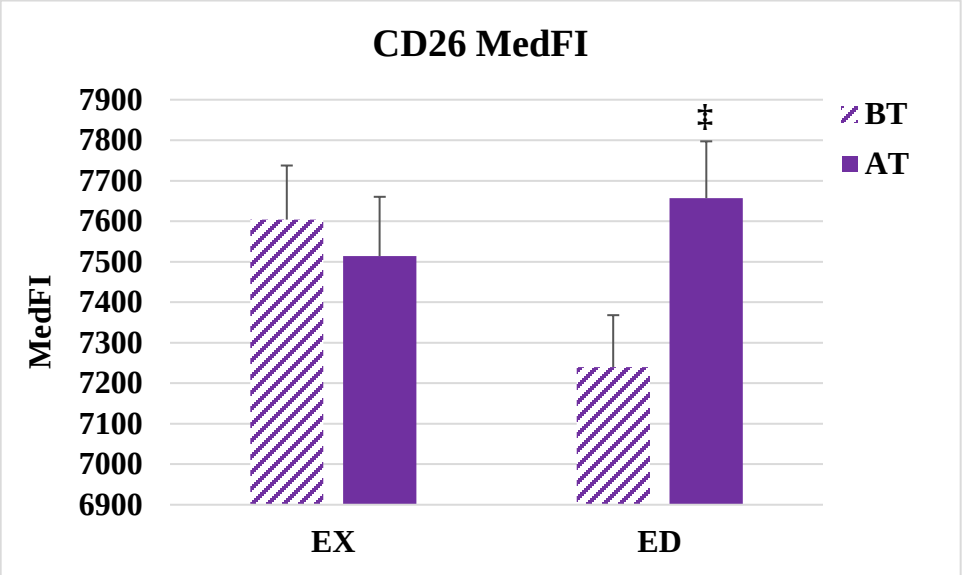

Figure 11. CD26 receptor expression (MedFI) before and after 12-weeks of intervention with time points collapsed. EX n=12, ED n=12. The ‡ indicates training differences within condition (AT>BT). Values are expressed as mean ± SE.

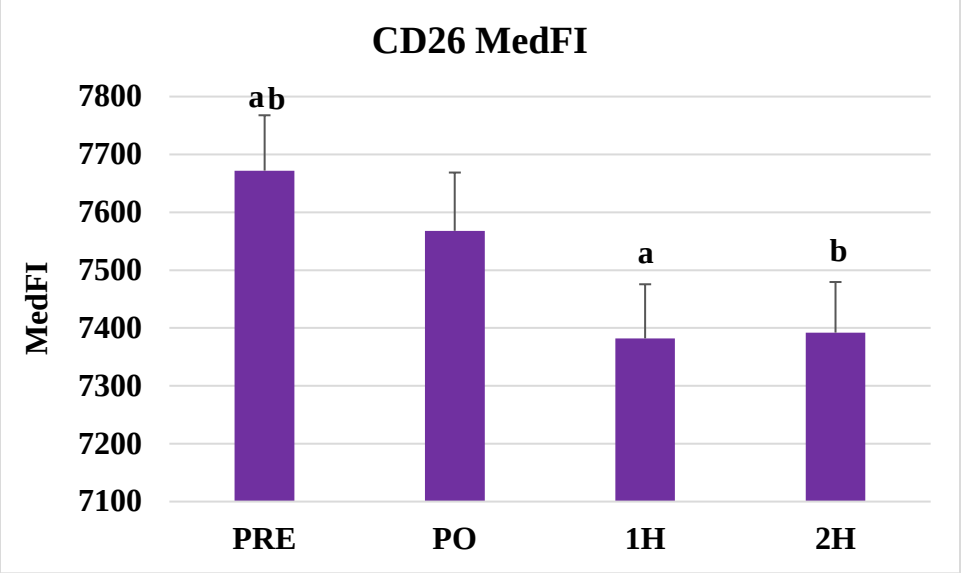

Figure 12. CD26 receptor expression (MedFI) with EX and ED collapsed across intervention. The * indicates main effect of time point, where both 1H and 2H are significantly lower than PRE. Values are expressed as mean ± SE.

Significant correlations between circulating Th17 lymphocytes and markers of adiposity are illustrated in Table 10. Th17 lymphocyte number was significantly correlated with BMI, body mass, percent android fat and HbA1c both before and after 12-weeks of intervention. In addition, Th17 lymphocyte number was significantly associated with systolic blood pressure and resting heart rate AT. Similarly, percent Th17 lymphocytes were significantly correlated with BMI, body mass, NLR and HbA1c both before and after 12-weeks of intervention.

	BMI	**Body mass**	**Android Fat %**	**HbA1c**	**SBP**	**RHR**
Th17 Number	0.352 0.099	0.415 0.049	0.435 0.038	0.666 0.0001	0.510 0.011	0.430 0.036
Th17 %	0.568 0.005	0.531 0.009	0.216 0.227	0.491 0.015	0.406 0.049	0.405 0.050

Table 10. Significant correlations for Th17 number and percent Th17 lymphocytes. Top values represent r value and bottom values represent *p*-values.

A significant stepwise linear regression (p=0.0001) revealed that HbA1c, BMI and RHR are significant predictors of Th17 number (R^2=0.675, SEE=8.09; Table 11; Figure 13).

Regression Model Th17 Number	**Standardized Beta Coefficients**	**%Variance Explained**	**Significance (*p*-value)**
BMI	0.361	12.5	0.013
HbA1c	0.611	45.3	0.0001
RHR	0.318	9.7	0.028

Table 11. Th17 lymphocyte number regression model.

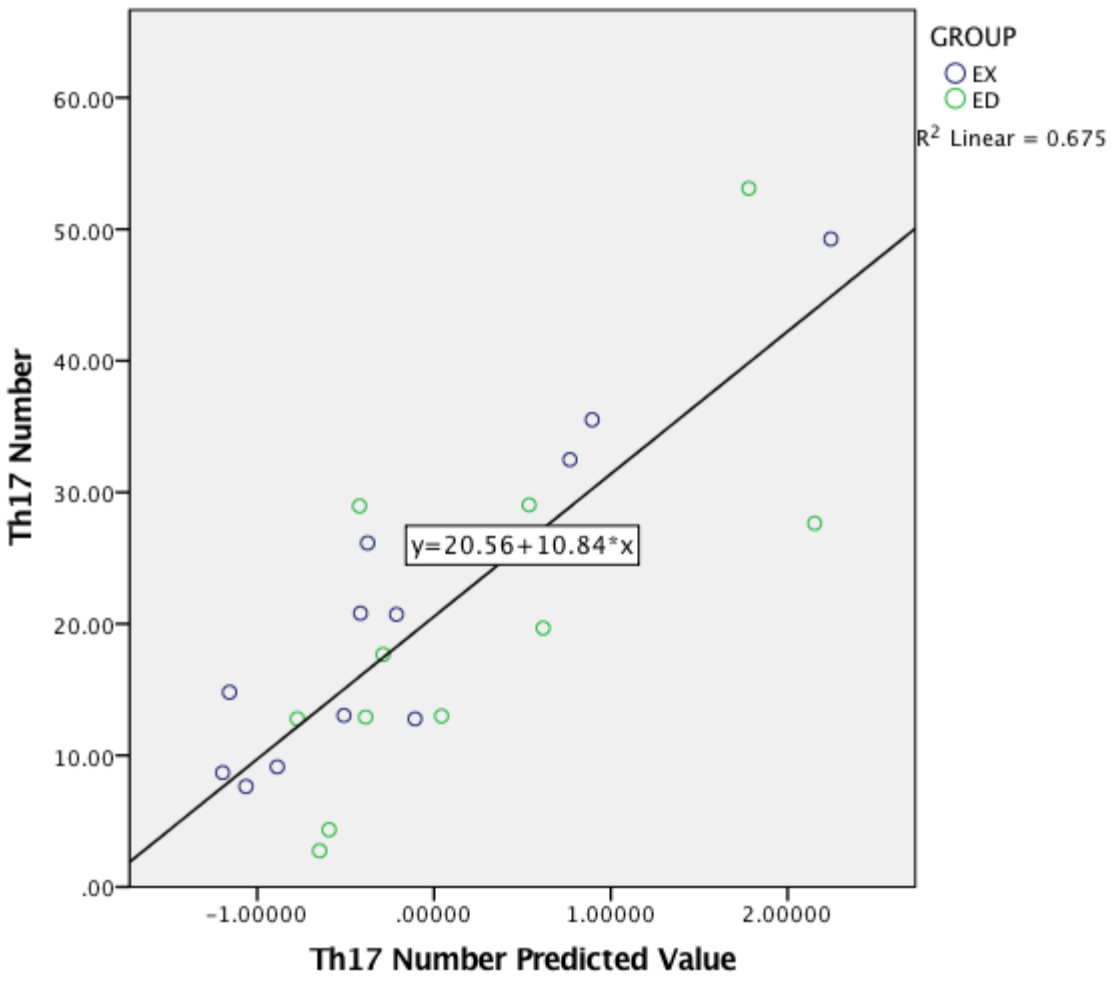

Figure 13. Th17 lymphocyte number regression model. The x-axis represents predicted Th17 lymphocyte number from the regression equation and the y-axis represents Th17 lymphocyte numbers from the flow cytometer.

Similarly, a significant stepwise linear regression equation (p=0.0001) revealed that BMI, HbA1c and NLR are significant predictors of percent Th17 cells (R^2=0.648, SEE=1.3; Table 12; Figure 14).

Regression Model Percent Th17 cells	Standardized Beta Coefficients	%Variance Explained	Significance (*p*-value)
BMI	0.473	32.2	0.004
HbA1c	0.557	24	0.001
NLR	0.316	8.6	0.044

Table 12. Percent Th17 lymphocytes regression model. Th17 cells are expressed as a percent of all $CD4^+$ lymphocytes (T-helper cells).

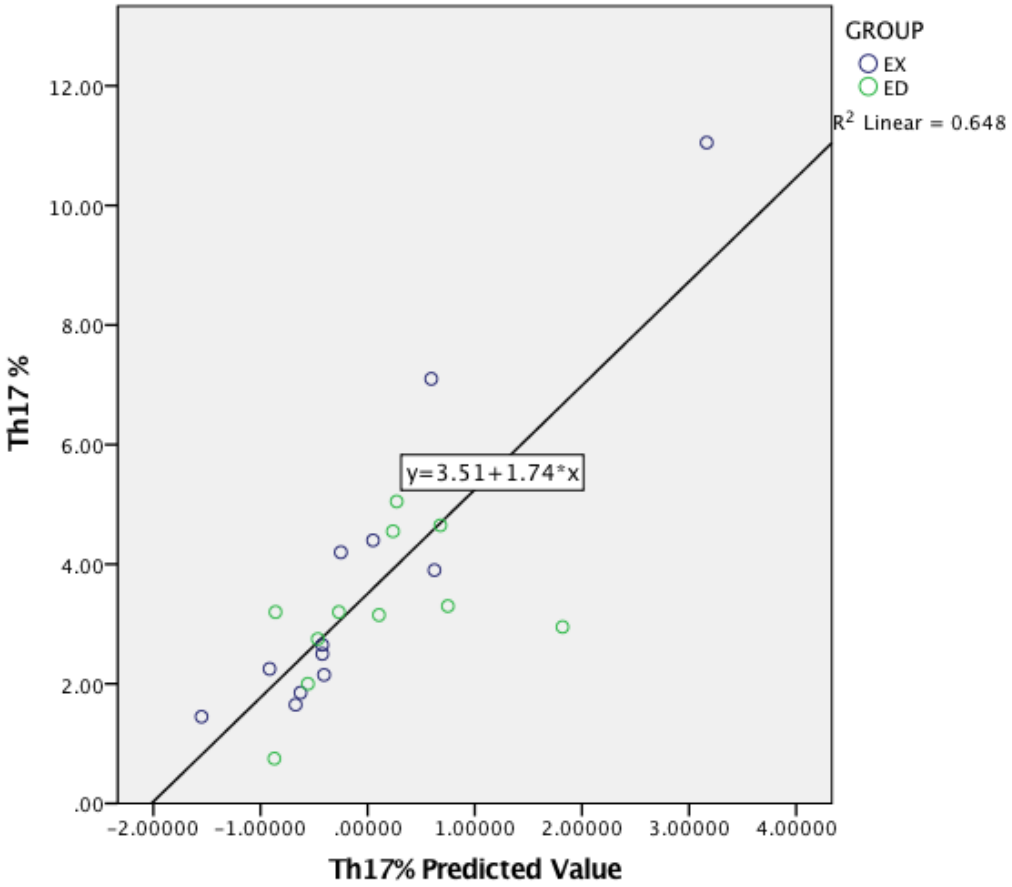

Figure 14. Percent Th17 lymphocyte regression model. The x-axis represents predicted percent Th17 lymphocyte from the regression equation and the y-axis represents percent Th17 lymphocyte from the flow cytometer.

Resting, Cross Sectional Analysis After Intervention

Trained EX vs. ED vs. Lean

Trained EX (n=12) completed a 12-week exercise (aerobic and resistance) training program, as previously described, ED (n=12) are sedentary non-exercise control participants and LN (n=8) are moderately active lean women. The mean age, height, resting heart rate (RHR), blood pressure and hemoglobin A1c (HbA1c) were not significantly different among trained EX, ED and LN (Table 13). The ANOVA identified differences among the groups in body mass, BMI, waist-to-hip ratio, percent lean mass, percent fat and maximum VO_2 (Table 11). Total bone mineral density (BMD), however, was greater in EX (p=0.017) when compared to LN and a tendency(p=0.132) for EX to have greater BMD than ED.

	EX n=12	**ED n=12**	**LN n=8**
Height (m)	163.8 ± 4.6	160.9 ± 8.0	163.2 ± 8.3
Body mass (Kg)	89.9 ± 16.7	83.6 ± 10.2	54.0 ± 5.1 #
BMI ($kg \cdot m^{-2}$)	33.5 ± 5.5	32.3 ± 2.8	20.3 ± 1.1 #
W/H ratio	0.90 ± 0.06	0.90 ± 0.07	0.76 ± 0.06 #
Lean Mass (%)	51.7 ± 4.6	51.0 ± 4.1	66.0 ± 4.8 #
Region Fat (%)	45.3 ± 4.7	45.8 ± 4.2	29.7 ± 5.2 #
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	24.3 ± 3.4	22.8 ± 4.9	29.6 ± 5.0 #
RHR (bpm)	59.2 ± 5.4	64.0 ± 8.5	62.8 ± 8.4
BMD ($gm \cdot cm^{-2}$)	1.20 ± 0.2 †	1.09 ± 0.1	1.03 ± 0.1 †
HbA1c (%)	5.47 ± 0.4	5.58 ± 0.41	5.43 ± 0.2
SBP (mmHg)	126 ± 9.1	122 ± 14.8	113 ± 10.3

Table 13. Descriptive variables for trained EX (n=12), sedentary ED (n=12) and LN (n=8). # indicates both EX and ED different from LN. † indicates differences among groups. Values are expressed as mean ± SD.

Blood and Flow Cytometry Variables

Leukocyte, lymphocyte, neutrophil number and percent, as well as neutrophil to lymphocyte ratio (NLR) were not different among groups (Table 14). Similarly, $CD4^+$ lymphocyte number and percent, as well as percent Th17 lymphocytes were not different among groups (Table 14). As previously mentioned, Th17 cells are expressed as a percent of total $CD4^+$ T-lymphocytes (percent of all T-helper cells). A tendency (p=0.072) for differences in Th17 lymphocyte number among groups at rest (Figure 15), where both overweight- obese groups (EX and ED) had elevated Th17 lymphocyte number when compared to LN (2.6 and 2.23-fold greater than LN, respectively).

Leukocytes (10^6 cells·mL^{-1})	**EX (Mean ± SE)**	**ED (Mean ± SE)**	**LN (Mean ± SE)**
Leukocyte Number	4.32 ± 0.13	5.12 ± 0.40	4.73 ± 0.35
Percent (%) Lymphocyte	33.1 ± 1.92	31.8 ± 1.80	32.5 ± 2.28
Neutrophil Number	2.31 ± 0.12	2.80 ± 0.29	2.60 ± 0.22
$CD4^+$ Number	0.61 ± 0.07	0.65 ± 0.11	0.47 ± 0.17
Percent $CD4^+$ Lymphocytes	41.7 ± 2.74	38.9 ± 4.76	29.5 ± 4.01
Percent Th17 Lymphocytes	3.76 ± 0.81	3.19 ± 0.34	2.29 ± 0.38

Table 14. Leukocyte variables at rest in trained overweight-obese women (EX), resting non-exercise overweight-obese women (ED) and lean women (LN). Values are expressed as mean ± SE.

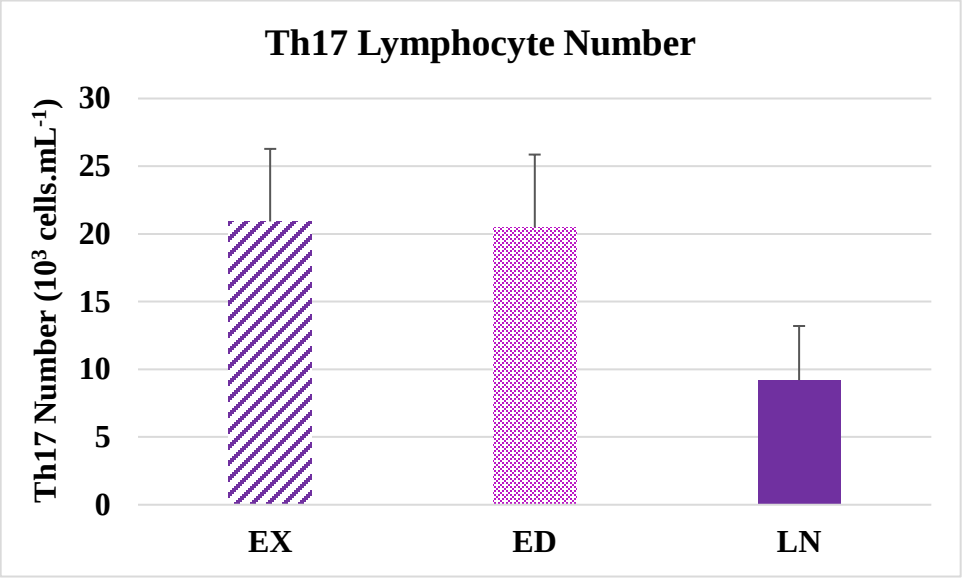

Figure 15. Resting circulating Th17 lymphocytes in trained obese, untrained obese and lean moderately active women. † indicates significantly different than LN. EX AT n=12 and ED AT n=12 vs. LN n=9. Values are expressed as mean ± SE.

Extracellular CD26 and CD196 receptor expression were significantly greater at rest in both EX and ED when compared to LN (Table 15). CD161 and CD194, however, extracellular density was greater in LN when compared to both EX and ED (Table 15). No differences between EX and ED were identified.

	EX	ED	LN
CD196 (MedFI)	2980 ± 187	2987 ± 223	2124 ± 193 #
CD194 (MedFI)	1381 ± 97	1406 ± 66	3894 ± 440 #
CD26 (MedFI)	7835 ± 155	7756 ± 154	2907 ± 151 #
CD161 (MedFI)	2804 ± 198	3322 ± 185	4102 ± 110 #

Table 15. Th17 phenotype-associated extracellular receptors in trained overweight-obese women (EX), untrained overweight-obese women (ED) and moderately active lean women (LN). # indicates both EX and ED different from LN. Values are expressed as mean ± SE.

Due to low detection ability of the multibead cytokine kit utilized to measure circulating cytokines, non-detectable resting circulating IL-6 concentration were assigned the lowest detection level (0.53 pg·mL^{-1}). Resting circulating IL-6 concentration in ED was significantly greater than LN (p=0.004), while the trained overweight-obese women (EX) were not different from LN (Figure 16). The ANOVA showed a tendency (p=0.077) for resting circulating IL-6 concentration to be greater in sedentary ED when compared to physically trained EX (Figure 16).

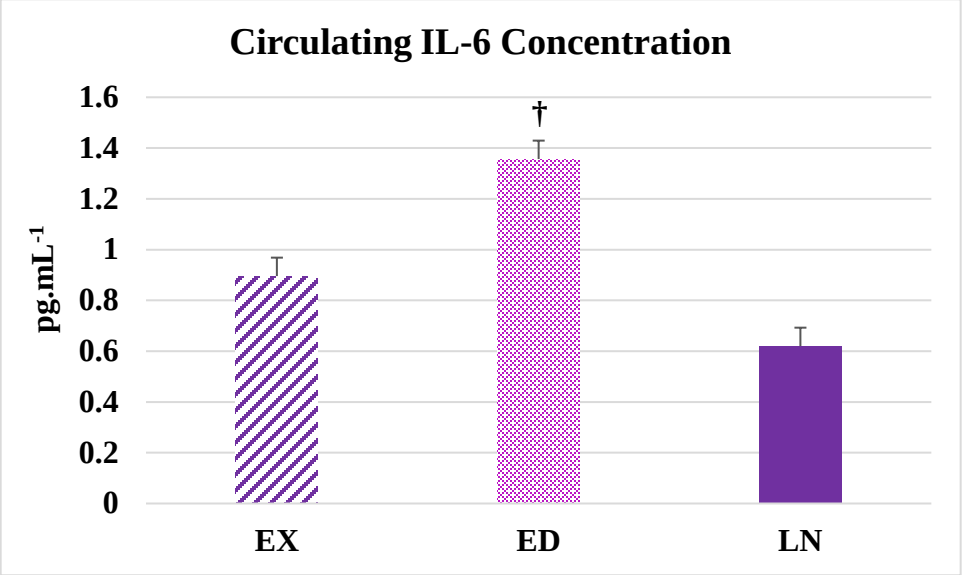

Figure 16. Resting circulating IL-6 in trained obese, untrained obese and lean moderately active women. † indicates significantly different than LN. EX n=12 and ED n=12 vs. LN n=8. Values are expressed as mean ± SE.

CHAPTER 5. DISCUSSION

Th17 lymphocytes play an important role in host defense against extracellular pathogens, primarily in mucosal tissues, as they help protect against bacteria and fungi [50]. Augmented differentiation and expansion, however, of Th17 cells leads to the pathogenesis of inflammatory and autoimmune diseases, including psoriasis, arthritis, cancer and multiple sclerosis. Even though T-helper lymphocytes have been well studied in immunology, less is known about specific T-helper cell lineages. Interestingly, Th17 lymphocytes can be activated and expand not only through pathogenesis or autoimmune diseases, but also in response to specific symptoms of the metabolic syndrome, particularly metabolic dysfunction. Current evidence indicates that chronic low-grade inflammation hypersensitizes Th17 lymphocytes to neoantigens, which induces hypertension, the leading cause of CVD and vascular dysfunction, via an autoimmune-dependent mechanism [24,25]. Obesity and obesity-dependent metabolic dysregulation (elevated IL-6, inflammatory macrophages and acetyl-coA carboxylase 1 (ACC1) activity) preferentially stimulate the Th17 lineage and IL-17 production in rodent models [20, 28, 32, 58, 62, 63]. A significant increase in circulating Th17-dependent cytokines (IL-17 and IL-23), as well as IL-17 and RORyt mRNA expression, were also reported in obese women [22, 57] and T2D patients [13] when compared to lean healthy controls, further supporting the implication of Th17 lymphocytes in obesity and the metabolic syndrome.

Based on the data presented here, it appears that resting, circulating Th17 lymphocyte number is elevated in obese post-menopausal women when compared to lean controls, without differences in extracellular receptor densities (MedFI). In addition, our study results identified significant correlations among key markers of adiposity (BMI) and blood glucose control (HbA1c) with circulating Th17 lymphocyte concentration. Linear regression analyses of Th17

lymphocytes identified BMI, HbA1c, resting heart rate and NLR as significant predictors of Th17 cell number and percent. In agreement with our results, various authors previously reported significant associations between Th17 lymphocytes and severity of diabetes in T2D patients [13, 28], as circulating IL-17 can inhibit glucose uptake in cultured human hepatocytes [31]. Xu et al. (2009) reported elevated circulating Th17 cells and enhanced secretion of IL-17 and IFN-y in T2D patients. The authors reported a skewed imbalance of the Th17/Treg ratio in T2D patients, which promotes chronic inflammation through elevated cytokine production. Fabbrini et al. (2013) reported that IL-17 and IL-22 receptor activation via cytokine binding in skeletal muscle and liver tissues impaired glucose homeostasis and contributed to metabolic dysfunction [22]. Based on the relationship between Th17 cell number and HbA1c, our results along with those of Xu and Chen suggest that Th17 lymphocytes may be significant determinants of glycemic control in obesity.

Current evidence indicates a feedforward relationship between adipocytes and Th17 cells in obesity and CVD [20]. Obesity along with excess salt and fatty acids intake induce adipocytes to secrete pro-inflammatory cytokines, which leads to low-grade systemic inflammation and a pro-Th17 lymphocyte milieu [13,20]. Although our results demonstrate a strong relationship between adiposity and the Th17 phenotype, the association among markers of adiposity (BMI, WHR, %Fat) with Th17 lymphocytes and Th17 lineage-dependent cytokines (IL-17 and IL-23) is not well defined in the literature, as results from several studies are mixed [22, 62, 63, 65]. Recent studies have shown that obesity is associated with elevated IL-17 production and enhanced severity of inflammation in IL-17-dependent human and rodent models [62, 63]. Sumarac-Dumanovic (2008) reported elevated circulating IL-17 and IL-23 in obese women without significant associations with markers of central adiposity. Moreover, morbidly obese women have elevated IL-17 mRNA

expression in visceral adipose tissue, with significant positive associations with resistin when compared to normal weight controls [65]. Resistin is an adipocyte-derived circulating signaling molecule that contributes to insulin resistance *in vivo* [63]. Resistin and other adipokines play a pro-inflammatory role in metabolism and may induce obesity-associated low-grade inflammation [63]. Circulating IL-17 serum concentration, however, was greater in the normal-weight women when compared to the morbidly obese group [65]. The authors explained that Th17 self-regulatory mechanisms in response to antigen stimulation limit their expansion in inflammatory sites of human diseases. Th17 lymphocytes are memory-like cells that typically reside in a quiescent state in peripheral tissues where they provide a first line of defense against pathogens [50]. Additionally, Th17 exhibit high plasticity with the Th1 phenotype where in the presence of IL-2 and TNF-alpha Th17 lymphocytes shift to an inflammatory Th1 profile [65]. Morbidly obese individuals' adipose tissue may blunt excessive Th17 lymphocyte proliferation as a protective mechanism. Additionally, adipose tissue-induced inflammatory milieu in morbidly obese individuals may induce the Th17 lymphocyte shift to the inflammatory "non-classical" Th17/Th1 cells, further promoting disease development. Fabbrini et al. (2013) reported augmented differentiation of Th17 lymphocytes in subcutaneous adipose tissue in obese individuals with metabolic dysfunction. Adipose tissue contributes significantly to the elevated production of pro-inflammatory cytokines, particularly IL-6 and TGF-B [62], promoting augmented Th17 differentiation in obese individuals [36]. Our findings showed elevated circulating resting IL-6 concentration in obese non-exercising participants when compared to lean controls, which is not surprising due to its role to promote Th17 differentiation. Unfortunately, due to IL-17 and IL-23 cytokine kit sensitivity, we were not capable of reporting circulating concentrations of the Th17-dependent cytokines. Our results support the work of Onishi et al. (2010), demonstrating a

positive feedback relationship between circulating IL-6, Th17 differentiation and IL-17 production in obesity. IL-6 expands the Th17 lineage [50] and is a key downstream gene target for IL-17 production, thus further promoting Th17 expansion. Our findings indicate a potential link between obesity and Th17 lymphocytes in post-menopausal women supporting the work of Summarac-Dumanovic and Fabbrini. Targeting the Th17 imbalance in obesity may re-establish homeostasis, leading to reduced low grade inflammation and obesity-associated diseases.

Physical exercise is recognized as a non-pharmacological model to reduce systemic inflammation. Unlike total leukocyte counts and "classic" pro- and anti-inflammatory mediators (IL-6, IL-10, TNF-α), specific T-helper phenotype changes in response to a single exercise bout have not been extensively investigated. Our results indicate that one exercise bout of moderate-vigorous intensity does not influence circulating Th17 lymphocyte number or percent. Based on the data reported here, there appears to be a diurnal response in Th17 lymphocytes, as Th17 cell number increased significantly at PO and remained elevated up to 2H in both groups. In contrast, Perry et al. (2012) reported an immediate and continued increase in circulating Th17 lymphocyte percent up to 1-hour after an acute exercise bout of moderate intensity in both healthy trained athletes and chronic lymphocytic leukemia patients. The disparities in results can be explained, at least partially, by the absence of a control group to account for diurnal variations of the Th17 phenotype and the physical activity status of the participants. Yu et al. (2013) described the diurnal regulation of Th17 cell differentiation in mice as light cycle exposure-dependent, which leads to high Th17 lymphocyte concentration and enhanced susceptibility to inflammatory disease in mice. One possible mechanism of the observed diurnal changes in Th17 lymphocyte concentration could be the secretion of hormones with known immunomodulatory effects, including cortisol, melatonin and norepinephrine.

Extracellular receptors are key for the function and recruitment of T-cells to sites of inflammation. Th17 lymphocytes express CD196 on the cell surface, even after prolonged antigen activation [50]. CD196 mediates chemotaxis of Th17 lymphocytes in response to CC chemokine ligand 20 (CCL20) and mediates the arrest of Th17 cells at specific sites in muscle or endothelium [56]. Arima et al. (2012), reported that sympathetic stimulation lead to elevated IL-6-dependent CCL20 expression in muscle cells, followed by chemotaxis of $CD196^+$ $CD4^+$ T-lymphocytes. Although not significant, a trend for a decrease in Th17 lymphocyte number at 1H may be explained by an upregulation of CD196 receptor expression post-exercise. The exercise group induced a 9.4% increase in CD196 expression when compared to 2.4% in the non-exercise education participants. Our results suggest that exercise-dependent sympathetic stimulation and increased secretion of myokine IL-6 may increase local CCL20 concentration and induce CD196/CCL20 dependent Th17 lymphocyte migration.

Although no exercise-dependent changes in surface receptor expression, our findings also indicate a diurnal response for CD26, where receptor density declined over time after the resting (PRE) blood sample. Dipeptidyl peptidase IV (CD26) is involved in T-cell activation and cell infiltration through cell adhesion and invasion to inflammatory sites. Interestingly, CD26 can be shed from Th17 lymphocyte membranes into circulating blood via proteolytic cleavage to exert enzymatic activities [31]. Nargis et al. (2015) reported significantly lower membrane-bound CD26 on Th17 lymphocytes in TD2 patients, suggesting an important role of CD26 proteolytic cleavage in glucose metabolism. Soluble CD26 plays a key role in blood glucose homeostasis as it regulates the inactivation of insulinotropic hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [31, 47]. GLP-1 and GIP are responsible for ~60% of postprandial insulin secretion, thus CD26 enzymatic activity stimulated greater glucose

concentration in circulating blood [8]. Ryskjaer et al. (2006), reported that plasma CD26 enzymatic activity was positively correlated with HbA1c and fasting blood glucose levels in patients with T2D. CD26 inhibitors prolong the insulinotropic effect of incretins (GLP1 and GIP) and induce macrophage polarization toward anti-inflammatory phenotypes in adipose tissue, mediating adipose tissue inflammation and insulin resistance [51]. During our 5-hour experimental trial, participants were fasted for approximately 15 hours at the 1H and 2H time points, possibly inducing the proteolytic cleavage of CD26 from the membrane of Th17 lymphocytes into circulation to regulate blood glucose.

Exercise training exerts beneficial changes in subclinical inflammation in circulation. We found that twelve weeks of combined exercise training significantly improved participants' cardiovascular fitness, as represented by estimated maximum $VO_{2\ and}$ resting heart, as well as 8RM strength among eight resistance exercises. The improvements in cardiovascular fitness and strength were observed without exercise-training dependent anthropometric (BMI, body mass, waist-to-hip ratio) or glucose control (HbA1c) improvements. Our findings are in accordance with previous studies [23, 54, 55]) that have shown fitness related improvements without changes in body composition or metabolic markers of obesity. Contrary to previously reported exercise training-dependent blunted leukocyte response to a single exercise bout, leukocyte responses were remarkably similar across time points before and after training. Participants in our study, however, performed the exercise bout at a greater absolute intensity and total volume load AT, as the participants 8RM was reassessed and heart rate reserve was re-calculated using AT resting heart rate.

Exercise training is known to reduce systemic inflammation (IL-6, TNF-a) and improve insulin sensitivity with minimal changes in body composition [54, 55]. In our study, twelve-weeks of

aerobic and resistance training did not alter Th17 lymphocyte number or percent in peripheral blood. The absence of a training response in Th17 lymphocytes may be due to several factors. First, human Th17 lymphocytes are long-lived proliferating effector memory T-cells with unique genetic and functional characteristics [50]. Th17 cells maintain core molecular signature resembling memory $CD8^+$ T-cells [45], thus highlighting their persistence as long-lived functional T-effector cells. Due to the relative short-term experimental training period (12-weeks) and the long half-life of the Th17 phenotype (2-8 years), a decrease in absolute number or percent of Th17 lymphocytes in peripheral blood may be unlikely [10].

Second, no training-dependent significant weight loss, changes in body composition or blood glucose control were reported for trained obese women. Long term effects of adiposity and poor dietary habits may override the short-term training benefits in cardiorespiratory fitness for Th17 lymphocytes (Table 12, Figure 15). The linear regression model reported body composition (BMI) and blood glucose control (HbA1c) as the main predictors of Th17 number and percent in circulation. Long-standing dietary and exercise habits exhibited by the lean women overrides the observed 12-week training improvements in the sedentary overweight-obese women.

Finally, the absence of an exercise-training response in Th17 lymphocytes may be due to Th17 activation mechanisms and their respective cytokine secretion after activation in the periphery. Various studies have previously reported significant reduction of circulating Th17-dependent cytokines (IL-17 and IL-23) in murine and human models after exercise training [23, 35]. In addition, Dalgas et al. (2015) reported an attenuated exercise-induced IL-17 release into peripheral circulation after 24-weeks of resistance training in multiple sclerosis patients. The anti-inflammatory effects of exercise training may not be reflected in absolute Th17 lymphocyte concentration, but instead in Th17-dependent cytokine production *in vivo*. Lowder et al. (2010)

reported a dampened T-cell receptor signal in response to antigen presenting cells, thus attenuating inflammation and hyperresponsiveness of Th17 cells in a murine asthma model. The tendency for a reduction in resting circulating IL-6 in trained overweight-obese women (EX) may potentially indicate a training-dependent reduction in systemic inflammation. The Th17 transcription factor RORγt is induced by IL-6 and subsequently increases the expression of circulating IL-17 *in vivo* [10]. Future studies will elucidate on the exercise training-induced Th17-dependent cytokine response.

Conclusion

Data presented herein support for the first time that Th17 lymphocyte number is greater in overweight-obese post-menopausal women when compared to lean controls. Concomitantly, we reported significant associations among key markers of adiposity and blood glucose control with circulating Th17 concentration, highlighting a potential key role of Th17 cells as significant determinants of glycemic control in obesity. The data presented here, in concordance with results presented by other authors (Fabbrini, Onishi, Cheng, Summarac-Dumanovic), suggests that Th17 lymphocytes and an elevated Th17/Treg ratio may be key markers of obesity and may be one of the reasons that obese individuals are at an increased risk of developing CVD and T2D.

No significant acute or exercise-training dependent changes in circulating Th17 lymphocytes were observed. The CD196 upregulation at the PO time point, lead primarily by the exercise group, may induce Th17 lymphocyte migration in response to CCL20 production in muscle tissue. The strong diurnal decrease in CD26 after a log fasting period, reflects the ubiquitous role of CD26 as a surface T-cell activation receptor and soluble enzymatic activity in glucose metabolism [31]. Proteolytic cleavage of Th17 membrane bound CD26 during fasted states

may be one of the mechanisms by which the adaptive immune system contributes to metabolic homeostasis.

Due to the long half-life of effector Th17 cells [10], the known exercise training-dependent positive effects in blood pressure and vascular function may be reflected in Th17 phenotype responsiveness to antigen presentation and changes in circulating cytokine concentrations. The observed trend in training-induced reduction in resting IL-6 concentration may be indicative of decreased ROR-*γt* induction and IL-17 production. The effects of an acute bout of exercise and exercise training on Th17 phenotype cytokines, IL-17 and IL-23, remains to be determined.

Overall, we demonstrated that Th17 lymphocytes are elevated in obese, sedentary post-menopausal women. Our results suggest that the Th17 phenotype is associated with the metabolic dysfunction and glucose control in obesity and may be one of the key reasons why obese individuals are at greater risks for CVD and T2D. Additional studies are required to explore the role of exercise training on Th17 lymphocyte activity and IL-17/IL-23 cytokine production.

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ABSTRACT

T-HELPER 17 RESPONSE TO AN ACUTE EXERCISE BOUT
AND TO 12-WEEKS EXERCISE TRAINING

by Maria Andrea Cardenas, M.S., 2018
Department of Kinesiology
Texas Christian University

Thesis Advisor: Melody Phillips, Associate Professor of Kinesiology

A causative link between adaptive immunity and pathogenesis of obesity-associated diseases has been established. **PURPOSE:** To examine the effects of adiposity and acute/chronic exercise on Th17 lymphocytes in overweight/obese post-menopausal women. **METHODS:** Forty-nine overweight/obese women were randomly assigned to the exercise (EX, n=27) or education (ED, n=22) groups. Thirteen lean, moderately active women were recruited to examine the influence of adiposity on resting circulating Th17 lymphocytes. EX performed a 25-min walk (75-80% HRR) and 2 sets of 8 resistance exercises (70-80% 1RM) with blood samples obtained at: pre-exercise, post-exercise, one-hour and two-hour post-exercise. Blood samples were obtained at the same time points in resting ED. **RESULTS:** Th17 lymphocyte number is greater in overweight-obese post-menopausal women when compared to lean controls (19.6±1.9 vs. 9.7±1.6, respectively). Th17 cells are associated with key markers of adiposity and blood glucose control. No significant acute or exercise-training dependent changes in Th17 lymphocytes were observed.

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EDUCATION

Texas Christian University, Fort Worth, TX 2016-2018

- **Master of Science in Kinesiology – Exercise Physiology, May 2018**
- Harris College Outstanding Academic Achievement Award – 4.0 GPA
- Thesis – "The effects of combined exercise training on Th17 lymphocytes in obese post-menopausal women"

University of Houston, Houston, TX 2013-2016

- **Bachelor of Science - Kinesiology: Exercise Science- Health Professions**
- Date of graduation - July 2016
- Summa Cum Laude - 3.91 GPA

RESEARCH & TEACHING EXPERIENCE

Texas Christian University, Fort Worth, TX 2016-present
Department of Kinesiology – Departmental Graduate Assistant

Research Assistant – Supervisor – Melody Phillips, Ph.D.

- Master thesis work involved the influence of exercise training on cellular modulators of atherosclerotic plaque formation, various markers of inflammation, and cardiovascular disease risk, specifically T-helper 17 lymphocytes (Th17) and Th17 phenotype-dependent cytokines (IL-6, IL-17, IL-23, IL-12), with a special interest in the autoimmune-associated model of Th17 cells and its link to hypertension in overweight/obese, post-menopausal women.

Teaching Assistant –

- Sport and Exercise Pharmacology (HLTH 30233)
- Anatomical Kinesiology (KINE 10603)

Primary instructor – Courses taught:

- Body Conditioning (PEAC 10411)
- Exercise Physiology Laboratory (KINE 30634)

University of Houston, Houston, TX 2014- 2016
Department of Health and Human Performance

Research Assistant – Supervisor – Thomas Lowder, Ph.D.

- Impact of physical activity on female individuals with a rare lung disease known as lymphagioleiomyomatosis (LAM).

PUBLICATIONS, PRESENTATIONS, & AWARDED GRANTS

2018 Exercise-induced Th17 Lymphocyte Response and their Relationship to Cardiovascular Disease Risk Factors in Obese, Post-menopausal Women.

- **Cardenas, M., BS. MS.,** Levitt, M., BS., Richie B., Lu S., Cook, C., Haynes, J., M.D., Orr, K., Kreutzer, A., MS., Mitchell, J., Ph.D., Phillips, M., Ph.D. *International Journal of Exercise Science.* 2: 10, 2018.
- Presented at American College of Sports Medicine Conference: Minneapolis, MN

2018 Texas Chapter of the American College of Sports Medicine Student Research Development Award, "The effects of exercise training on circulating Th17 cells and IL-17 in obese post-menopausal women", $750.00.

2017 Adult Learning and Pediatric Cardiology Education: what works and what doesn't.

- Cabrera, A., M.D, **Cardenas, M., BS.,** Maskatia, S., M.D., & Brown, D., M.D. Adult Learning and Pediatric Cardiology Education: what works and what doesn't. *Progress in Pediatric Cardiology.* "In press".

2017 HIT vs. LSD: Four Days of Intensive Training does not Influence Lactoferrin, but LSD Increases resting IL-6 while Attenuating the Acute Exercise Response, yet HIT Elevates Salivary Cortisol Levels.

- **Cardenas, M., BS.**, Levitt, M., Burgess, B., Haston, C., Jaitner, A., Mitchell, J., Ph.D., Phillips, M., Ph.D. HIT vs. LSD: Four Days of Intensive Training does not Influence Lactoferrin, but LSD Increases resting IL-6 while Attenuating the Acute Exercise Response, yet HIT Elevates Salivary Cortisol Levels. *International Journal of Exercise Science.* 2: 49, 2017.
- Presented at Texas American College of Sport Medicine Conference: Waco, TX.

2017 Self-reported Moderate-high Intensity Physical Activity Does Not Reduce Inflammatory Markers in Overweight/Obese Individuals.

- **Cardenas M., BS.,** Levitt, M., Steck K., Orr K., Kreutzer A., Phillips M., Ph.D. Self-reported Moderate-high Intensity Physical Activity Does Not Reduce Inflammatory Markers in Overweight/Obese Individuals. *Annals of Research in Sport and Physical Activity,* July 2017.
- Presented at the International Society for Exercise and Immunology (ISEI) Symposium.
- Honorable Mention for research poster and presentation

2017 TCU Research and Creative Activities Fund (RCAF) grant, "The effects of 12-Week exercise Training on the Number and Percentage of Th17 Cells in Obese Post-Menopausal Women", $4,000.

2017 TCU HCNHS Student Research Grant, "The effects of exercise training on Th17 cells and IL-17 plasma concentration in obese post-menopausal women", $500.

2016 The effects of high intensity training on myokine response to acute exercise.
Maria A. Cardenas, Michael Levitt, Melody D. Phillips, Brooke Burgess, Cheryl Haston.
HIT does not alleviate intensified training-induced reductions in muscle cytokine response to acute exercise. *The Physiologist.* 59: 6, 2016
Presented at The Integrative Biology of Exercise VII Conference: Phoenix, Arizona.

2015 Acute Heart Falling Syndrome. "Guia Clinica para el manejo de SICA en pediatria."
- Co-writer with Dr. Antonio Cabrera of *"Acute Heart Failing Syndrome"*
SIAC Journal of Cardiology under the pediatrics section. Pignatelli, R., M.D., **Cardenas, M. A., BS.,** & Cabrera, A., M.D. (December, 2015). Guia Clinica para el manejo de SICA en pediatria. *Sociedad Interamericana de Cardiologia*.

OBSERVATION EXPERIENCE

Clinica Sanitas Venezuela, Caracas, Venezuela January 2016
- Dr. Carlos Torrealba
- Assisted in a coronary bypass surgery with stitching and leg incision, where the alternative shunt being used was the femoral artery

CVICU unit, Texas Children's Hospital, Houston, Texas March 2015- 2016
- Weekly observations as an intern in the pediatric cardiovascular ICU under Dr. Dickerson, Dr. Mott, and Dr. Cabrera.
- Assisting in morning and afternoon rounds, treating and caring for critically ill patients

Heart Failure unit, Texas Children's Hospital, Houston, Texas 2014 – 2016
- Dr. Antonio Cabrera

NCAA ATHLETICS

University of Houston Women's Tennis Team, Houston, Texas 2013- 2016
- Character, Teamwork, Time Management Skills
- Accustomed to performing under high pressure situations
- Effectively balanced academic and athletic time commitments

Member of the Houston Leadership Academy (SAAC) 2014- 2016
- Developed personal and team leadership and decision-making skills

VOLUNTEER EXPERIENCES

One Shirt One Body	2016
Soap for Hope	2015
Houston Food Bank	2015-2016
INVEDIN ambassador	2012-present
Ronald McDonald House	2013
Green Cross Venezuela	2017

HONORS, AWARDS, & ACADEMIC ACHIEVEMENTS

Athletic Scholarship Recipient- Auburn University	2012
Athletic Scholarship Recipient – University of Houston	2013-2016
ITA Scholar Athlete – University of Houston	2014 – 2016
Academic MVP- University of Houston	2015
Dean's List – University of Houston	2012 – 2016
Graduate Academic Scholarship Recipient- Texas Christian University	2016-2018
Harris College Outstanding Academic Achievement Award	2018

SKILLS & CERTIFICATIONS

Kinesio Taping, BSN Medical	2014
Basic Life Support BLS- CPR certified	2017
Protecting Human Research Participants - National Institute of Health	2016
encore Operator Training	2017
Radiation Safety Training - Texas Christian University	2016
Fluent in English, Spanish, and French	