HISTAMINE AND CARDIOVASCULAR ADAPTATION TO ENDURANCE EXERCISE

by

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A DISSERTATION

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DISSERTATION ABSTRACT

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Adaptations associated with repeated aerobic exercise come in many forms and act synergistically to increase the amount of oxygen an individual can consume during exercise. Moderate intensity aerobic exercise causes increased histamine concentrations within the active skeletal muscle that is released from mast cell degranulation or *de novo* formation through the enzyme histidine decarboxylase (Steven A. Romero et al., 2016). Histamine activates H_1 and H_2 receptors on vascular endothelial cells and vascular smooth muscle cells of the working skeletal muscle to acutely cause vasodilation and promote increased blood flow. Exercise induced histamine release not only affects post-exercise hemodynamics, but also influences glucose delivery to skeletal muscle, mediates the normal inflammatory response to aerobic exercise, as well as mRNA expression following a single bout of exercise (S A Romero et al., 2016). Outside of the exercise-induced mRNA response, histamine release also mediates other signaling mechanisms that are indirectly involved with adaptation to exercise training such as angiogenesis and red blood cell maturation (Byron, 1877; Qin et al., 2013). Taken together, there is ample evidence that by repeatedly activating or antagonizing histamine receptors during exercise, physiological adaptations to repeated exercise stress could be modulated. The overall purpose of this dissertation was to determine the role of histamine receptor

activation in adaptations to aerobic exercise training. We hypothesized histamine receptor antagonists would blunt positive physiologic adaptation, or gains in aerobic capacity, by effecting the ability to deliver and utilize oxygen during subsequent exercise. The results indicate that the ability to deliver oxygen through increased blood volume via hematopoiesis did not contribute to increased aerobic capacity and was not affected by histamine receptor blockade. However, the ability to deliver oxygen through increased angiogenesis (assessed via capillary density and corroborated by peak vasodilatory capacity) and utilize oxygen through increased SDH activity (corroborated by VO_{2peak}) contribute to increased exercise capacity but that these adaptations occur either independent of the effects of histamine receptor activation or that adaptation is generated by redundant mechanisms in a highly resilient manner.

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CHAPTER I

INTRODUCTION

Statement of the Problem

The measurement of maximal oxygen consumption (VO_{2Max}) is used not only to assess exercise performance, but also as a marker of population-based cardiorespiratory fitness, cardiovascular disease, and mortality, which all show positive adaptation with continued exercise training (Lamonte et al., 2006; Lee, Hsieh, & Paffenbarger, 1995; Pedersen & Saltin, 2015). Physical inactivity is a known, but modifiable, risk factor that can contribute to lifestyle-related diseases, including many causes of preventable death (F. Booth, Roberts, & Laye, 2012). However, only 50% of the American population exercise regularly with only 33% of the exercising population that perform the recommended amount of physical activity each week (Benjamin et al., 2017). Since exercise is medicine that the general population does not want to consume, all efforts should be made to ensure the beneficial adaptations associated with repeated exercise are realized. Therefore, the need to maximize the positive effects of exercise training is of the upmost importance and strategies to enhance post-exercise recovery such as altered nutrition, temperature, or supplements are commonly employed (Dalleck, 2017). The efficacy of post-exercise recovery strategies remains questionable and generally cannot be applied across a population to promote positive responses, therefore factors that modulate training induced adaptation need further exploration (Meredith J. Luttrell & Halliwill, 2015; Padilla & Mickleborough, 2007).

Adaptations associated with repeated aerobic exercise come in many forms and act synergistically to increase the amount of oxygen an individual can consume during

exercise. The concept that there is a limit to the transport of oxygen from the environment to be used for energy production, termed maximal oxygen consumption, started nearly a century ago and is still being utilized today (Hill, Long, & Lupton, 1924). Maximum oxygen consumption is now a commonly used tool in exercise physiology to assess fitness levels, and can also be used in the general population to assess clinical risk of multiple preventable diseases (F. Booth et al., 2012). The ability to adapt to exercise training is highly variable across individuals and that variability is not well understood (Green, Spence, Rowley, Thijssen, & Naylor, 2012; Moreau & Ozemek, 2017; Peake et al., 2015). Exploiting physiological responses to acute exercise can provide insight into the process of physiological adaptation and help further understand how to promote positive physiologic adaptations across the population.

Many of the signaling mechanisms thought to be responsible for the beneficial physiological adaptations to exercise training remain elevated during the period of recovery from exercise and could be exploited to modulate adaptation (Meredith J. Luttrell & Halliwill, 2015; Steven A. Romero, Minson, & Halliwill, 2017). Histamine receptor activation is known to be the primary driver of post-exercise hypotension (Barrett-O'Keefe, Kaplon, & Halliwill, 2013); thus, modulation of histamine receptor activation during exercise, and subsequent reduction in post-exercise hypotension, could influence adaptation to repeated exercise stress (Meredith J. Luttrell & Halliwill, 2015). Exercise induced histamine release not only affects post-exercise hemodynamics, but also influences glucose delivery to skeletal muscle, mediates the normal inflammatory response to aerobic exercise, as well as mRNA expression following a single bout of exercise (S A Romero et al., 2016). Outside of the exercise-induced mRNA response,

histamine release also mediates other signaling mechanisms that are indirectly involved with adaptation to exercise training such as angiogenesis and red blood cell maturation (Byron, 1877; Qin et al., 2013). Taken together, there is ample evidence that by repeatedly activating or antagonizing histamine receptors during exercise, physiological adaptations to repeated exercise stress could be modulated.

Purpose and Hypothesis

The overall purpose of this dissertation was to determine the role of histamine receptor activation in adaptations to aerobic exercise training. Specifically, following six weeks of aerobic cycling exercise training in young healthy inactive volunteers, we determined the effect of histamine receptor antagonists on improvements in:1) aerobic capacity, 2) angiogenesis and blood volume, and 3) skeletal muscle SDH activity. The overall hypothesis of this dissertation is that histamine receptor antagonists will blunt positive physiologic adaptation, or gains in aerobic capacity, by affecting the ability to deliver and utilize oxygen during exercise. Specifically, following six weeks of aerobic cycling exercise training in young healthy inactive volunteers, we hypothesized that histamine receptor antagonists will blunt exercise induced gains in: 1) aerobic capacity and maximal work, 2) capillarization and blood volume, and 3) skeletal muscle mitochondrial succinate dehydrogenase activity.

Significance

The research objectives outlined in this dissertation represent an advance in the basic scientific and mechanistic literature of adaptation to aerobic exercise training through a pathway responsible for robust post-exercise hemodynamic responses and gene expression responses to acute aerobic exercise. The mechanisms by which repeated

exercise stress are translated into beneficial physiological adaptation are not fully understood. However, histamine receptor activation during exercise has been clearly implicated. By blocking histamine receptor activation, we have gained novel insight into what role this signaling pathway has in the physiologic mechanisms that drive adaptation to aerobic exercise training. This dissertation also advances our knowledge concerning the role of histamine receptor activation in recovery from exercise and how this distinct physiological state can be used as a tool to maximize the benefits of aerobic exercise. Additionally, this study furthers the knowledge of disease states like hypertension and the use of exercise as a non-pharmacological approach to chronically reduce blood pressure.

CHAPTER II

REVIEW OF LITERATURE

Introduction.

The maximum amount of oxygen an individual can consume during exercise, termed VO_{2Max}, is calculated as cardiac output multiplied by arterio-venous oxygen difference. The equation for VO_{2Max} can be broken down into two parts: oxygen delivery and oxygen utilization. VO_{2Max} is determined by the combined capacities of the nervous system to recruit muscle (motor units), the cardiovascular and pulmonary system to extract and deliver oxygen from the environment to the contracting skeletal muscle, and the increase in skeletal muscle metabolism to consume oxygen. Simply, oxygen is extracted from the environment, transported by blood, and utilized by working muscle cells. Repeated exercise stress causes physiologic adaptations of multiple body systems that lead to the ability to consume more oxygen and perform more work during subsequent exercise (F. W. Booth, Ruegsegger, Toedebusch, & Yan, 2015). The exact chemical and physical signals derived from exercise that drive adaption are still unknown and require further exploration. Isolating and manipulating these signals provides insight into unknown mechanisms underlying adaption to chronic exercise training.

It is known that histamine is released during exercise and histamine receptor activation is an obligatory step in the normal response to an acute bout of aerobic exercise (Halliwill, Buck, Lacewell, & Romero, 2013; Steven A. Romero et al., 2017). When histamine receptor activation is antagonized, normal molecular responses to exercise are modulated and post-exercise reductions in blood pressure are attenuated (S A Romero et al., 2016). These physiological responses to histamine receptor antagonism

play a major role in how the body responds to exercise and allude to the lack of understanding regarding the role of histamine receptors in adaption to exercise training. (M J Luttrell & Halliwill, 2017; S A Romero et al., 2016). This chapter will address exercise-induced histamine release, the physiologic responses to normal adaptation to chronic exercise, and highlight the importance of histamine receptor activation in these processes with an emphasis on how manipulating the histamine signaling pathway can augment the adaptive response to exercise training.

Acute Exercise and Histamine.

Evidence that histamine is released during exercise comes from a line of research in Dr. Halliwill's laboratory exploring post-exercise hypotension. This research found that histamine is created, stored, and released within skeletal muscle. Histamine activates H_1 and H_2 receptors on vascular endothelial cells and vascular smooth muscle cells to cause vasodilation and promote increased blood flow. Previously active skeletal muscle vasculature will remain dilated for hours following exercise and result in reduced arterial pressure during that time. The increased blood flow and reduced arterial pressure following exercise is attenuated by blocking histamine H_1 and H_2 receptors (Mccord & Halliwill, 2006; J.L. McCord, Beasley, & Halliwill, 2006; Steven A. Romero et al., 2016). Additionally, blocking histamine H_1 and H_2 receptors during exercise modulates the normal change in skeletal muscle mRNA expression related to endothelial and vascular function, metabolism, angiogenesis, cell maintenance, and inflammation (S A Romero et al., 2016). Due to the wide range of exercise related factors affected by

histamine receptor activation, chronic blockade of histamine receptors during exercise will likely affect normal exercise adaptation.

Histamine concentrations are difficult to measure in blood because of its short half-life *in vivo* of approximately 100 s and its rapid degradation via enzymatic processes of di-amine oxidase or histamine-N-methyltransferase (M J Luttrell & Halliwill, 2017). Studies that have shown attenuation of sustained post-exercise vasodilation and post-exercise hypotension with histamine receptor antagonism have failed to demonstrate that whole blood or plasma histamine concentrations increase during or following whole body exercise (Lockwood, Wilkins, & Halliwill, 2005; J.L. McCord & Halliwill, 2006; Jennifer L McCord, Beasley, & Halliwill, 2006). Additionally, studies that utilize small muscle mass exercise, like unilateral knee kicking, show no vasodilation in the unexercised leg (Barrett-O'Keefe et al., 2013; S A Romero et al., 2016). Histamine is released locally in the active skeletal muscle and does not spillover into the systemic circulation, which was confirmed by use of muscle microdialysis during exercise (S A Romero et al., 2016). This evidence suggests that histamine exerts its influence locally and that blocking its actions will have little to no effect on systemic exercise adaptations, but greater effects on the local exercised skeletal muscle.

Mechanisms of Histamine Release.

The mechanisms of local production and release of histamine in exercising skeletal muscle include mast cell degranulation and *de novo* formation through the enzyme histidine decarboxylase (Steven A. Romero et al., 2016). Both mechanisms act through the enzyme histidine decarboxylase (HDC) to contribute to the local production

and release of histamine in exercising skeletal muscle. The histamine molecule is a biogenic amine and synthesized by the pyridoxal phosphate (activated vitamin B6) containing L-histidine decarboxylase from the amino acid L-histidine (Criado, Criado, Maruta, & Filho, 2010; M J Luttrell & Halliwill, 2017). Once histamine has been released, it acts as either an autocrine or paracrine signal and binds to one of its 4 known (H_1-H_4) receptor subtypes. All 4 histamine receptor subtypes are G protein-coupled receptors but signal through different second messengers (Parsons & Ganellin, 2006). Activation of H_1 receptors acts through a G-protein coupled (G_q) receptor that activates phospholipase-C and culminates in the formation of inositol triphosphate (IP_3) and diacylglycerol (DAG), leading to increased intracellular calcium (Criado et al., 2010). Activation of H_2 receptors acts through a G-protein coupled (G_s) receptor that causes increased cyclic adenosine monophosphate (cAMP) and leads to smooth muscle relaxation.

The exercise-related factors that trigger histamine release are still unclear, but it has been thought that factors such as increased heat production, oxidative stress, vibration, pH changes, shear stress, and cytokines cause mast cell degranulation leading to histamine release (Halliwill et al., 2013). Research within the Halliwill lab has ruled out oxidative stress as a potential trigger of histamine release with exercise, as a potent antioxidant did not attenuate post-exercise hypotension (S A Romero et al., 2015). Vibration from muscular movement does not seem to be a likely trigger of histamine release, because no sustained vasodilation is observed following passive movement (unpublished results). Increased muscle temperature as a byproduct of muscular contraction is a promising candidate for histamine release as local heat therapy causes

increased interstitial histamine concentration, which is similar to that observed during exercise (Romero et al., 2016, unpublished results). Other causes of exercise-induced histamine release could occur, and more research is needed to understand the signal for histamine production during exercise.

Actions of Histamine.

Histamine H_1 and H_2 receptors are located on vascular smooth muscle cells and cause differing responses depending on the type and location of the receptor (Marshal, 1984). When histamine is directly introduced into the circulation, it causes increased blood flow through small resistance coronary arteries regardless of metabolic needs (Kern, 1991). Intravenous injection of histamine causes hypotension, which is acutely mediated through H_1 receptors and sustained by slower activation of H_2 receptors, which causes vasoaction for longer periods of time (Marshal, 1984). In endothelial or smooth muscle cells surrounding the vasculature, activation of histamine H_1 and H_2 receptors ultimately causes vasodilation through reduced smooth muscle cytosolic calcium concentration.

Histamine is synthesized and released by different cell types (mast, basophils, platelets) in humans and is a potent mediator of numerous physiological reactions (Criado et al., 2010). Depending on the location and type of receptor, histamine causes vasodilation, increased gut motility, bronchoconstriction, increased cardiac contractility, and induces shock-like symptoms (Parsons & Ganellin, 2006). Histamine induced vasodilation is reduced when H_1 receptors are antagonized but no effect was observed with H_2 receptor blockade, suggesting H_1 receptors drive this tissue specific vasodilatory

response in coronary resistance arteries (Vigorito et al., 1987). In humans, activation of H_1 and H_2 receptors causes constriction of large conduit arteries when exposed to histamine, primarily through H_1 receptors (Daneshmand, Keller, Canver, Canver, & Canver, 2004). In humans and other animal models, histamine H_1 receptors found on the smooth muscle of epicardial coronary arteries cause vasodilation when exposed to histamine, however, activation of histamine H_1 receptors in large conduit coronary arteries causes vasoconstriction (Miller & Bove, 1988; Toda, 1983; Vigorito, Poto, Picotti, Triggiani, & Marone, 1986). In animal models, activation of histamine H_2 receptors found on the smooth muscle of epicardial coronary resistance arteries causes vasoconstriction, but their actions are reversed in larger conduit arteries (Miller & Bove, 1988). Together, these results demonstrate there are differing responses of activation of histamine receptors based on their anatomical location within species, tissues, and cell types.

Adaptation to Repeated Exercise Stress.

The term adaptation refers to the idea that repeated low levels of stress upregulate cellular pathways that improve the capacity of an organism to resist greater stress (Calabrese et al., 2007). On a molecular level, gene expression is increased in response to acute exercise stress, which causes increased synthesis of respective proteins, and facilitate gradual structural remodeling that leads to long-term functional improvements (Perry et al., 2010). On a systemic level, repeated exercise stress will improve oxygen delivery and utilization, enhancing one's ability to maintain homeostasis in the face of increasing metabolic challenges. Adaptations to exercise that are oxygen delivery centric

include blood volume expansion, improved vascular function, and angiogenesis. Adaptation to exercise that are oxygen utilization centric include increased mitochondrial volume density and oxidative function. Additionally, adaptations to exercise will eventually cause improved overall health status via decreased blood pressure, improved body composition, reduced risk of cardiovascular disease, and reduced all-cause mortality (Lamonte et al., 2006; Lee et al., 1995; Pedersen & Saltin, 2015).

Angiogenesis. With chronic dynamic exercise, there is remodeling of the vascular system in skeletal muscles subjected to training in order to increase oxygen delivery. This remodeling includes an increase in the diameter of feed arteries and also the quantity of arterioles and capillaries within the exercised skeletal muscle. (Green et al., 2012). Simply, exercise promotes growth and expansion of the capillary network, known as angiogenesis, within skeletal muscle (Green, Hopman, Padilla, Laughlin, & Thijssen, 2017; Green & Smith, 2017; Prior, Yang, & Terjung, 2004). Angiogenesis causes increased oxygen delivery by increasing the surface area for diffusion of oxygen from red blood cells, which allows more oxygen to be consumed and higher levels of exercise to be accomplished (Green & Smith, 2017; D. H J Thijssen, De Groot, Smits, & Hopman, 2007). The growth or decline of the skeletal muscle capillary network is dependent upon pro- and anti-angiogenic growth factors that are stimulated in various ways, one being activity level (Olfert & Birot, 2011). The upstream mechanisms of exercise induced angiogenesis are unclear, however shear stress and hypoxia are both thought to drive the formation of new capillaries in skeletal muscle with chronic exercise (Egginton, 2009, 2011).

New capillaries are formed via sprouting or intussusceptive (splitting) angiogenesis, with much more information available on sprouting (Haas, Lloyd, Yang, & Terjung, 2012). Sprouting angiogenesis has 5 major steps that include: increased capillary permeability, endothelial cell proliferation, degradation of the vessel basement membrane, extension of endothelial cells, and vessel perfusion (Haas et al., 2012). Splitting angiogenesis is thought to occur by growth of new capillaries within the existing vasculature, which creates two separate vessels in parallel with each other that eventually become independent vessels (Olfert & Birot, 2011). Both sprouting and splitting angiogenesis have been shown to occur in response to increased blood flow which occurs during exercise (Egginton, Zhou, Brown, & Hudlická, 2001). Blood flow in the previously active skeletal muscle normally remains elevated following exercise and could be a continued stimulus for angiogenesis. However, blood flow in the previously active skeletal muscle following exercise is reduced with antihistamine consumption, which could limit the angiogenic stimulus (Barrett-O'Keefe et al., 2013; J.L. McCord & Halliwill, 2006).

Capillary remodeling and angiogenesis in skeletal muscle is complex and dependent on the balance of pro- and anti-angiogenic factors. When the balance is in favor of pro-angiogenic factors, the capillary network will grow and conversely when the balance tips to anti-angiogenic factors the capillary network will decline (Olfert & Birot, 2011). Acute aerobic exercise upregulates both pro- and anti-angiogenic growth factors within the previously active skeletal muscle such as vascular endothelial growth factor (VEGF), nitric oxide synthase, thrombospondin 1 and endostatin (Richardson et al., 1999; S A Romero et al., 2016). Importantly, histamine receptor blockade modulates the

balance of pro- and anti-angiogenic factors following acute exercise by blunting both nitric oxide synthase and thrombospondin 1 mRNA (S A Romero et al., 2016). The blunting of both a pro- and anti-angiogenic factor with histamine receptor blockade may influence vascular adaptations, like angiogenesis, with exercise training.

Activation of histamine receptors during aerobic exercise upregulates the pro-angiogenic growth factor vascular endothelial growth factor (Ghosh, Hirasawa, & Ohuchi, 2001), which is attenuated with histamine receptor blockade (S A Romero et al., 2016). The synergistic effect of histamine on vascular endothelial growth factor acts through H_1 receptors as it is attenuated with histamine H_1 receptor blockade (Lu et al., 2013). Additionally, histamine has been shown to directly stimulate angiogenesis through activation of histamine receptors and is independent from growth factors like vascular endothelial growth factor (Qin et al., 2013). Taken together, histamine receptor activation and histamine itself could be a signal for angiogenesis associated with aerobic exercise training. Since antagonism of histamine receptors attenuates the upregulation of vascular endothelial growth factor, it could blunt the angiogenic potential of exercise training.

Blood Volume Expansion. Chronic dynamic exercise causes an expansion of red blood cell and plasma volume to enhance the ability to deliver oxygen and increase aerobic exercise capacity (David Montero et al., 2017). Exercise training-induced blood volume expansion is evident by increased red blood cell and plasma volumes found in endurance trained individuals when compared to sedentary counterparts, regardless of sex differences (Michael N. Sawka, Convertino, Eichner, Schnieder, & Young, 2000). The primary role of red blood cells is the transport of respiratory gasses that diffuse across

alveolar or capillary barriers and bind to hemoglobin to be transported as bulk blood flow and used in oxidative phosphorylation or removed from the body. Red blood cells also participate in supporting exercise by buffering blood pH, clearing metabolic waste products, and releasing molecules like ATP or nitric oxide to modulate vascular conductance (Mairbäurl, 2013). Expansion of red blood cell and plasma volume with exercise training is a fundamental adaptation and primary driver of the enhanced ability to consume oxygen via increased oxygen delivery through increased cardiac preload and maximal cardiac output (David Montero et al., 2015). Improvements in oxygen consumption following exercise training are not solely due to passive increases in blood volume and rely on the combined adaptations of vascular oxygen delivery mechanisms and blood volume expansion (Warburton et al., 2004).

Exercise training-derived increases in blood volume cause increased VO_{2Max} that is reversed when red blood cell volume is experimentally manipulated to pre-exercise training levels through phlebotomy (Bonne et al., 2014). Additionally, sedentary individuals, who have no history of exercise training but higher than average VO_{2Max}, have enhanced red blood cell volume, demonstrating the importance of red blood cell volume and oxygen delivery in determining maximal oxygen consumption during exercise (Martino, Gledhill, & Jamnik, 2002). Red blood cell volume expansion usually occurs slowly over weeks to months of exercise training, while plasma volume expansion occurs rapidly and can change within hours (Michael N. Sawka et al., 2000). Within the first 2 weeks of exercise training, all blood volume expansion can be accounted for by plasma volume expansion, however it takes up to 3 weeks of exercise training before red blood cell volume expansion is observed. Red blood cell volume expansion continues

with additional exercise training and has maximal values around a 10% increase from pre-training (Michael N. Sawka et al., 2000).

Studies that utilize exercise training have reported inconsistent expansion of red blood cell volume ranging from no change following months of training (Shoemaker, Green, Coates, Ali, & Grant, 1996), to a 10% increase in red cell volume following 6-weeks of exercise training (Bonne et al., 2014). It is likely that inconsistencies in training intensity, duration, and population are driving these differences in response (David Montero et al., 2017; Michael N. Sawka et al., 2000). Current meta-analyses demonstrate that young sedentary individuals will show moderate (4%) red blood cell volume expansion in response to endurance exercise training averaging 15 weeks, with duration and intensity of exercise training playing a major role in the scale of response (D. Montero & Lundby, 2017). When exercise intensity is controlled, continuous exercise training and high intensity interval training have similar effects on blood volume expansion across similar time periods (Warburton et al., 2004). Additionally, exercise training related factors like initial training status of the population, exercise intensity, exercise modality, and posture during exercise can all factor into the scale of blood volume expansion observed (Michael N. Sawka et al., 2000).

Red blood cell production is a heavily regulated process that starts with hematopoietic stem cells located within bone marrow that have the unique ability to produce any mature blood cell type in a process termed hematopoiesis. The hormone erythropoietin is required for common myeloid progenitor cells to mature into red blood cells (M.N. Sawka, 1999). The principle factor regulating red cell volume expansion is the hormone erythropoietin, which is stimulated by factors like hypoxia and reduced

central venous pressure that occur during exercise (M.N. Sawka, 1999). Erythropoietin is a glycoprotein hormone produced in the kidney and stimulates proliferation and maturation of erythroid progenitor cells found in bone marrow, which ultimately leads to increased release of red blood cells into the circulation (Jelkmann, 2011). Plasma erythropoietin levels will increase around 30% in untrained individuals following 60 min of cycle exercise at 50-70% VO_{2Max}. However acute plasma erythropoietin responses to exercise will decrease with continued exercise training (David Montero et al., 2017). These findings suggest that sedentary individuals will have a robust erythropoietin response to exercise that will decrease with further exercise exposure at the same intensity and could shine light on the inconsistent red blood cell volume expansion found across the literature.

Mature red blood cells and mast cells are derived specifically from common myeloid progenitors and as the stem cell matures it undergoes changes in gene expression that move it closer to a specific cell type (Szade et al., 2018). The level of the enzyme histidine decarboxylase is very high in bone marrow compared to other tissue types and histamine release in bone marrow initiates additional histamine induction through enzymatic activity (Hirata, 1975). Additionally, hematopoietic stem cells express histamine H_2 receptors that initiate changes in the cell cycle when activated (Byron, 1877). This indicates that antihistamine use could inhibit hematopoiesis that would normally be associated with exercise training.

Mitochondrial Biogenesis. There is a well-established expansion of the oxidative capacity of trained skeletal muscles to enhance the ability to utilize oxygen with chronic aerobic

exercise (F. W. Booth et al., 2015; Howald, 1985). It has been known since the 1960s that exercise training results in increased mitochondrial protein levels involved with ATP production when compared with sedentary controls (Holloszy, 1967). Mitochondrial biogenesis is defined as an increase in skeletal muscle mitochondrial number and volume, with increased organelle protein content (Egan & Zierath, 2013). Mitochondria found in skeletal muscle form two distinct populations, subsarcolemmal and intermyofibrillar, based on their location within the myocyte. Intermyofibrillar mitochondrial populations are in close proximity to the contractile apparatus, while subsarcolemmal mitochondria are responsible for energy demands of maintaining the cell membrane (F. W. Booth et al., 2015). The stimulus for mitochondrial biogenesis is muscle contraction; thus, mitochondrial biogenesis is fiber type specific such that muscle fibers not recruited during exercise training will display limited or no mitochondrial adaptations (Hood, 2001).

Mitochondrial biogenesis is a complicated process that involves the coordinated transcription of two genomes and assembly of large protein and enzyme complexes into a functional electron transport chain (Hood, Irrcher, Ljubicic, & Joseph, 2006). Muscular contraction is the primary signal for mitochondrial biogenesis and can cause altered rate of gene transcription, mRNA degradation, mitochondrial protein import, or protein folding, all of which can influence protein level and functional oxygen consumption capacities (F. W. Booth et al., 2015; Hood, 2001). A single bout of endurance exercise has been shown to upregulate the expression of the inducible coactivator protein peroxisome proliferator-activated receptor gamma coactivator 1-alpha, which regulates the coordinated expression of mitochondrial proteins and has been positively correlated

with increased skeletal muscle mitochondria quantity (F. W. Booth et al., 2015; Lin, Handschin, & Spiegelman, 2005). Continuous exercise stimulus is needed to maintain elevated mitochondrial volume density as mitochondrial protein and enzyme levels will start to decline following 7 days of disuse (Henriksson & Reitman, 1977). Mitochondrial content will increase up to 100% and remain at that elevated steady state following 6-weeks of exercise training depending on intensity and duration of exercise (Hoppeler, 1986). Moderate improvements in VO_{2Max} (around 5-20%) with 6-weeks of endurance exercise training can be explained by the large increases (50-100%) in mitochondrial content and the ability to utilize oxygen. Interestingly, moderate improvements in VO_{2Max} fail to explain large enhancements in exercise performance within the same time frame (Levine, 2008; Vollaard et al., 2009).

Increases in mitochondrial biogenesis can be assessed using multiple methods including direct assessment of organelle volume in relation to total cellular volume (mitochondrial volume density), total count of mitochondrial DNA copy number, or expression of inducible coactivators like peroxisome proliferator-activated receptor gamma coactivator 1-alpha that regulate the expression of mitochondrial proteins (Hood, 2001). Additionally, mitochondrial content can be assessed by the maximal velocity of reactions of marker enzymes like citrate synthase or succinate dehydrogenase under supraphysiological substrate concentration conditions in vitro (Henriksson & Reitman, 1977; Proctor, Sinning, Walro, Sieck, & Lemon, 1995). Changes in mitochondrial volumes estimated morphometrically are highly correlated with changes in enzyme maximum velocity values, which allows functional measurements of potential oxygen utilization (Reichmann, Hoppeler, Mathieu-Costello, von Bergen, & Pette, 1985).

Influences on Adaptation to Exercise Training.

Hormesis refers to a process in which low doses of stress, which are damaging at high doses, induces a beneficial effect on an organism (Mattson, 2008; Peake et al., 2015). Hormesis embodies the basic concept of adaptation and can be a useful tool when thinking about adaptation to chronic exercise stress (Radak, Chung, & Goto, 2005). The concept of hormesis is likely why moderate levels of physical activity cause reduced illness and mortality, while high doses of exercise increase those risks (Lee et al., 1995; Peake et al., 2015). The exact chemical or physical signals from exercise that drive adaptation are still unknown but thought to occur during exercise, which drive an adaptive response within hours following exercise. The recovery from exercise could therefore be a window of opportunity to modulate the exercise derived signals that drive adaptation (Meredith J. Luttrell & Halliwill, 2015).

The gold standard measurement of cardiorespiratory fitness is the measurement of maximal oxygen consumption during exercise, or VO_{2Max}, and can be applied across the population. There is a robust response in VO_{2Max} to aerobic physical fitness programs that allow for increased ability to consume oxygen, along with multifaceted health benefits. However, there is high variation (up to 50%) in individual responses to exercise training with some individuals exhibiting reduced ability to increase cardiorespiratory fitness measured as VO_{2Max} (Bouchard et al., 2011). In young healthy sedentary individuals, VO_{2Max} is thought to be limited by cardiac output, or the ability to deliver oxygen to working skeletal muscle in the face of basal metabolic demand and maintaining homeostasis (González-Alonso & Calbet, 2003; Lundby, Montero, & Joyner, 2016; Saltin & Strange, 1992). However, the ability to utilize oxygen in skeletal muscle through

oxidative metabolism can also be a limiting factor in some populations and must be explored to understand the full picture of adaptation to exercise training (Joyner & Coyle, 2008; David Montero & Díaz-Cañestro, 2015). To overcome variation associated with responses to exercise training, the combination of continuous and high intensity interval exercise will demonstrate more robust increases in VO_{2Max} across a population (Bacon, Carter, Ogle, & Joyner, 2013). Therefore, studies aiming to understand the basic biology of exercise-induced training adaptations need to utilize methods designed for the most robust responses in cardiovascular fitness.

Antioxidant supplementation is a common strategy employed to promote enhanced health effects associated with aerobic exercise training. Antioxidants like vitamin C and E are supplemented to help counteract the production of reactive oxygen species from mitochondrial oxidative phosphorylation (Ristow et al., 2009). However, antioxidant supplementation may interfere with exercise induced cell signaling is skeletal muscle and blunt or block adaptations to exercise training (Padilla & Mickleborough, 2007). Direct evidence shows that daily antioxidant supplementation with vitamin C and E diminish increases in markers of mitochondrial biogenesis following exercise training, however no clear interactions were detected for improvements of VO_{2Max} (Paulsen et al., 2014). These results imply that normally functioning cell signaling pathways are essential in maximizing exercise induced adaptations to training and anything that modulates normal cell signaling may blunt positive adaptations.

Adaptation and Histamine Receptor Activation.

Histamine is an important signaling molecule that is released during exercise, and activation of its receptors drive multiple post-exercise responses. Histamine receptors are located in multiple cell types, found throughout the body, and cause a multitude of physiological responses. Histamine receptor antagonism causes decrements in exercise performance in competitive cyclists, suggesting histamine has an inherent role in endurance exercise capacity (Ely et al., 2019). Additionally, histamine receptor antagonism during muscle damaging exercise results in increased markers of muscle damage, while minimizing strength loss normally associated with this exercise modality, suggesting histamine may play a protective role in exercise induced muscle damage (Ely et al., 2017). Blocking histamines actions could influence physiological adaptation to exercise training in multiple ways including reduced expression of nitric oxide synthase, post-exercise blood flow, angiogenesis, and red blood cell formation. Normally functioning cell signaling pathways are essential in maximizing exercise induced adaptations to training and anything that modulates normal cell signaling may blunt positive adaptations (Padilla & Mickleborough, 2007).

Histamine is an established endothelial-dependent vasodilator that acutely modulates smooth muscle tone by activation of endothelial nitric oxide synthase and formation of nitric oxide. Histamine H_1 and H_2 receptor blockade has been shown to decrease expression of endothelial nitric oxide synthase following acute exercise (S A Romero et al., 2016). A histamine-induced upregulation of endothelial nitric oxide synthase gene expression could result in a sustained enhancement of nitric oxide production, which would help increase oxygen delivery during exercise. This represents a

clear pathway of histamine receptor activation and positive adaptations to endurance exercise training that could be blunted with chronic use of histamine receptor antagonists.

Histamine receptor activation is essential for normally observed post exercise increased blood flow of the previously active skeletal muscle. Specifically, histamine H_2 receptor activation on vascular smooth muscle causes sustained vasodilation in the previously active skeletal muscle (J.L. McCord & Halliwill, 2006; Jennifer L McCord et al., 2006; Pellinger, Simmons, Maclean, & Halliwill, 2010). Antagonism of histamine H_1 and H_2 receptors consistently reduces post-exercise vasodilation by ~80% and post-exercise hypotension by ~65% following 60 min cycling exercise at 60% VO_{2Peak} (Lockwood et al., 2005; J.L. McCord & Halliwill, 2006; Jennifer L McCord et al., 2006). Histamine receptor activation induced blood flow patterns and blood pressure reductions following exercise could be a stimulus for positive adaptations to endurance exercise training that could be blunted with chronic use of histamine receptor antagonists

Both sprouting and splitting angiogenesis have been shown to occur in response to increased blood flow (Egginton et al., 2001), which occurs during exercise. Blood flow is also elevated following exercise due to activation of histamine receptors (Barrett-O'Keefe et al., 2013; J.L. McCord & Halliwill, 2006). Histamine has been shown to directly stimulate angiogenesis through activation of histamine receptors and is independent from growth factors like vascular endothelial growth factor (Qin et al., 2013). Histamine receptor blockade modulates the balance of pro- and anti-angiogenic factors following acute exercise by blunting both nitric oxide synthase and thrombospondin 1 gene expression (S A Romero et al., 2016). The blunting of both a pro- and anti-angiogenic factor with histamine receptor blockade may influence vascular

adaptations, like angiogenesis, with exercise training. Activation of histamine receptors during aerobic exercise upregulated the pro-angiogenic growth factor vascular endothelial growth factor (Ghosh et al., 2001) and is attenuated with histamine receptor blockade (S A Romero et al., 2016). The synergistic effect of histamine on vascular endothelial growth factor could be attenuated with histamine H_1 receptor blockade (Lu et al., 2013). Taken together, histamine receptor activation and histamine itself could be signals for angiogenesis that occurs with aerobic exercise training. Since antagonism of histamine receptors attenuates post-exercise vasodilation following acute exercise, it could also blunt the angiogenic potential of exercise training.

CHAPTER III

EXPLANATION OF THE METHODOLOGY

Overview of Project.

Studies conducted in this dissertation were approved by the Institutional Review Board at the University of Oregon (Protocol #10272017.031) and conducted in accordance with guidelines set forth by the Office for Protection of Human Subjects at the University of Oregon. Written informed consent was obtained from all subjects subsequent to a verbal briefing of experimental design, total time commitments, and testing procedures. The double-blind placebo controlled research protocol included young, recreationally active men and women undergoing a 6-week exercise training intervention (exercising 3-4 times per week, totaling 21 sessions) with the experimental group consuming over-the-counter antihistamine drugs and a control group consuming a placebo 1h prior to each exercise session. There were 6 data collection study days that included 2 separate study days for pre- and post-training measurements (4 total) and 1 study day after every 7 exercise sessions, or 2 weeks, depending on subject schedules. All study days were conducted on the University of Oregon Campus in Esslinger Hall apart from muscle biopsy procedures, which were completed in Pacific Hall. Figure 3.1 shows an overview of the research timeline.

Subject Characterization.

Seventeen (11 women) healthy subjects participated in this study (age 25 ± 4 yrs, height 171.8 ± 9.3 cm, weight 73.8 ± 11.8 kg, body mass index 25 ± 3.2 m^2, mean ± SD), Table 3.1. Sixteen (10 women) subjects completed the study as 1 subject withdrew from

the study after the second study day due to scheduling conflicts, thus those data were not included in any analysis.

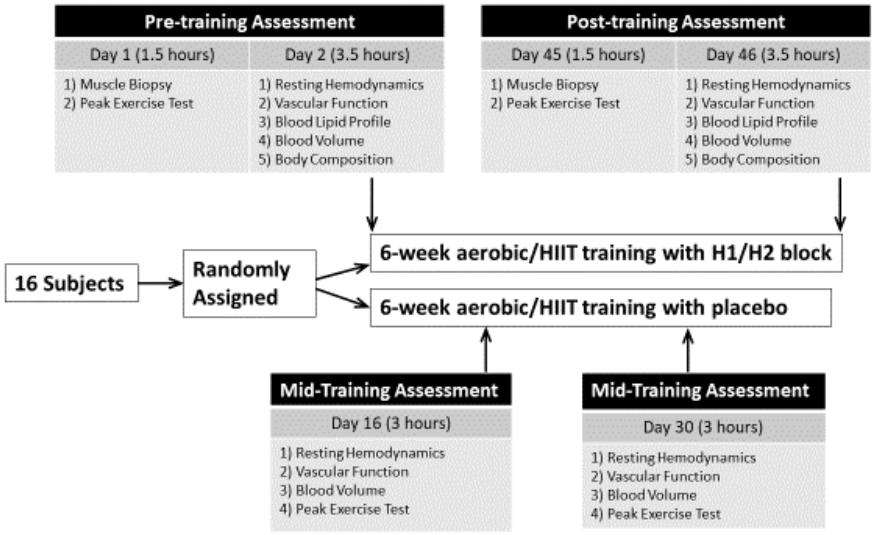


Figure 3.1. Overview of the research timeline.

Subjects were deemed healthy following a standard health screening. Once written informed consent was obtained, subjects completed a self-reported questionnaire (Baecke sport index) to determine their average activity level for the 12 months prior to participating in the study in order to classify subjects as sedentary to recreationally active (Baecke, Burema, & Frijters, 1982). All subjects were required to abstain from supplements, caffeine, and alcohol for at least 12 h, and exercise for at least 24 h prior to all study days. Additionally, food logs were kept 24 h prior to starting the study and replicated before all subsequent data collection days.

Subjects reported to the laboratory after an overnight fast for muscle biopsies and following a 4-h fast for all other data collection study days. All data collection study days took place in a thermoneutral (18-22^{o}C) lab environment and were completed 24-48 h after the most recent exercise session. Data collection start time varied across subjects

due to scheduling conflicts; however, experimental start-time was held constant within a subject to minimize circadian influence on physiologic variables. None of the subjects were using any over the counter or prescription medications at the time of the study, except for oral contraceptives. Women completed a pregnancy test prior to all data collection study days, and due to the design of the exercise training program, were studied irrespective of menstrual cycle phase. Each vascular function testing day (study day 2, 16, 30, 46) started with measurements of body mass and height before the subject lay quietly in the supine position on a padded exam table for 20 min before vascular measurements started. During this time, subjects were briefed on experimental procedures and instrumented with a 3-lead ECG and brachial blood pressure cuff.

Table 3.1. Subject Characteristics

	Control	Blockade
n	9	8
Age (yrs)	25 ± 5	24 ± 4
Height (cm)	175.2 ± 8.4	169.8 ± 9.4
Weight (kg)	73.2 ± 13.6	74.2 ± 10.8
Body Mass Index (kg m^{-2})	23.7 ± 3.0	26.0 ± 3.2
Baecke sport index (arbitrary units)	2.5 ± 0.6	2.5 ± 0.8
Physical activity index (MET hr^{-1} $week^{-1}$)	22.2 ± 13.9	35.9 ± 10.5
60% power output (W)	124 ± 37	123 ± 36

Values are mean ± SD, MET, metabolic equivalents.

Histamine Receptor Blockade and Placebo.

Common over-the-counter oral antihistamine drugs were used in the experimental arm of this study. Histamine H_1 and H_2 receptors were blocked using a dose of 540 mg fexofenadine and 300 mg ranitidine. Oral fexofenadine has been shown to selectively block H_1 receptors (time to peak plasma concentration 1.15 h and half-life 12 h), while oral ranitidine has been shown to selectively block H_2 receptors (time to peak plasma concentration 2 h and 2.5 h half-life) (Garg, 1985; Russell, 1998). Fexofenadine and ranitidine do not appear to cross the blood-brain barrier into the central nervous system or possess sedative actions (Brunton, Chabner, & Knollman, 2011). Furthermore, these drugs do not have any direct cardiovascular effects in the absence of histamine receptor stimulation (J.L. McCord & Halliwill, 2006). The combination of fexofenadine and ranitidine reduces sustained post-exercise vasodilation by ~80% following moderate intensity aerobic exercise on a cycle ergometer (Lockwood et al., 2005; Mccord & Halliwill, 2006; J.L. McCord et al., 2006).

Placebo pills were manufactured by a compounding pharmacy (Creative Compounds, Wilsonville, OR) and contained the inactive ingredients of the fexofenadine and ranitidine tablets. Subjects consumed the antihistamine medication, or placebo, with water 1 h prior to every exercise session, but not before data collection study days.

Exercise Testing and Training Protocol.

Peak oxygen consumption. Subjects performed an incremental cycle exercise test (Lode Excalibur, Groningen, The Netherlands) to exhaustion, four times (following muscle biopsies, and/or once every data collection day (study day 1, 16, 30, 45))

throughout the study. The exercise test consisted of 1-min workload increments until exhaustion to determine maximal oxygen uptake (VO_{2peak}). Specifically, subjects expired breath was collected through a one-way valve connected to a pneumotach and breathed normally during a 2-min resting period sitting quietly on the bike. After the rest period, subjects began pedaling at a self-selected cadence for a 4-min warm-up period of low intensity cycling (40-130 W) determined as 1.5 times body weight (kg). Following the 4-min warm-up, subjects started the maximal exercised test and continued cycling while workloads increased at 20 or 30 W every min until volitional fatigue. Selection of the workload increment was subjective based on sex and training status, with the goal of producing exhaustion within 9-12 min. Whole body oxygen consumption (VO_2) was measured with a mixing chamber (Parvomedics, Sandy, UT, USA). The peak test was stopped and a cool-down period (40-60 W) of 3-5 min was initiated once subject reached exhaustion. Criteria for ending the peak test include; respiratory exchange ratio of 1.10 or greater, age-predicted heart rate max (220-age) was reached, pedaling cadence dropped below ~60 rpm, and perceived subjective exhaustion (rating of perceived exertion) on the Borg scale of 19-20 (Borg, 1970; Poole & Jones, 2017). The peak test was ended once 3 of 4 criteria were met or the subject could not continue pedaling. Additionally, subjects completed a supramaximal exercise test (110% work rate, 90-120 s) to verify peak O_2 consumption following the cool down (Poole & Jones, 2017). Subjects continued to pedal and cool down for 3-5 min following the supramaximal exertion.

Exercise Training Intervention. This study utilized a combination of exercise approaches including continuous exercise (CE) of moderate intensity aerobic cycling for 1 h and high intensity interval training (HIIT) for 30 min. There were 21 exercise

sessions (18 CE, 3 HIIT) through the 6-week exercise program with intensity prescription assessed every 7 exercise sessions. Continuous exercise has been shown to elicit a robust vasodilatory response during recovery from exercise that is reduced by ~80% via H_1 and H_2 histamine receptor blockade (Lockwood et al., 2005; Mccord & Halliwill, 2006; J.L. McCord et al., 2006). High intensity interval training was included to ensure increases in VO_{2peak} with training as some individuals will not show, or have blunted, adaptation to continuous exercise alone (Bacon et al., 2013).

Moderate Exercise Intensity. Continuous exercise consisted of a 5 min warm-up and cool-down at 30% VO_{2peak} separated by a workload that produced 60% VO_{2peak} and was estimated from the following equation:

[Target VO_2 (ml/min)/12] – 25 = estimated workload (watts)

Equation 3.1 Estimated Workload

Steady state continuous exercise VO_2 was confirmed to be 60% of peak within the first 2 exercise session after each assessment of VO_{2peak}. The workload was adjusted, if needed, to achieve a workload that produced steady-state 60% VO_{2peak}. This workload was used on each of the continuous exercise sessions until VO_{2peak} was re-assessed.

High Intensity Interval Training Intensity. High intensity interval training consisted of 5-min warm-up and cool-down periods at 30% VO_{2peak} separated by a 20-min period of 3:1 interval training at 90% and 30% VO_{2peak} for a total of five 3-min intervals at 90% VO_{2peak}. Equation 3.1 was used to determine all cycle intensities during exercise sessions.

Hemodynamic Measurements.

Heart Rate. Heart rate was monitored on all study visits (data collection days and exercise sessions) using a combination of instruments. On all vascular study days, heart rate was monitored continuously while the subject was supine, using a three-lead electrocardiograph (Datex-Ohmeda Cardiocap/5, GE Healthcare, Tewksbury, MA, USA) and recorded using data acquisition software (Windaq; Dataq Instruments, Akron, OH, USA). During all exercise testing and training sessions, heart rate was also measured with commercially available Polar chest straps (Polar Electro, New York, NY, USA) connected to a Polar watch sensor and recorded manually every minute during exercise testing or every 5-10 min during exercise sessions.

Arterial Blood Pressure. Arterial blood pressure was measured in the right arm at heart level while the subject was supine using an automated sphygmomanometer (Tango+, SunTech Medical, Raleigh, N.C., USA). Arterial blood pressure was measured in triplicate after 20 min of supine rest to begin each vascular study day and in accordance with guidelines set forth by the American Heart Association (Muntner et al., 2019). Additionally, arterial blood pressure was taken following each dynamic arterial compliance measurement to be used in that calculation. Mean arterial pressure was calculated as:

$$MAP = Diastolic\ BP + [(Systolic\ BP - \ Diastolic\ BP) \div 3]$$

Equation 3.2 Mean Arterial Pressure

Arterial Stiffness.

Arterial stiffness was assessed via measures of dynamic arterial compliance and pulse wave velocity. Dynamic arterial compliance was assessed in the common carotid and common femoral arteries while pulse wave velocity was assessed between the common carotid and femoral arteries and the brachial and pedal arteries. All vascular function measurements were measured using either Doppler ultrasonography, applanation tonometry, or both.

Doppler ultrasound applies the principles of sound wave reflection and Doppler shift to make measurements of arterial diameter and blood velocity simultaneously to estimate blood flow. This study utilized the Doppler ultrasound to make measurements of arterial diameter. A Phillips iE33 ultrasound was used with a 9 MHz linear array probe to image arterial diameter. The ultrasound probe contains a piezoelectric element that converts electric signals into mechanical vibrations and emits ultrasonic sound waves. The ultrasound probe also detects the reflected ultrasonic sound waves and converts them back into electrical signals (Franklin, Schlegel, & Rushmer, 2020). Video recordings of arterial diameter were transferred to an external hard drive for offline analysis using commercial wall-tracking software (Brachial Analyzer for Research v6.11.9, Medical Imaging Applications LLC, Coralville, IA, USA) to make vessel diameter measurements.

Applanation tonometry measures changes in pressure using pencil-like pressure transducers (PCU-2000; Millar, Inc., Houston, Tx, USA) and can be applied over any artery of interest. Tracings were recorded using Windaq (Dataq, Inc.) and the foot (diastolic to systolic transition, fig 3.1) of the pressure tracing was marked to determine the time difference between sites. Velocity was calculated as distance over time, where

distance was measured between the carotid and femoral probes, or the sum of the brachial probe to the sternal notch, to anterior superior iliac spine, to ankle probe minus the distance between the brachial probe to sternal notch. Measures of dynamic arterial compliance and pulse wave velocity were assessed across multiple sites on the body to estimate stiffness of one or both conduit and peripheral segments of the arterial tree.

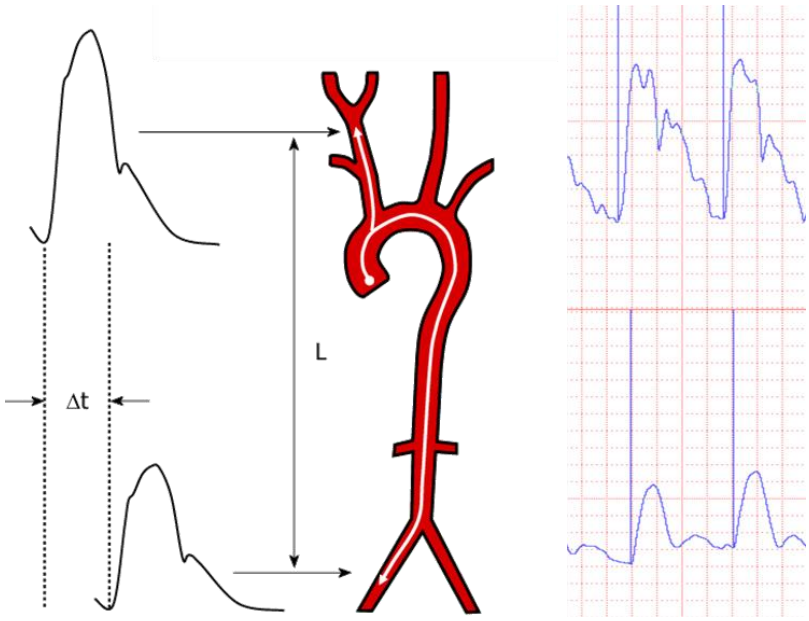


Figure 3.2. Example carotid-brachial pulse wave velocity measurement. Distance measurement with time differential (left) and sample of pulse tracings from which time differential is measured (right) used for pulse wave velocity. Adapted from (Zanoli, Rastelli, Inserra, & Castellino, 2015).

Common carotid and femoral dynamic arterial compliance were measured using high-resolution Doppler ultrasound (Phillips iE33) with a 9 MHz linear array probe and concurrent applanation tonometry on the contralateral side of the body. Sampling was initiated through DUC2 software and sampled in 40-s simultaneous recording from both instruments. All ultrasound recordings were performed on the right side of the body. Changes in arterial diameter were assessed through ultrasound images, which were recorded at 46 Hz and saved to the ultrasound system before being transferred to a

computer with custom software (Brachial Analyzer) for analysis. Changes in pressure were measured using applanation tonometry and recorded via Windaq data acquisition (Dataq, Inc.) at 100 Hz and analyzed using the valley to peak pressure differential, fig 3.2. The change in pressure was assessed relative to changes in diameter in order to calculate cross-sectional compliance and β-stiffness using the following equations:

$$DAC = [(\Delta D/D)/2\Delta P] * \pi D^2$$

Equation: 3.3 Dynamic arterial compliance

$$\beta\text{-index} = \text{Ln}\,(SBP/DBP) * D/\Delta D$$

Equation: 3.4 β-stiffness index

Pulse Wave Velocity. Pulse wave velocity is the most robust and reproducible measurement of arterial stiffness (Laurent et al., 2006; Rourke, Staessen, Vlachopoulos, Duprez, & Plante, 2002). Decreases in pulse wave velocity represent a less stiff artery, or better cardiovascular health (Rourke et al., 2002). Arterial pulse wave velocity has been shown to decrease (less stiff) with exercise training (Sugawara et al., 2005). Carotid-femoral pulse wave velocity is a measurement of conduit artery stiffness and is predictive of cardiovascular outcomes in various disease conditions (Ben-Shlomo et al., 2014; Vlachopoulos, Aznaouridis, & Stefanadis, 2014). Brachial-ankle pulse wave velocity provides information about peripheral artery stiffness (Sugawara et al., 2005) as well as some information about conduit artery stiffness.

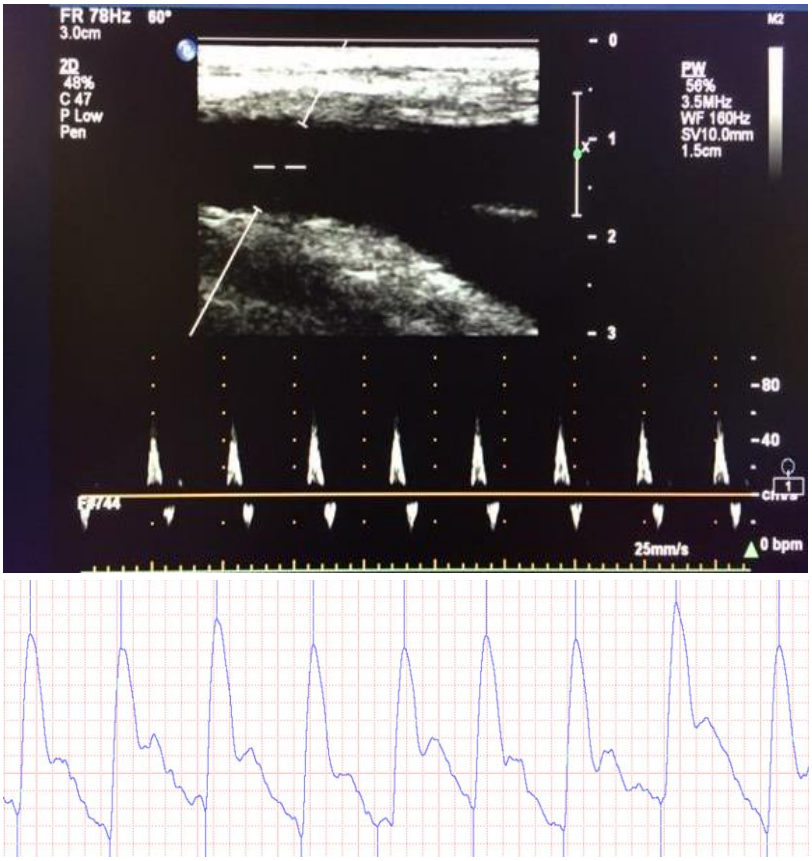


Figure 3.3. Example of femoral dynamic arterial compliance measurement. Sample ultrasound measurement of the common femoral artery (top) and sample pressure tracings with peak and valley marked for analysis (bottom) used for measurement of dynamic arterial compliance.

Peak Reactive Hyperemia. A measurement of vascular function termed flow mediated dilation was collected 4 times throughout this study according to established methodology (Dick H.J. Thijssen et al., 2011) in order to examine well established increases in vascular function associated with aerobic exercise training and its role in oxygen delivery during exercise and improved exercise performance. However, technical issues associated with ultrasound video file size impacted the ability to observe peak dilation following arterial occlusion, which usually occurs about 30 s following release of the occlusion cuff. Due to these technical difficulties flow mediated dilation measurements had to be excluded, however peak reactive hyperemia at the onset of occlusion cuff release was still obtained. The measurement of peak reactive hyperemia

can give some insight into microvascular adaptation with exercise training as increased reactive hyperemia could be indicative of more complex capillary networks allowing for increased bulk blood flow through the conduit artery. Peak reactive hyperemia was measured using high-resolution Doppler ultrasound (Phillips iE33) with a 9 MHz linear array probe to detect blood velocity and image arterial diameter. The Phillips iE33 ultrasound was interfaced with a computer running custom software (DUC2) to measure velocity through audio recordings. Ultrasound recordings were performed on the right side of the body. Arterial diameter was assessed through ultrasound images, which were recorded at 46 Hz and saved to the ultrasound system before being transferred to a computer with custom software (Brachial Analyzer) for analysis.

Artery Lumen Diameter. Resting carotid, and femoral artery lumen diameter were measured via duplex ultrasonography. A linear-array vascular ultrasound probe (9MHz, Phillips iE33, Andover, MA., USA) was used for this study. An insonation angle of 60^{o} was used to accesses artery lumen diameter across multiple locations. Video recordings of arterial diameter were transferred to an external hard drive for offline analysis using commercial wall-tracking software (Brachial Analyzer for Research v6.11.9, Medical Imaging Applications LLC, Coralville, IA, USA) to make vessel diameter measurements. Measurements of lumen diameter were taken from at least 10 cardiac cycles at end-diastole and the mean values are reported.

Other Acceptable Methods. In addition to measures of arterial stiffness, measures of vascular function to assess the ability of the artery to vasodilate and increase blood flow that positively correlate with increase oxygen consumption during exercise could have been explored. One such measurement is flow mediated dilation, which is a non-

invasive means to assess endothelium-dependent vasodilation (Celermajer, Sorensen, Bull, Robinson, & Deanfield, 1994). Flow-mediated dilation uses Doppler ultrasound to image changes in arterial diameter in response to 5 min of arterial occlusion. Hyperemia follows the occlusion release, which increases shear stress on the blood vessel walls and translates to larger arterial diameter. Larger changes in arterial diameter indicate greater endothelial function and is also a well-established predictor of cardiovascular risk and future cardiovascular events (Shechter, Shechter, Koren-Morag, Feinberg, & Hiersch, 2014).

Blood Volume.

Hemoglobin, hematocrit, and carbon monoxide bound to hemoglobin (carboxyhemoglobin (COHb)), were measured for the purpose of estimating blood and plasma volume. Blood and plasma volume were assessed with a carbon monoxide (CO) uptake method as previously described (Burge & Skinner, 1995). The CO uptake method was used for pre and post exercise training measurements (Day 2 and 46), while blood samples were collected to measure hematocrit and hemoglobin across all 4 data collection study days (2, 16, 30, and 46). The CO uptake blood volume technique is based on the dilution of a tracer, inhaled CO, which binds to hemoglobin. Once CO is bound to hemoglobin (HbCO) its color changes (Burge & Skinner, 1995; David Montero et al., 2017; Siebenmann, Keiser, Robach, & Lundby, 2017) and can be detected spectrophotometrically with the diode-array spectrophotometer OSM3 hexoximeter (OSM3 hexoximeter, Radiometer, Copenhagen, Denmark). Hematocrit was measured with the microcapillary (75 mm) centrifuge method (Autocrit Ultra 3, Becton Dickson,

USA) and spun for 10 min at 11,700 rpm. Hemoglobin was measured with the OSM3 hexoximeter (day 2 and 46) and hemocue (day 2, 16, 30, 46) (Wilburn Medical, Kernersville, NC, USA). All blood samples were collected in heparinized syringes or vacutainers and transferred to capillary tubes or microcuvettes when necessary. Subjects sat in a phlebotomy chair for at least 20 min in the same body position before every blood draw for the volume measurements.

Carbon Monoxide Uptake Method. The CO uptake method used a dosage of 1mL CO per kg body weight. This dosage is predicted to raise HbCO by ~6.5% to levels around 8% (Burge & Skinner, 1995). A closed rebreathing circuit was utilized for this measurement and consisted of a glass structure connected to 2 1 L non-diffusible anesthesia bags and mouthpiece. The rebreathing circuit was pre-filled with 100% O_2 and equipped with a soda-lime CO_2 scrubber to prevent buildup of CO_2 in the circuit. Subjects were instructed to exhale most of their breath to the environment before going onto the rebreathing circuit mouthpiece, that was still open to the environment, and exhaling to residual volume. Once the subject had expired to residual volume, a glass syringe filled with a calibrated volume of pure CO was connected to an open port. Within 1 s of breathing down to residual volume, the subject inhaled maximally, and the CO was injected into the system. After reaching inspiratory capacity, the subject held their breath for 10 s before breathing normally for the remainder of the 2 min period, after which the subject breathed back down to residual volume and came off the mouthpiece. Blood samples were obtained before and 7 min after the CO was added to the rebreathing circuit in order to measure the change in HbCO% in accordance with previously described methods (Burge & Skinner, 1995).

$$nCO = 1000\ X \left[\frac{\left(\frac{PB}{760}\right) X\ Vco}{(0.08206) X\ (273 + T)}\right]$$

Equation: 3.5 Molar Amount of Carbon Monoxide

$$nHB = \frac{nCO\ X\ 25}{\Delta HbCO}$$

Equation: 3.6 Hemoglobin Mass

$$Vrbc = \left[\frac{644\ X\ Hct}{Hb}\right] X\ nHb$$

Equation: 3.7 Hemoglobin Mass Bound to Carbon Monoxide in Milliliters

$$BV = \frac{Vrbc\ X\ 100}{Hct\ X\ Fcell\ ratio}$$

Equation: 3.8 Blood Volume

$$PV = BV - Vrbc$$

Equation: 3.9 Plasma Volume

These series of equations are used to calculate blood volume, plasma volume, and hemoglobin mass where *n*CO is the molar amount of carbon monoxide added to the rebreathing system (mmol), P_B is the barometric pressure (mmHg), V_{CO} is the volume of carbon monoxide (ambient temperature and pressure, dry) added to the rebreathing system (Liters), T is the room temperature (^oC), *n*Hb is the hemoglobin mass (mmol), ΔHbCO is the difference in carbon monoxide bound to hemoglobin levels measured

before and after rebreathing (percentage), V_{RBC} is the volume of red blood cells (milliliters), Hct is hematocrit (percentage), Hb is hemoglobin in grams per liter, BV is blood volume in milliliters, PV is plasma volume in milliliters, and F_{cell} ratio is the venous to body hematocrit correction factor.

Venous Blood Sampling. During each study day, either a butterfly needle (day 16 and 30) or venous catheter (day 2 and 46) was used to draw venous blood samples. A catheter was utilized for each CO uptake experiment to sample blood at specific times before and after CO administration. After disinfecting the antecubital region of the arm, an intravenous catheter was placed by inserting a 20- or 22-gauge needle into the vein. Once the catheter was in place, the needle was removed, the catheter was connected to a saline locked extension set and held in place with a clear adhesive cover (Tagaderm, 3M, Maplewood, MN, USA). After each blood draw, the sampling line and catheter were cleared with non-lactated, non-dextrose saline solution (0.9%) and closed to both the sampling line and the environment. Blood samples were obtained from the sampling line into a sterile heparinized syringe and immediately analyzed.

Other Acceptable Methods. Several techniques based on tracers and dyes (Evans blue dye, chromium, carbon monoxide) can be used to measure vascular volumes. These methods are based on the Fick principle with a known volume introduced into the system and measurements made at certain time points following, depending on the tracer or dye. Different tracers or dyes are useful in measuring specific components of the blood and are most useful depending on what question is being asked. The carbon monoxide technique was chosen for this study because the tracer (carbon monoxide) binds to hemoglobin, which is then measured by the change in color caused by this reaction. Since

hemoglobin carries most of the oxygen in the blood, this method was the best choice as this dissertation was based around increases in oxygen consumption during exercise.

Changes in blood and plasma volume can be estimated from changes in hematocrit and hemoglobin using the method of Dill and Costill (Dill & Costill, 1974). Changes in PV are generally calculated indirectly from values of hemoglobin and hematocrit. The underlying assumption when using the Dill and Costill method to calculate relative changes is that peripheral circulating erythrocyte volume does not change and is comparable to total erythrocyte volume (Stewart, Warburton, Hodges, Lyster, & McKenzie, 2003). However, circulating erythrocyte volume was assumed to change through the course of exercise training so this technique was not used (Warburton et al., 2004).

Body Composition.

Body composition was measured on every two weeks (day 2, 16, 30, 46) by 7-site skinfold analysis in accordance with best practices (Reilly, Wilson, & Durnin, 1995). Skinfolds are measured using large calipers to determine skin and adipose tissue thickness around certain anatomical landmarks. The seven sites used were triceps, pectoralis, midaxillary, subscapular, abdomen, suprailliac, and quadriceps. Skinfold measurements are all made on the right side of the body and measured in duplicate with third measurements made if a difference of over 2 mm was observed. Additionally, body weight was measured at the beginning of every data collection day. Subjects were asked to remove their shoes and step onto a scale (Sartorius EB6CE-I, Precision Weighing Balances, Bradford, MA, USA).

Other Acceptable Measurements. Measurements of body composition can be made with several techniques including hydrostatic weighing, dual-energy x-ray absorptiometry, or air displacement plethysmography. Hydrostatic weighing is the gold standard for determining body composition followed closely by dual-energy x-ray absorptiometry. Both methods require expensive equipment and put the subject at a higher risk when compared to skinfold analysis. Since body composition was included in this dissertation as good practice with the exercise training intervention and was not a main variable of interest, we were able to use the less accurate but least invasive technique to determine body composition.

Skeletal Muscle Biopsy.

All skeletal muscle biopsies were performed on the University of Oregon campus in Pacific Hall by an expert muscle physiologist, Dr. Hans C. Dreyer. Prior to the procedure, subjects refrained from exercise for 24 h and arrived in lab fasted overnight. Biopsies were taken from the left vastus lateralis muscle under sterile technique. To start, the skin and underlying fascia were anesthetized using 1% lidocaine hydrochloride (Hospira Worldwide, Lake Forest, IL, USA) and subject perception of pain was verbally acquired to ensure the biopsy area was effectively anesthetized. Once verbal confirmation of local anesthetic was obtained, a small incision made in the skin and underlying fascia before introduction of a 5 mm Bergstrom biopsy needle to the vastus lateralis muscle. A 20-cc syringe and tubing were attached to the aspiration port on the Bergstrom needle in order to apply negative pressure to assist the biopsy. After the vastus lateralis muscle sample was collected, a series of Steri-Strips (3M, Maplewood, MN, USA) were placed

over the incision and covered with a sterile transparent dressing (Tagaderm). The tissue sample was blotted with gauze, and any visible adipose tissue was removed before being mounted on cork, covered in medium (Optimal Cutting Temperature Compound (OCT)) and immediately fresh-frozen in melting isopentane cooled with liquid nitrogen and stored at -80^{o}C until analysis. The muscle sample mounted on cork and fresh frozen was used to access either the maximum velocity of the succinate dehydrogenase reaction in single type-identified muscle fibers (SDH_{max}) or capillary density.

Succinate Dehydrogenase Activity: Vastus lateralis muscle biopsy samples were cut in transverse serial sections at 6 µm thickness using a cryostat (Reichert Jung Frigocut 2800 Cryostat, Reichert Microscope Services, Depew, NY, USA), maintained at a temperature of -30^{o}C. Sections were used for a quantitative assessment of the maximum velocity of succinate dehydrogenase reaction using a previously described quantitative histochemical procedure (Blanco, Sieck, & Edgerton, 1988; Enad, Fournier, & Sieck, 1989; Fogarty, Mathieu, Mantilla, & Sieck, 2020; Proctor et al., 1995). Sections were cut and placed on a cover slip where they were immediately visualized to determine cross-section orientation of cells, maintained at 5^{o}C with a control solution covering the section on the slide. Once the orientation of the section was confirmed, serial sections were cut from the sample at 6 µm for SDH_{max} measurements and at 10 µm thickness to identify capillaries (haematoxylin and eosin, H&E stain) and fiber type (immunoreactivity to myosin heavy chain, MyHC, antibodies), as described below in detail.

For SDH_{max} determinations, a baseline image was acquired (Olympus IX71, Olympus America, Melville, NY) with a camera and image capture system running software (Elements C-ER, Nikon, Tokyo, Japan) for image processing. Images were

acquired using a 20x objective (1.0 NA) and captured in a 1200 x 1200-pixel array, with similar acquisition parameters across samples. An interference filter (570 nm) was used to limit the spectral range of the light source to the optimal absorbance wavelength for nitroblue tetrazolium diformazan (the SDH reaction indication). Once the baseline image was acquired, the control solution was removed via suction and a solution containing 80 μM succinate (to maximize substrate availability for the SDH reaction) was added. Thereafter, images were acquired every 15s for a 10-min period with no changes to the environmental conditions or image processing system throughout the experiment, fig 3.3.

Both control (no succinate) and succinate (80 nM) solutions contained 1.5 mM nitro blue tetrazolium (NTB – reaction indicator), 5 mM EDTA, 0.2 mM PMS, and 0.1 mM azide in 0.1 M phosphate buffer (pH = 7.6). The maximum concentration of succinate for the succinate dehydrogenase activity reaction was previously determined (Blanco et al., 1988; Enad et al., 1989) and validated for human muscle fibers (Proctor et al., 1995). In the quantitative histochemical procedure, the progressive precipitation of diformazan from the reduction of nitro blue tetrazolium is used as the reaction indicator (Blanco et al., 1988; Proctor et al., 1995). The linearity of the accumulation of NBT diformazan in individual vastus lateralis muscle fibers was confirmed across a 20-min period. Based on the linearity of the SDH reaction, a final end-point time of 10 min was selected to avoid any saturation of the imaging system. The SDH_{max} was calculated using the Beer-Lambert-Bouguer law:

$$\text{NTB reduction} = OD/kL$$

Equation: 3.10 Beer-Lambert-Bouguer Law

Where OD is the average optical density within a region of interest (in this case the boundary of a muscle fiber), *k* is the molar extinction coefficient of nitro blue tetrazolium diformazan (26, 478 mol/cm) and L is the path length of light absorbance (6 µm) at 570 nm (peak absorbance wavelength for nitro blue tetrazolium diformazan).

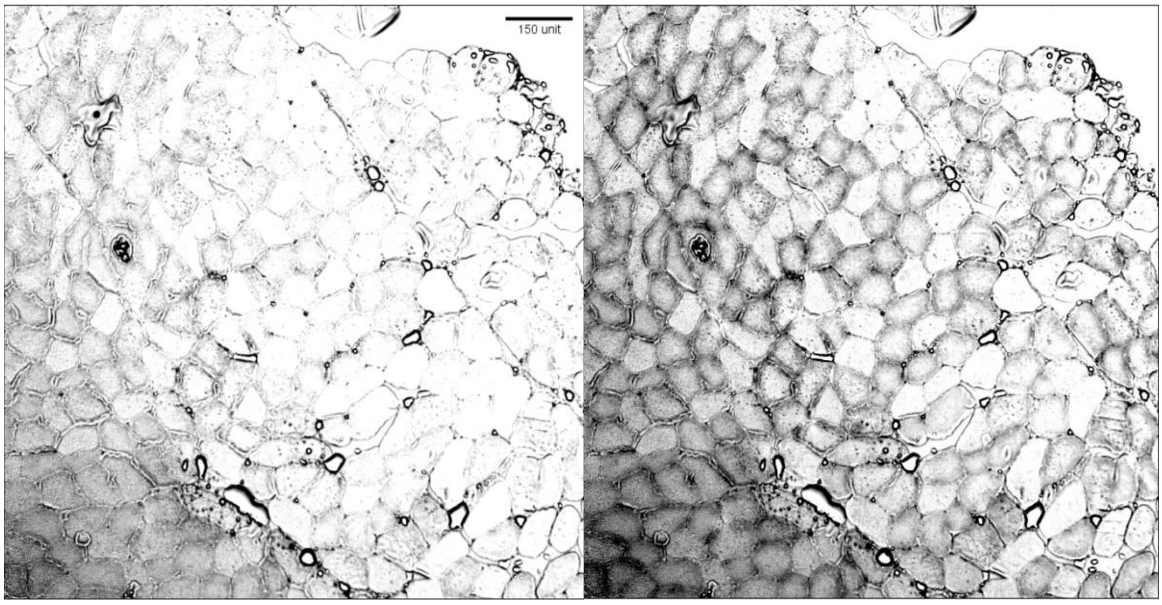


Figure 3.4. Baseline succinate dehydrogenase activity (left) and following 10min of reaction (right) used to quantify the change in optical density. Scale bar indicates 150 µm.

Based on calculations using the Beer-Lambert-Bouguer law, the maximum velocity of the succinate dehydrogenase reaction within individual type-identified muscle fibers was expressed as millimoles of fumarate L tissue^{-1} min^{-1}.

Capillary Density: The assessment of capillary density was assessed using a Hematoxylin and Eosin staining procedure as previously described ("Guidelines for Hematoxylin & Eosin Staining," 2001). Alternate 10 µm serial sections adjacent to those used for the succinate dehydrogenase activity SDH_{max} and fiber type analysis, were placed on a cover slip and allowed to dry for 15 min to prevent lifting of the section.

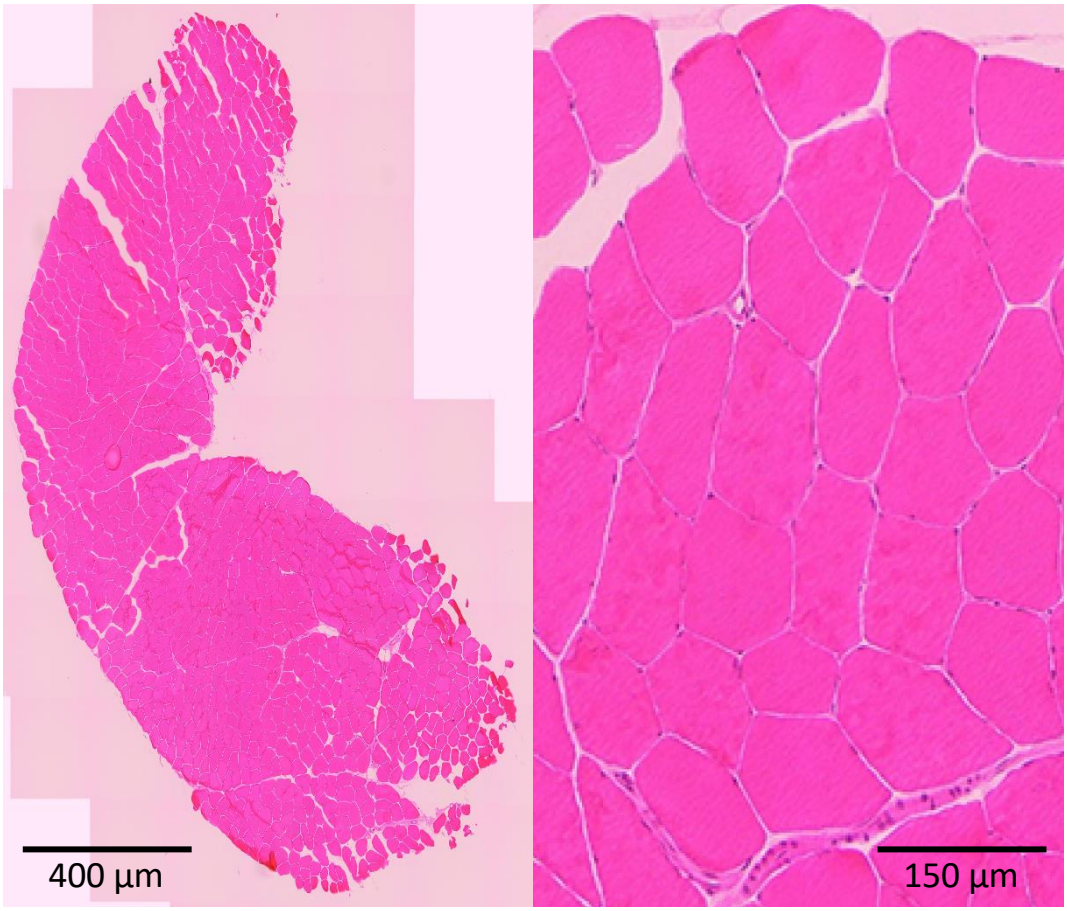


Figure 3.5. Example of Hematoxylin and Eosin stained transverse section of vastus lateralis muscle used to identify capillaries (left) zoomed in on same sample to visualize capillaries (right) used to quantify the number of capillaries in contact with each muscle fiber.

Samples were fixed in 10% buffered formalin solution for 20 s before being stained in hematoxylin (Gill's III, Millipore Sigma GHD332-1L, St. Louis, MO, USA) for 2 min. Staining was stopped by detaining slides in 1% acid alcohol (1ml HCL/100ml 70% EtOH) for 10 s then rinsed in tap water for 5 min. A bluing reagent (0.3% ammonia water) was applied for 1 min before the slides were rinsed in distilled water to color the hematoxylin stain. The Eosin stain was applied by serial washes in 70% then 95% ethanol for 60 s each before staining slides in eosin-Y (Polysciences Cat309859, Warrington, PA, USA) for 60s. The slides were then dehydrated by serial washes in

duplicate of 95% and 100% ethanol for 60s before the slides were cleared in xylene for 5 min. Slides were imaged using a Motic slide scanner (Motic, Richmond, British Columbia, Canada) with a 20x aperture according to manufacturer specifications, fig 3.4.

Muscle Fiber Types: Alternate 10 µm serial sections adjacent to those used for determination of SDH_{max} and H&E staining of capillaries, were placed on a cover slip and allowed to dry for 15 min to prevent lifting of the section. A total of 5 sections were cut from each sample and mounted onto a single slide to assess all fiber types. Samples were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 5 min prior to starting immunofluorescence staining protocols. Slides were washed thoroughly in 0.1 M phosphate buffer before being incubated overnight at $4^{o}C$ with each section covered in a unique primary antibody.

A total of 4 primary antibodies were used to determine immunoreactivity of myosin heavy chain (MyHC) isoforms expressed by different muscle fiber types: (1) A specific skeletal muscle $MyHC_{Slow}$ antibody (Novus, NBP2-50299 Mouse IgG, Centennial, CO, USA) was used at a 1:10 dilution to identify slow type I muscle fibers. (2) A specific fast skeletal muscle MyHC 2A antibody (SC-71 Developmental Studies Hybridoma Bank, Mouse IgG, Iowa City, IA, USA) was used at a 1:1 dilution to identify fast type IIa muscle fibers. (3) A specific skeletal muscle $MyHC_{2X}$ antibody (Novus, NBP1-22811 Mouse IgG, Centennial, CO, USA) was used at a 1:10 dilution to identify fast type IIx muscle fibers. (4) A non-specific skeletal muscle $MyHC_{Fast}$ antibody (Vector VP-M665, Mouse IgG, Burlingame, CA, USA) was used at a 1:10 dilution to identify all fast type II muscle fibers and by exclusion any fibers expressing $MyHC_{2B}$ (type IIb muscle fibers for which there is no specific antibody). After the primary antibodies

incubated overnight, the slides were washed thoroughly in 0.1 M phosphate buffer and then all sections were incubated for 2 h in secondary antibody at a 1:200 dilution (Cy3 donkey anti-mouse IgG, ThermoFisher Scientific, Waltham, MA, USA) to assist in detection during imaging. Finally, the samples were washed in 0.1M phosphate buffer and allowed to dry before confocal microscopy.

Transverse vastus lateralis muscle sections were imaged using a 20x oil-immersion objective (NA 1.0) on an Olympus FV2000 laser confocal microscope capable of simultaneous multilabel florescence imaging. Images were captured in a 1200 x 1200-pixel array, with similar acquisition parameters across preparations. Type-identified fibers were paired with the sections used to determine SDH_{max} and capillary density.

Statistics.

The statistical analysis for this research was performed using Prism software (GraphPad Prism 8, CA, USA). All subject characteristics are reported as mean ± SD and all additional data are reported as mean ± SEM. A Two-way ANOVA and Tukey *post hoc* test were used to compare groups and factor. There were repeated measurements over time with subject, drug, and time analyzed. Significance was set at $P<0.05$ for all analyses.

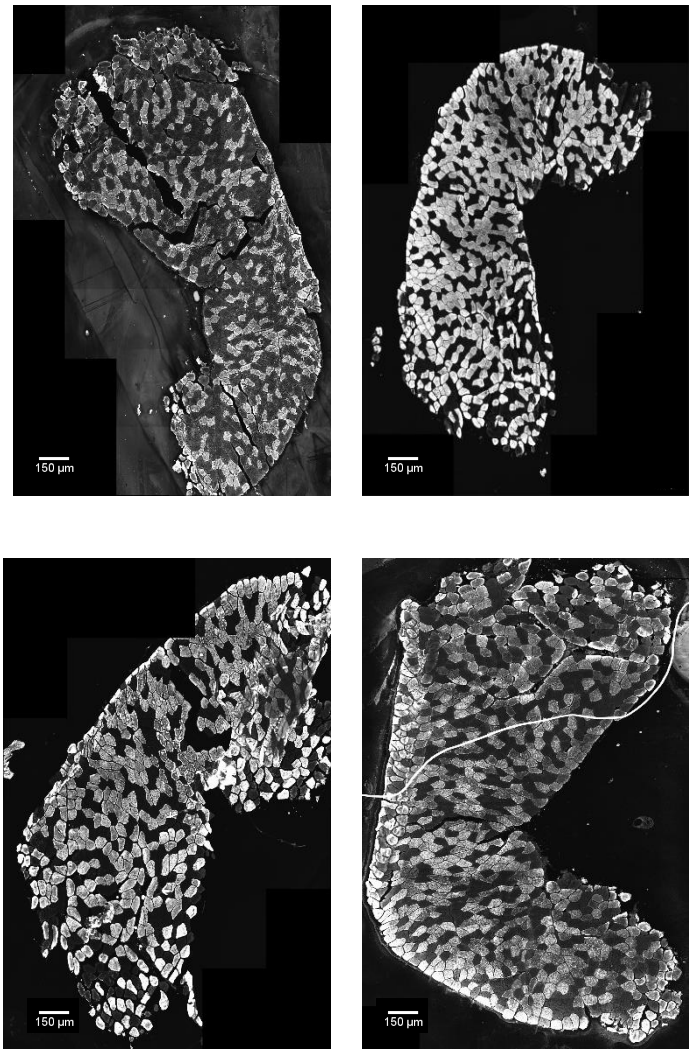


Figure 3.6. Example of slow fiber type stain (top left), fast fiber type stain (top right), all but 2x fiber type stain (bottom left), fast 2a fiber type stain (bottom right) used to identify fiber type for succinate dehydrogenase and capillary quantification.

CHAPTER IV

Aerobic Exercise, Training Effects, and Vascular Health

This study was made possible through the contributions of Brendan W. Kaiser, Joshua E. Mangum, Emily A. Larson, Michael A. Francisco, Sydney Kobak, Christopher T. Minson, and John R. Halliwill. Brendan, Josh, Emily, and Sydney provided much needed assistance with data collection throughout the study. John Halliwill and Chris Minson assisted in the intellectual development of the project. I was involved with all aspects of this project including the development of the protocol, funding through fellowship applications, completed all data collection, compiled the data, analysis of the data, and developed the manuscript.

INTRODUCTION

Aerobic exercise capacity increases with repeated exercise stress and can be quantified by measuring oxygen consumption during graded exercise tests (VO_{2Max}). Increases in oxygen consumption with exercise training are typically associated with an increase in exercise performance, reflected by the amount of work accomplished at peak exercise. Exercise training not only promotes increased ability to preform greater work during subsequent exercise, but also promotes beneficial adaptation associated with vascular health such as increased vascular function and decreased arterial stiffness (Green, 2009; David Montero & Lundby, 2016). The observation that aerobic exercise training protects against premature cardiovascular disease and mortality is robust (Keteyian et al., 2008). The beneficial vascular health adaptations associated with aerobic exercise training cause reduced cardiovascular risk and lower rates of all-cause mortality

that are more protective than predicted based on traditional risk factors like hypertension or blood lipid levels (Green, 2009; Joyner & Green, 2009). Additionally, variables like arterial diameter and compliance are independent risk factors for the development of cardiovascular disease, which will show improvements with continuous aerobic exercise training (Tanaka et al., 2000). Moderate intensity aerobic exercise training results in improvements in aerobic capacity for the duration of training (Bacon et al., 2013), while also improving measures of vascular health and decreasing risk of future cardiovascular disease.

Moderate intensity aerobic exercise causes increased histamine concentrations within the active skeletal muscle that is released from mast cell degranulation or through *de novo* formation through the enzyme histidine decarboxylase (Steven A. Romero et al., 2016). Histamine activates H_1 and H_2 receptors on vascular endothelial cells and vascular smooth muscle cells to acutely cause vasodilation and promote increased blood flow. Previously active skeletal muscle vasculature will remain dilated for hours following exercise and result in reduced arterial pressure during that time. The increased blood flow and reduced arterial pressure following exercise is attenuated by blocking histamine H_1 and H_2 receptors (Mccord & Halliwill, 2006; J.L. McCord et al., 2006; Steven A. Romero et al., 2016). Additionally, blocking histamine H_1 and H_2 receptors during exercise modulates the normal change in skeletal muscle mRNA expression related to endothelial and vascular function, metabolism, angiogenesis, cell maintenance, and inflammation (S A Romero et al., 2016).

Blocking histamine's actions during exercise has potential to blunt enhancements in markers of vascular health, like pulse wave velocity or dynamic arterial compliance,

normally associated with aerobic exercise training. Histamine is an established endothelial-dependent vasodilator that acutely modulates smooth muscle tone by activation of endothelial nitric oxide synthase and formation of nitric oxide. Blockade of histamine H_1 and H_2 receptors has been shown to decrease expression of endothelial nitric oxide synthase following acute exercise (S A Romero et al., 2016), which could potentially lead to reduced levels of nitric oxide and a blunted vasodilator tone manifested as reduced arterial compliance or increased arterial pulse wave velocity (Fitch, Vergona, Sullivan, & Wang, 2001). A histamine-induced upregulation of endothelial nitric oxide synthase gene expression could result in a sustained enhancement of nitric oxide production, which would benefit the previously mentioned measures of vascular health.

It is unknown if chronically consuming antihistamines prior to exercise cause differences in the rate or scope to exercise-induced cardiovascular adaptations. The purpose of this study was to determine the role of histamine receptor activation in adaptations to aerobic exercise training. Specifically, following six weeks of aerobic cycling exercise training in young healthy inactive volunteers, we determined the effect of histamine receptor antagonists on improvements in aerobic capacity and measures of vascular health. The hypothesis of this study is that in young healthy inactive volunteers, histamine receptor antagonists will blunt aerobic exercise-induced gains in VO_{2Peak} and maximal work, as well as beneficial changes in vascular health associated with aerobic exercise training.

METHODS

Subjects

This study was approved by the Institutional Review Board at the University of Oregon. Written informed consent was obtained from all subjects prior to participation and the study conformed to the principles of the Declaration of Helsinki. Seventeen (6 men, 11 women) healthy, non-smoking individuals participated in this study and were considered recreationally active based on exercise habits over the previous 12 months (Baecke, Burema, & Frijters, 1982). Subjects were deemed healthy following a standard health screening. Sixteen (10 women) subjects completed the study while one subject withdrew from the study after the second study day due to scheduling conflicts. The data from this subject was not included in any analysis. All subjects were required to abstain from supplements, caffeine, and alcohol for at least 12 h, and exercise for at least 24 h prior to all study days. None of the subjects were using any over the counter or prescription medications at the time of the study, with the exception of oral contraceptives. Women were studied irrespective of menstrual cycle phase and had a negative pregnancy test prior to all study days. Subjects reported to the laboratory following a 4 h fast and all study days took place in a thermoneutral lab environment. Data collection start-time varied across subjects due to scheduling conflicts; however, experimental start-time was held constant within a subject to minimize circadian influence on physiologic variables. With the exception of the first study day, all data collection occurred between 24-48 h after the most recent exercise session.

Experimental Design

The research protocol consisted of a double-blind placebo-controlled exercise training study. Subjects underwent a 6-week exercise training intervention (exercising 3-4 times per week, totaling 21 sessions) with an experimental group receiving antihistamines (BLK) and a control group receiving a placebo (CTL) prior to each exercise session. There were 6 study days that included 2 separate study days for pre- and post-training measurements and 1 study day after every 7 exercise sessions, or 2 weeks, depending on subject schedules. Study days are referred to as "vascular testing days" or "exercise testing days" based on the measurements that were made. Subjects started and ended the study with exercise testing days and both mid-point study days (following 2-weeks of training) consisted of both vascular and exercise testing. All study days were conducted on the University of Oregon Campus in Esslinger Hall. Prior to starting the first study day, subjects were block-randomly assigned to the control or blockade group.

Each exercise testing study day started with measurement of height and weight. The subject was then instrumented with a Polar chest-strap heart rate monitor and Hans-Rudolph two-way non-rebreathing valve with headgear and nose clip. Subjects sat quietly on the bike for 2 min before warming up for 5 min and starting the incremental exercise test. Once peak oxygen consumption was reached, subjects cooled down for 3-4 min before completing a supra-maximal exercise effort to verify that VO_{2Peak} had been attained.

Each vascular function testing day started with measurements of body mass and height before the subject lay quietly in the supine position on a padded exam table for 20 min prior to initiating vascular measurements. During this time, subjects were

instrumented with a 3-lead electrocardiogram and brachial blood pressure cuff. Following the 20-min supine resting period, heart rate and blood pressure were measured in triplicate. Vascular measurements started with dynamic arterial compliance measurements of the carotid and femoral arties, followed by pulse wave velocity measurements of the carotid-femoral segments and the brachial-ankle segments

Histamine Receptor Blockade and Placebo.

Common over-the-counter oral antihistamine drugs were used in the experimental arm of this study. Histamine H_1 and H_2 receptors were blocked using a dose of 540 mg fexofenadine and 300 mg ranitidine. Placebo pills were manufactured by a compounding pharmacy (Creative Compounds, Wilsonville, OR) and contained the inactive ingredients of the fexofenadine and ranitidine tablets. Subjects consumed the antihistamine medication, or placebo, with water 1 h prior to every exercise session, but not before data collection study days.

Exercise Testing and Training Protocol.

Peak oxygen consumption. Subjects performed an incremental cycle exercise test (Lode Excalibur, Groningen, The Netherlands) to exhaustion, four times (study day 1, 16, 30, 45) throughout the study. The exercise test consisted of 1-min workload increments until exhaustion to determine maximal oxygen uptake (VO_{2peak}). Specifically, subjects expired breath was collected through a one-way valve connected to a pneumotach and mixing chamber and breathed normally during a 2-min resting period sitting quietly on the bike. After the rest period, subjects began pedaling at a self-selected cadence for a 4-

min warm-up period of low intensity cycling (40-130 W) determined as 1.5 times body weight (kg). Following the 4-min warm-up, subjects started the maximal exercised test and continued cycling while workloads increased at 20 or 30 W every min until volitional fatigue. Selection of the workload increment was subjective based on sex and training status, with the goal of producing exhaustion within 9-12 min. Whole body oxygen consumption (VO_2) was measured with a mixing chamber (Parvomedics, Sandy, UT, USA). The peak test was stopped and a cool-down period (40-60 W) of 3-5 min was initiated once subject reached exhaustion. Criteria for ending the peak test include; respiratory exchange ratio of 1.10 or greater, age-predicted heart rate max (220 - age) was reached, pedaling cadence dropped below ~60 rpm, and perceived subjective exhaustion (rating of perceived exertion) on the Borg scale of 19-20 (Borg, 1970; Poole & Jones, 2017). The peak test was ended once 3 of 4 criteria were met or the subject could not continue pedaling. Additionally, subjects completed a supramaximal exercise test (110% work rate, 90-120 s) to verify peak O_2 consumption following the cool down (Poole & Jones, 2017). Subjects continued to pedal and cool down for 3-5 min following the supramaximal exertion.

Exercise Training Intervention. This study utilized a combination of exercise approaches including continuous exercise (CE) of moderate intensity aerobic cycling for 1 h and high intensity interval training (HIIT) for 30 min. There were 21 exercise sessions (18 CE, 3 HIIT) through the 6-week exercise program with intensity prescription assessed every 7th exercise session. Continuous exercise has been shown to elicit a robust vasodilatory response during recovery from exercise that is reduced by ~80% via H_1 and H_2 histamine receptor blockade (Lockwood et al., 2005; Mccord & Halliwill, 2006; J.L.

McCord et al., 2006). High intensity interval training was included to ensure increases in VO_{2peak} with training as some individuals will not show, or have blunted, adaptation to continuous exercise alone (Bacon et al., 2013).

Moderate Exercise Intensity. Continuous exercise consisted of a 5 min warm-up and cool-down at 30% VO_{2peak} separated by a workload that produced 60% VO_{2peak} for 50 min. Steady state continuous exercise VO_2 was confirmed to be 60% of peak within the first 2 exercise session after each assessment of VO_{2peak}. The workload was adjusted, if needed, to achieve a workload that produced steady-state 60% VO_{2peak}. This workload was used on each of the continuous exercise sessions until VO_{2peak} was re-assessed.

High Intensity Interval Training Intensity. High intensity interval training consisted of 5-min warm-up and cool-down periods at 30% VO_{2peak} separated by a 20-min period of 3:1 interval training at 90% and 30% VO_{2peak} for a total of five 3-min intervals at 90% VO_{2peak}.

Hemodynamic Measurements.

Heart Rate. Heart rate was monitored on all study visits (data collection days and exercise sessions) using a combination of instruments. On all vascular study days, heart rate was monitored continuously while the subject was supine, using a three-lead electrocardiograph (Datex-Ohmeda Cardiocap/5, GE Healthcare, Tewksbury, MA, USA) and recorded using data acquisition software (Windaq; Dataq Instruments, Akron, OH, USA). During all exercise testing and training sessions, heart rate was measured with commercially available Polar chest straps (Polar Electro, New York, NY, USA)

connected to a Polar watch sensor and recorded manually every minute during exercise testing or every 5-10 min during exercise sessions.

Arterial Blood Pressure. Arterial blood pressure was measured in the right arm at heart level while the subject was supine using an automated sphygmomanometer (Tango+, SunTech Medical, Raleigh, N.C., USA). Arterial blood pressure was measured in triplicate after 20 min of supine rest to begin each vascular study day and in accordance with guidelines set forth by the American Heart Association (Muntner et al., 2019). Additionally, arterial blood pressure was taken following each dynamic arterial compliance measurement to be used in that calculation

Body Composition.

Body composition was measured every two weeks (day 2, 16, 30, 46) by 7-site skinfold analysis in accordance with best practices (Reilly et al., 1995). Skinfolds are measured using large calipers to determine skin and adipose tissue thickness around certain anatomical landmarks. The seven sites used were triceps, pectoralis, midaxillary, subscapular, abdomen, suprailliac, and quadriceps. Skinfold measurements were all made on the right side of the body and measured in duplicate with third measurements made if a difference of over 2 mm was observed. Additionally, body weight was measured at the beginning of every data collection day. Subjects were asked to remove their shoes and step onto a scale (Sartorius EB6CE-I, Precision Weighing Balances, Bradford, MA, USA).

Artery Lumen Diameter

Carotid, and femoral artery lumen diameter were measured via duplex ultrasonography. A linear-array vascular ultrasound probe (9MHz, Phillips iE33, Andover, MA., USA) was used for this study. An insonation angle of 60^{o} was used to accesses artery lumen diameter across multiple locations. Video recordings of arterial diameter were transferred to an external hard drive for offline analysis using commercial wall-tracking software (Brachial Analyzer for Research v6.11.9, Medical Imaging Applications LLC, Coralville, IA, USA) to make vessel diameter measurements. Measurements of lumen diameter were taken from at least 10 cardiac cycles at end-diastole and the mean values are reported.

Arterial Stiffness.

Arterial stiffness was assessed via measures of dynamic arterial compliance and pulse wave velocity. Dynamic arterial compliance was assessed in the common carotid and common femoral arteries while pulse wave velocity was assessed between the common carotid and femoral arteries and the brachial and pedal arteries. All vascular function measurements were measured using either doppler ultrasonography, applanation tonometry, or both.

Doppler ultrasound applies the principles of sound wave reflection and doppler shift to make measurements of arterial diameter and blood velocity simultaneously to estimate blood flow. This study utilized the Doppler ultrasound to make measurements of arterial diameter. A Phillips iE33 ultrasound was used with a 9 MHz linear array probe to image arterial diameter. The ultrasound probe contains a piezoelectric element that

converts electric signals into mechanical vibrations and emits ultrasonic sound waves. The ultrasound probe also detects the reflected ultrasonic sound waves and converts them back into electrical signals (Franklin et al., 2020). Video recordings of arterial diameter were transferred to an external hard drive for offline analysis using commercial wall-tracking software (Brachial Analyzer for Research v6.11.9, Medical Imaging Applications LLC, Coralville, IA, USA) to make vessel diameter measurements.

Applanation tonometry measures changes in pressure using a pencil like pressure transducers (PCU-2000; Millar, Inc., Houston, Tx, USA) and can be applied over any artery of interest. Tracings were recorded using Windaq (Dataq, Inc.) and the foot of the pressure tracing was marked to determine the time difference between sites. Velocity was calculated as distance over time, where distance was measured between the carotid and femoral probes, or the sum of the brachial probe to the sternal notch, to anterior superior iliac spine, to ankle probe minus the distance between the brachial probe to sternal notch. Measures of dynamic arterial compliance and pulse wave velocity were assessed across multiple sites on the body to estimate stiffness of one or both conduit and peripheral segments of the arterial tree.

Common carotid and femoral dynamic arterial compliance were measured using high-resolution Doppler ultrasound (Phillips iE33) with a 9 MHz linear array probe and concurrent applanation tonometry on the contralateral side of the body. Sampling was initiated through custom DUC2 software and sampled in 40-s simultaneous recording from both instruments. All ultrasound recordings were performed on the right side of the body. Changes in arterial diameter were assessed through ultrasound images, which were recorded at 46 Hz and saved to the ultrasound system before being transferred to a

computer with custom software (Brachial Analyzer) for analysis. Changes in pressure were measured using applanation tonometry and recorded via Windaq data acquisition (Dataq, Inc.) at 100 Hz and analyzed using the valley to peak pressure differential. The change in pressure was assessed relative to changes in diameter in order to calculate cross-sectional compliance and β-stiffness.

Statistics.

The statistical analysis for this research was performed using Prism software (GraphPad Prism 8, CA, USA). All subject characteristics are reported as mean ± SD and all additional data are reported as mean ± SEM. A Two-way ANOVA and Tukey *post hoc* test were used to compare groups and factor. There were repeated measurements over time with subject, condition, and sex analyzed. Significance was set at $P < 0.05$ for all analyses.

RESULTS

Subject Characteristics

Sixteen (6 men, 10 women) healthy, non-smoking individuals participated in this study. Subject's demographic and anthropometric characteristics obtained from their first study day include age, height, weight, body mass index, Baecke sport index, Physical activity index, and their 60% cycling power output, and are presented in Table 4.1 separated by group. Demographic and anthropometric characteristics were obtained each study day and did not change throughout the course of the study with pre-study grouped

values (age 25 ± 4 yrs, height 171.8 ± 9.3 cm, weight 73.8 ± 11.8 kg, body mass index 25 ± 3.2 m^2, mean ± SD).

Exercise Responses. The amount of oxygen consumed at peak exercise (VO_{2Peak}) throughout exercise training is shown in Figure 4.1. Pre-training VO_{2Peak} was not different between control (2.63 ± 0.27 L min^{-1}) and blockade (2.79 ± 0.23 L min^{-1}) conditions. There was a main effect of time as both groups VO_{2Peak} increased with training ($P<0.001$); however no difference was observed between groups ($P=0.721$) or interaction of condition (histamine receptor blockade) and time (training) ($P=0.737$). The control group increased VO_{2Peak} above pre-training values at 2, 4, and 6 weeks ($P<0.05$), whereas the blockade group increased VO_{2Peak} above pre-training values only at 4 and 6 weeks ($P<0.05$) (Figure 4.1).

The maximal amount of work (Watts) performed during peak exercise throughout exercise training is shown in Figure 4.2. Pre-training work was not different between control (227.5 ± 27.3 Watts) and blockade (227.5 ± 21.1 Watts) groups. There was a main effect of time (training) as maximal work performed increased in both groups ($P<0.001$); however no difference was observed between groups ($P>0.999$) nor was there an interaction of condition (histamine receptor blockade) and time (training) ($P>0.999$). Both groups increased work performed above pre-training values at 2, 4, and 6 weeks ($P<0.05$) (Figure 4.2).

Resting Hemodynamics. Resting heart rate was not different between groups and did not change with exercise training (Figure 4.3). Pre-training resting heart rate was not different between control (61.7 ± 3.2 bpm) and histamine receptor blockade (65.1 ± 4.7 bpm) conditions.

Table 4.1. Subject Characteristics

	Control	Blockade
n	8	8
Age (yrs)	25 ± 5	24 ± 4
Height (cm)	175.2 ± 8.4	169.8 ± 9.4
Weight (kg)	73.2 ± 13.6	74.2 ± 10.8
Body Mass Index (kg m^{-2})	23.7 ± 3.0	26.0 ± 3.2
Baecke sport index (arbitrary units)	2.5 ± 0.6	2.5 ± 0.8
Physical activity index (MET hr^{-1} $week^{-1}$)	22.2 ± 13.9	35.9 ± 10.5
60% power output (W)	124 ± 37	123 ± 36

Values are mean ± SD, MET, metabolic equivalents.

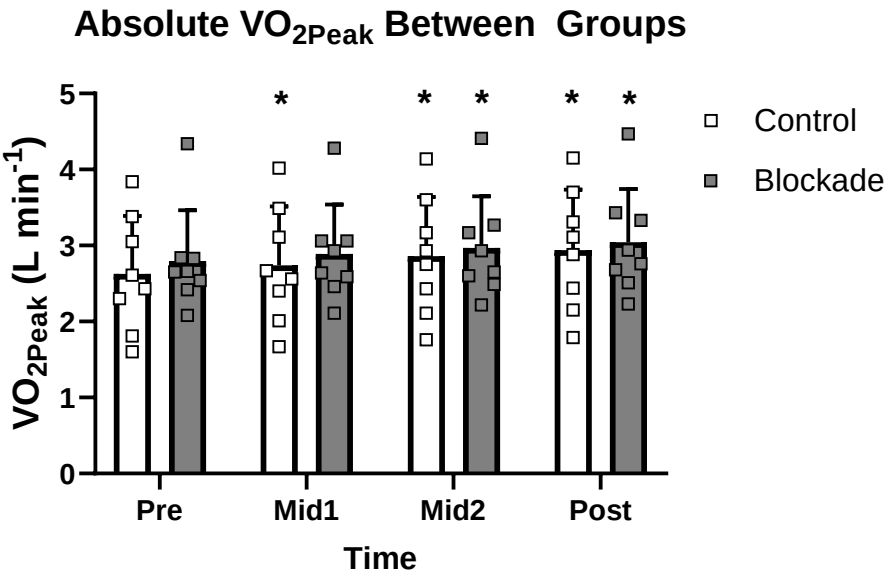


Figure 4.1. Absolute VO_{2Peak} attained at each time point throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * $P < 0.05$ vs Pre across both groups (main effect of time). There was no main effect of condition nor was there a condition x time interaction.

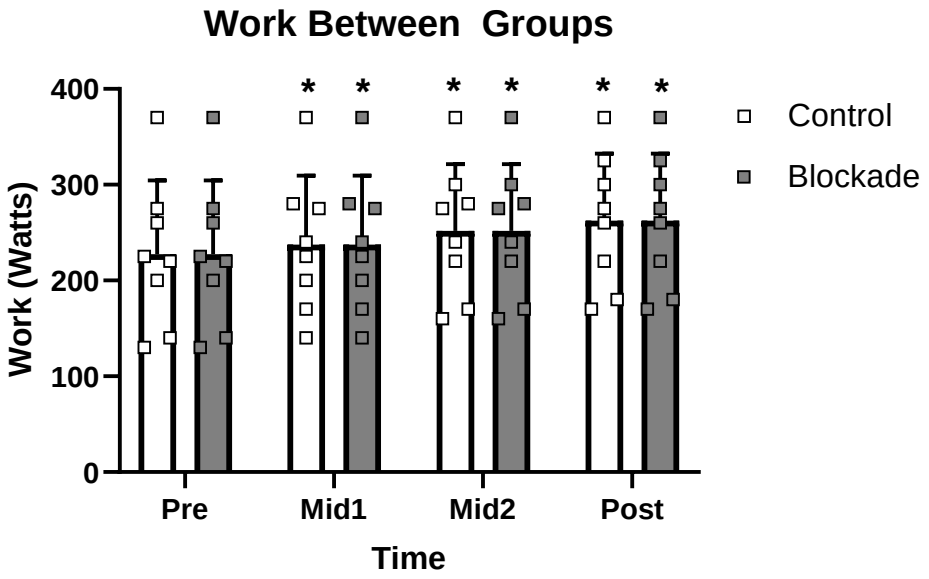


Figure 4.2. Maximal work attained at each time point throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * P < 0.05 vs Pre across both groups (main effect of time). There was no main effect of condition nor was there a condition x time interaction.

There was no main effect of time (training) for either group (P=0.546) or interaction of condition (histamine receptor blockade) and time (training) (P=0.296). Resting mean arterial pressure was not different between groups and did not change over time (Figure 4.4). Pre-training resting mean arterial pressure was not different between control (86.3 ± 4.8 mmHg) and histamine receptor blockade (86.6 ± 2.6 bpm) conditions. There was no main effect of time (training) for either group (P=0.783) or interaction of condition (histamine receptor blockade) and time (training) (P=0.605).

Body Composition. Pre-training body composition measured as percent body fat was not different between control (23.3 ± 2.8 %) and histamine receptor blockade (24.3 ± 3.0 %) conditions (P=0.447). There was no main effect of time (training) for either experimental groups (P=178). Body composition following exercise training was not different between groups (P=0. 938).

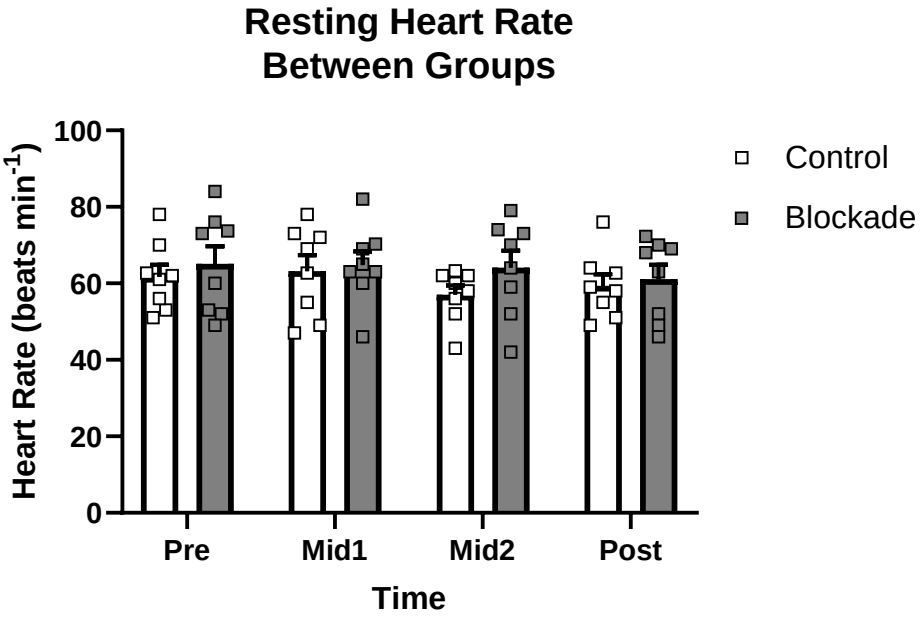


Figure 4.3. Resting heart rate following 20 min of supine rest attained at each time point throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. There was no main effect of time, no main effect of condition, nor was there a condition x time interaction.

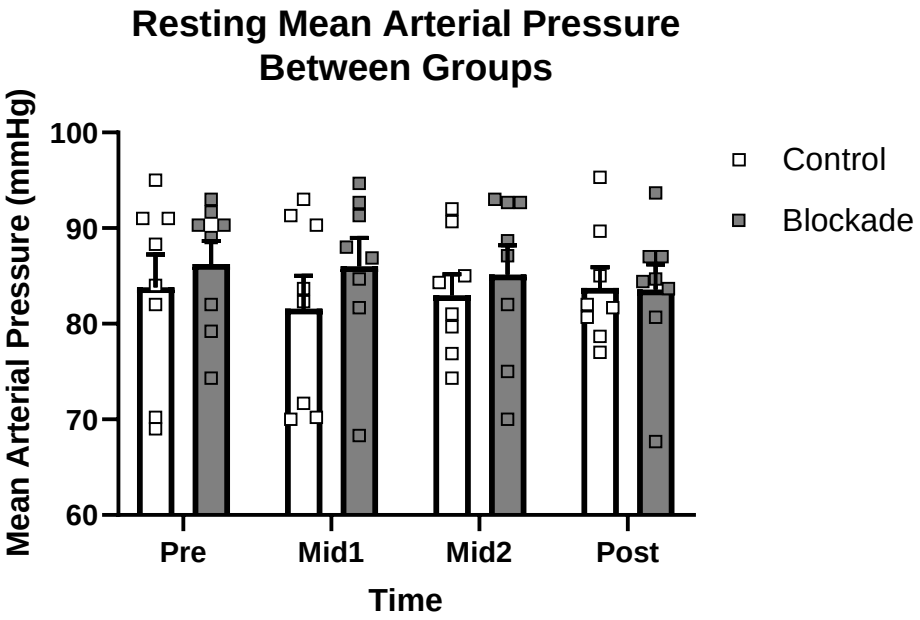


Figure 4.4. Resting mean arterial pressure following 20 min of supine rest attained at each time point throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. There was no main effect of time, no main effect of condition, nor was there a condition x time interaction.

Arterial Diameter. Resting carotid and femoral artery diameter, separated by experimental condition, and expressed in millimeters are shown in Figure 4.5. Pre-training resting carotid artery lumen diameter was not different between control (5.95 ± 0.19 mm) and histamine receptor blockade (6.21 ± 0.16 mm) conditions (P=0.238). There was a main effect of time (training) for both experimental groups (P<0.0001) as carotid artery lumen diameter was about 7% increased following exercise training. Resting carotid artery lumen diameter were increased from pre- (5.95 ± 0.19 mm) to post-exercise training (6.33 ± 0.24 mm) in the control group, and from pre- (6.21 ± 0.16 mm) to post-exercise training (6.79 ± 0.13 mm) in the histamine blockade group (P<0.0001). The increase in carotid artery lumen diameter following exercise training was not different between groups (P=0.267) (Figure 4.5A).

Pre-training resting femoral artery lumen diameter was not different between control (7.58 ± 0.44 mm) and histamine receptor blockade (8.67 ± 0.32 mm) conditions (P=0.086). There was a main effect of time (training) for both experimental groups (P<0.0001) as carotid artery lumen diameter was about 5% increased following exercise training. The control group femoral artery lumen diameter were increased from pre- (7.58 ± 0.44 mm) to post-exercise training (8.13 ± 0.45 mm) in the control group, and from pre- (8.67 ± 0.32 mm) to post-exercise training (9.06 ± 0.36 mm) in the histamine blockade group (P<0.0001). The increase in carotid artery lumen diameter following exercise training was not different between groups (P=0.462) (Figure 4.5B).

Vascular Function. Dynamic arterial compliance of the carotid artery was not different between groups and did not change over time. Pre-training dynamic arterial compliance

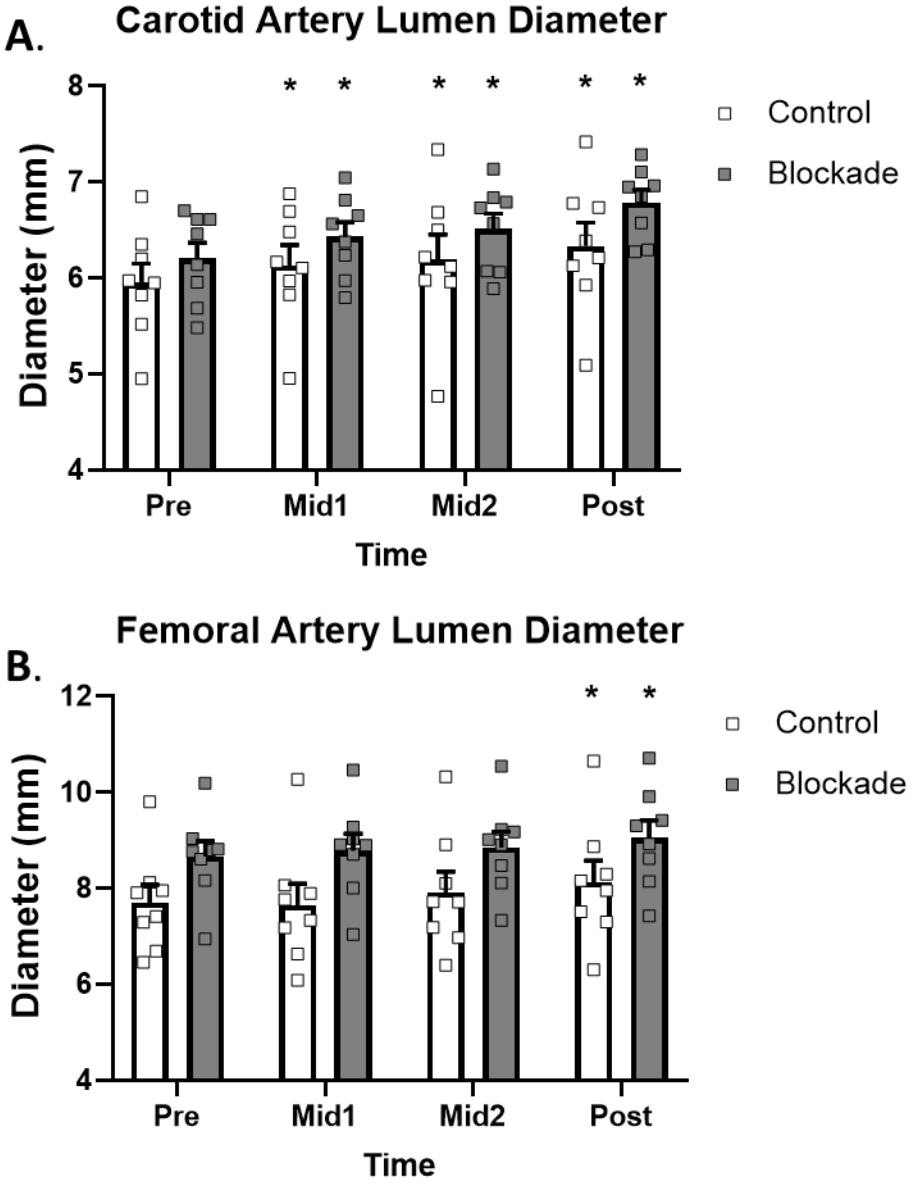


Figure 4.5. Artery lumen diameter attained at each time point throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * $P < 0.05$ vs Pre across both groups (main effect of time). There was no main effect of condition nor was there a condition x time interaction.

was not different between control (0.213 ± 0.05 mm^2 $mmHg^{-1}$) and histamine receptor blockade (0.169 ± 0.04 mm^2 $mmHg^{-1}$) conditions. There was no main effect of time (training) for either group (P=0.392) or interaction of condition (histamine receptor

blockade) and time (training) (P=0.759). Dynamic arterial compliance of the femoral artery was not different between groups and did not change with training. Pre-training dynamic arterial compliance was not different between control (0.328 ± 0.41 mm^2 $mmHg^{-1}$) and histamine receptor blockade (0.211 ± 0.06 mm^2 $mmHg^{-1}$) conditions. There was no main effect of time (training) for either group (P=0.399) or interaction of condition (histamine receptor blockade) and time (training) (P=0.644).

Stiffness represented as β-stiffness index of the carotid artery was not different between groups and did not change with training. Pre-training β-stiffness index was not different between control (7.394 ± 0.84 ratio (no units)) and histamine receptor blockade (7.326 ± 0.77 ratio (no units)) conditions. There was no main effect of time (training) (P=0.697) for either groups (P=0.694) or interaction of condition (histamine receptor blockade) and time (training) (P=0.800). Stiffness represented as β-stiffness index of the femoral artery was not different between groups and did not change with training. Pre-training β-stiffness index was not different between control (13.32 ± 4.29 ratio (no units)) and histamine receptor blockade (14.47 ± 3.76 ratio (no units)) conditions. There was no main effect of time (training) for ether group (P=0.316) or interaction of condition (histamine receptor blockade) and time (training) (P=0.757).

Pulse wave velocity of the carotid to femoral artery was not different between groups and did not change with training. Pre-training carotid-femoral pulse wave velocity was not different between control (698.7 ± 32.8 cm s^{-1}) and blockade (691.7 ± 32.6 cm s^{-1}) conditions. There was no main effect of time (training) for either group (P=0.509) or interaction of condition (histamine receptor blockade) and time (training) (P=0.522). Pulse wave velocity of the brachial to pedal (ankle) artery was not different between

groups and did not change with training. Pre-training pulse wave velocity was not different between control (944.6 ± 50.8 cm s^{-1}) and histamine receptor blockade (823.2 ± 28.1 cm s^{-1}) conditions. There was no main effect of time (training) for either group (P=0.280) or interaction of condition (histamine receptor blockade) and time (training) (P=0.649).

DISCUSSION

The purpose of this study was to determine the role of histamine receptor blockade on physiological adaptations induced by aerobic exercise training. We hypothesized that histamine receptor antagonists will blunt gains in aerobic capacity and measures of vascular health. The main finding of the present study was that combined blockade of histamine H_1 and H_2 receptors had no effect on the adaptations in exercise responses or vascular health induced by 6-weeks of endurance exercise training. These results suggest that chronically consuming antihistamines prior to exercise does not affect the adaptive ability to demonstrate improvements in measures of vascular health normally associated with exercise training. Additionally, consuming antihistamines prior to exercise does not affect the improvements in aerobic capacity that are observed following moderate intensity and duration exercise training.

Exercise Capacity

Exercise training resulted in increased aerobic capacity as evident by an increase in VO_{2Peak} and work performed following 6-weeks of combined continuous and high intensity exercise training, with no differences in these adaptations caused by histamine receptor blockade. The ability to adapt to exercise training is highly variable across

individuals and that variability is not well understood (Green et al., 2012; Moreau & Ozemek, 2017; Peake et al., 2015). This issue was addressed by the combination of continuous moderate intensity exercise and high intensity interval exercise used during the 6-week exercise training protocol. The current subject population demonstrated some variability in aerobic capacity improvements, ranging from about 2-21% increase in VO_{2Peak}, which is well within the expected variability for this exercise training duration and intensity combination (Bacon et al., 2013; Hautala, Kiviniemi, & Tulppo, 2009; David Montero & Díaz-Cañestro, 2015). On average there was about 11% improvement in VO_{2Peak} following the 6-week exercise training protocol, which is in line with what other comparable exercise training studies have found (Bonne et al., 2014; Hautala et al., 2009; D. H J Thijssen et al., 2007; Vollaard et al., 2009). The control group tended to demonstrate more improvement in VO_{2Peak} than the blockade group (13% vs 9% respectively), however with the high variability of improved aerobic capacity normally observed, the difference in VO_{2Peak} improvement is negligible and we conclude that the improvement in whole body oxygen consumption was not altered in a meaningful way between groups.

Vascular Health

Longitudinal studies have shown aerobic exercise training promote beneficial vascular health outcomes and reduce all-cause mortality, which is thought to occur in part through shear stress mediated improvements in endothelial nitric oxide availability (Green, 2009). While the current study did not directly measure bioavailable nitric oxide, histamine receptor antagonism has been shown to decrease expression of endothelial nitric oxide synthase following acute exercise (S A Romero et al., 2016). Histamine is an

established endothelial-dependent vasodilator that acutely modulates smooth muscle tone by activation of endothelial nitric oxide synthase and formation of nitric oxide. This led us to hypothesize that normal improvements in measures of vascular function would be blunted in the blockade group compared to the control following the exercise training protocol. Measuring flow mediated dilation, a gold standard measurement of vascular function, would have given insight into this question, however after making flow mediated dilation measurements in both the brachial and femoral arteries, technical problems with data acquisition prevented us from a possible answer. Thus, vascular health measures of arterial diameter, dynamic arterial compliance, and pulse wave velocity were also made and can give additional insight into histamine receptor blockade and vascular adaptations associated with exercise training.

Arterial lumen diameter

Exercise training causes changes in blood flow throughout the body including increased blood flow to active skeletal muscle. These chronic changes in blood flow can cause changes in the lumen diameter of the arterial segments subjected to increased blood flow (Brownlee & Langille, 1991). This flow-induced adaptation is thought to occur in order to maintain normal shear stress since larger vessels will be able to accommodate higher blood flow with relatively lower shear stress (Dinenno et al., 2001). The current study saw increased artery lumen diameter in both the carotid and femoral arteries across both experimental groups. The carotid artery lumen increased by about 7% and the femoral artery lumen increased by about 5%, which are in line with what other comparable exercise training studies have reported (Dinenno et al., 2001; D. H J Thijssen et al., 2007). The lack of difference between experimental groups most likely indicates

that activation of histamine receptors and post-exercise vasodilation are not major contributors to conduit vessel remodeling with exercise training. It may also be explainable by the observation that histamine receptor blockade does not reduce blood flow during exercise (Ely et al., 2020), and therefore the elevated blood flow and shear pattern during exercise may have far greater influence than the post-exercise flow and shear pattern.

Arterial stiffness

Dynamic arterial compliance. Arterial compliance refers to the amount of arterial expansion and recoil that occurs with cardiac contraction and relaxation and is determined by both structural and functional properties of the artery (Tryggestad & Short, 2014). Dynamic arterial compliance is decreased in sedentary individuals and multiple disease states, but will improve with continuous exercise training (Tanaka et al., 2000). Additionally, exercise training has been shown to increase total systemic arterial compliance in sedentary young subjects (Cameron & Dart, 1994). In the current study, measurements of arterial stiffness via dynamic arterial compliance did not change throughout the exercise training protocol. These results differ from those reported by previous studies in which arterial compliance was shown to decrease (improve) with acute cycle exercise (Kingwell et al., 1997) and after 4 weeks of exercise training at exercise intensities similar to those used in the present study (Cameron & Dart, 1994). The differences between the current results and those previously reported with similar exercise paradigms are likely due to methodological differences and measurements of total systemic vs single artery compliance. Additionally, previous measurements of total systemic compliance were made primarily from aortic waveform analysis via tonometry

and did not include diameter measurements. More recent observations, with similar methodology, have reported that arterial compliance improves with exercise training but in a different subject populations (>60 years old) who may have compromised compliance to start with, thus amplifying improvements with exercise training (D. H J Thijssen et al., 2007). Based on dynamic arterial compliance, the results of the present study suggest that in young healthy individuals who do not have compromised arterial compliance, this moderate exercise training protocol was not robust enough to illicit measurable changes.

Pulse Wave Velocity. Arterial stiffness measured as pulse wave velocity describes the velocity at which the arterial pulse propagates through the arterial tree. Pulse wave velocity is the most robust and reproducible measurement of arterial stiffness (Laurent et al., 2006; Rourke et al., 2002). Carotid-femoral pulse wave velocity is a measurement of conduit artery stiffness and is predictive of cardiovascular outcomes in various disease conditions (Ben-Shlomo et al., 2014; Vlachopoulos et al., 2014). Brachial-ankle pulse wave velocity provides information about peripheral artery stiffness (Sugawara et al., 2005) as well as some information about conduit artery stiffness. Pulse wave velocity is inversely related to compliance and is expected to decrease with exercise training, indicating less stiff arteries (Sugawara et al., 2005). Pulse wave velocity has been shown to decrease acutely with exercise (Kingwell et al., 1997) and with exercise training (Sugawara et al., 2005)(Sugawara et al., 2005)(Sugawara et al., 2005)(Sugawara et al., 2005)(David Montero & Lundby, 2016; Sugawara et al., 2005). In both the carotid-femoral and ankle-brachial pulse wave velocity measurements, we observed that velocity tended to decrease with exercise training; however, there was no statistical change with

training or with histamine receptor blockade. Based on pulse wave velocity, the results of the present study suggest that in young healthy individuals who do not have compromised arterial compliance, this moderate exercise training protocol was not robust enough to illicit measurable changes.

Methodological considerations

Measurements of vascular function through flow-mediated dilation would greatly contribute to a better understanding of the role of vascular health in adaptation to exercise training. Histamine is an established endothelial-dependent vasodilator through histamine H_1 receptors, that, when bound, activates endothelial nitric oxide synthase, which produces nitric oxide causing vasodilation of the surrounding smooth muscle (Li et al., 2003). In young healthy individuals, activation of endothelial H_1 receptors through binding of histamine cause vasodilation and increased blood flow. Histamine receptor blockade has been shown to decrease expression of nitric oxide synthase following acute exercise (S A Romero et al., 2016). Additionally, histamine and nitric oxide have differing pro-and anti-inflammatory properties in cultured human cells (Li et al., 2003; Tanimoto et al., 2007) proving that histamine's complex role in vascular health and disease is still being dissected. Due to the direct link between histamine and the endothelial-dependent release of nitric oxide, further research is needed to explore vascular function through flow mediated dilation and its role in how histamine mediated adaptation to aerobic exercise training.

Conclusion

Exercise training resulted in increased aerobic capacity evident by increased oxygen consumption and increased peak work following 6-weeks of combined

continuous and high intensity exercise training. Due to the lack of changes in pulse wave velocity or dynamic arterial compliance throughout exercise training, we conclude that in young healthy individuals who have nominal levels of arterial stiffness and do not have compromised arterial compliance, this moderate exercise training protocol was not robust enough to illicit measurable changes in clinical measures of arterial stiffness.

CHAPTER V

Histamine and Cardiovascular Adaptation to Endurance Exercise – Oxygen Delivery

This study was made possible through the contributions of Brendan W. Kaiser, Joshua E. Mangum, Emily A. Larson, Michael A. Francisco, Sydney Kobak, Christopher T. Minson, Hans C. Dreyer, Matthew Fogarty, Gary C. Sieck, and John R. Halliwill. Brendan, Josh, Emily, and Sydney provided much needed assistance with data collection throughout the study. Hans and Matthew helped collect and experiment with human skeletal muscle samples. John Halliwill and Chris Minson assisted in the intellectual development of the project. I was involved with all aspects of this project including the development of the protocol, funding through fellowship applications, completed all data collection, compiled the data, analysis of the data, and developed the manuscript.

INTRODUCTION

Aerobic exercise capacity increases with repeated exercise stress and can be quantified by measuring oxygen consumption during graded exercise tests (VO_{2Max}). In young healthy sedentary individuals, VO_{2Max} is thought to be limited by cardiac output, or the ability to deliver oxygen to working skeletal muscle in addition to basal metabolic demand while maintaining homeostasis (González-Alonso & Calbet, 2003; Lundby et al., 2016; Saltin & Strange, 1992). Adaptations to exercise training that are oxygen delivery centric include blood volume expansion and angiogenesis (Bonne et al., 2014; Laughlin et al., 2012). Moderate intensity aerobic exercise training will result in improvements in

the ability to deliver oxygen during subsequent exercise and will continue to improve aerobic capacity through the duration of training (Bacon et al., 2013). Moderate intensity aerobic exercise causes increased histamine concentrations within the active skeletal muscle that is released from mast cell degranulation or through *de novo* formation through the enzyme histidine decarboxylase (Steven A. Romero et al., 2016). Blocking histamine H_1 and H_2 receptors during exercise modulates the normal change in skeletal muscle mRNA expression related to endothelial and vascular function, metabolism, angiogenesis, cell maintenance, and inflammation (S A Romero et al., 2016).

Blocking the actions of histamine during exercise has potential to blunt enhancements in oxygen delivery, like increased red blood cell volume, normally associated with aerobic exercise training. The level of the enzyme histidine decarboxylase is very high in bone marrow compared to other tissue types, and histamine release in bone marrow initiates additional histamine induction through enzymatic activity (Hirata, 1975). Additionally, hematopoietic stem cells express histamine H_2 receptors that initiate changes in the cell cycle when activated; thus, antihistamines could inhibit hematopoiesis, specifically red blood cell formation, that would normally be associated with exercise training (Byron, 1877). These histamine-related mechanisms could mediate endurance exercise-induced adaptations in oxygen delivery, which could thus be blunted with chronic use of histamine receptor antagonists.

Blocking the actions of histamine during exercise has potential to blunt enhancements in oxygen delivery through reduced signals for angiogenesis. Exercise promotes growth and remodeling of the capillary network, known as angiogenesis, within skeletal muscle. Angiogenesis is needed to support adaptation to exercise as it augments

blood flow to decrease transit time and increases surface area for increased diffusion of oxygen from red blood cells in skeletal muscle. Both sprouting and splitting angiogenesis have been shown to occur in response to increased blood flow which occurs during exercise (Egginton et al., 2001). Blood flow in the previously active skeletal muscle normally remains elevated following exercise and could be a continued stimulus for angiogenesis. However, blood flow in the previously active skeletal muscle following exercise is reduced with antihistamine consumption, which could limit the angiogenic stimulus (Barrett-O'Keefe et al., 2013; J.L. McCord & Halliwill, 2006).

Acute aerobic exercise upregulates both pro- and anti-angiogenic growth factors within the previously active skeletal muscle such as vascular endothelial growth factor (VEGF), nitric oxide synthase, thrombospondin 1 and endostatin (Richardson et al., 1999; S A Romero et al., 2016). Importantly, histamine receptor blockade modulates the balance of pro- and anti-angiogenic factors following acute exercise by blunting both nitric oxide synthase and thrombospondin 1 mRNA (S A Romero et al., 2016). The blunting of both a pro- and anti-angiogenic factor with histamine receptor blockade may influence vascular adaptations, like angiogenesis, with exercise training. Additionally, activation of histamine receptors during aerobic exercise upregulates the pro-angiogenic growth factor vascular endothelial growth factor (Ghosh et al., 2001), which is attenuated with histamine receptor blockade (S A Romero et al., 2016). The synergistic effect of histamine on vascular endothelial growth factor acts through H_1 receptors as it is attenuated with histamine H_1 receptor blockade (Lu et al., 2013). Additionally, histamine has been shown to directly stimulate angiogenesis through activation of histamine receptors and is independent from growth factors like vascular endothelial growth factor

(Qin et al., 2013). Taken together, histamine receptor activation and histamine itself could be a signal for angiogenesis associated with aerobic exercise training. Since antagonism of histamine receptors attenuates the upregulation of vascular endothelial growth factor, it could blunt the angiogenic potential normally associated with exercise training.

It is unknown if chronically consuming antihistamines prior to exercise cause differences in the rate or scope to exercise-induced cardiovascular adaptations. The purpose of this study was to determine the role of histamine receptor activation in adaptations to aerobic exercise training. Specifically, following six weeks of aerobic cycling exercise training in young healthy inactive volunteers, we determined the effect of histamine receptor antagonists on improvements in aerobic capacity, blood volume, and angiogenesis. The hypothesis of this study is that in young healthy inactive volunteers, histamine receptor antagonists will blunt aerobic exercise-induced gains in VO_{2Peak} and maximal work, as well as beneficial changes associated with increased oxygen delivery such as increased red blood cell volume and angiogenesis.

METHODS

Subjects

This study was approved by the Institutional Review Board at the University of Oregon. Written informed consent was obtained from all subjects prior to participation and the study conformed to the principles of the Declaration of Helsinki. Seventeen (6 men, 11 women) healthy, non-smoking individuals participated in this study and were considered recreationally active based on exercise habits over the previous 12 months

(Baecke, Burema, & Frijters, 1982). Subjects were deemed healthy following a standard health screening. Sixteen (10 women) subjects completed the study while one subject withdrew from the study after the second study day due to scheduling conflicts. The data from this subject was not included in any analysis. All subjects were required to abstain from supplements, caffeine, and alcohol for at least 12 h, and exercise for at least 24 h prior to all study days. None of the subjects were using any over the counter or prescription medications at the time of the study, with the exception of oral contraceptives. Women were studied irrespective of menstrual cycle phase and had a negative pregnancy test prior to all study days. Subjects reported to the laboratory following a 4 h fast and all study days took place in a thermoneutral lab environment. Data collection start-time varied across subjects due to scheduling conflicts; however, experimental start-time was held constant within a subject to minimize circadian influence on physiologic variables. With the exception of the first study day, all data collection occurred between 24-48 h after the most recent exercise session.

Experimental Design

The research protocol consisted of a double-blind placebo-controlled exercise training study. Subjects underwent a 6-week exercise training intervention (exercising 3-4 times per week, totaling 21 sessions) with an experimental group receiving antihistamines (BLK) and a control group receiving a placebo (CTL) prior to each exercise session. There were 6 study days that included 2 separate study days for pre- and post-training measurements and 1 study day after every 7 exercise sessions, or 2 weeks, depending on subject schedules. Study days are referred to as “vascular testing days” or

"exercise testing days" based on the measurements that were made. Subjects started and ended the study with exercise testing days and both mid-point study days (following 2-weeks of training) consisted of both vascular and exercise testing. All study days were conducted on the University of Oregon Campus in Esslinger Hall. Prior to starting the first study day, subjects were block-randomly assigned to the control or blockade group.

Each exercise testing study day started with measurement of height and weight. The subject was then instrumented with a Polar chest-strap heart rate monitor and Hans-Rudolph two-way non-rebreathing valve with headgear and nose clip. Subjects sat quietly on the bike for 2 min before warming up for 5 min and starting the incremental exercise test. Once peak oxygen consumption was reached, subjects cooled down for 3-4 min before completing a supra-maximal exercise effort to verify that VO_{2Peak} had been attained. Each blood volume testing day started with measurements of body mass and height before the subject sat upright in a phlebotomy chair for 20 min before blood volume measurements were made.

Histamine Receptor Blockade and Placebo.

Common over-the-counter oral antihistamine drugs were used in the experimental arm of this study. Histamine H_1 and H_2 receptors were blocked using a dose of 540 mg fexofenadine and 300 mg ranitidine. Placebo pills were manufactured by a compounding pharmacy (Creative Compounds, Wilsonville, OR) and contained the inactive ingredients of the fexofenadine and ranitidine tablets. Subjects consumed the antihistamine medication, or placebo, with water 1 h prior to every exercise session, but not before data collection study days.

Exercise Testing and Training Protocol.

Peak oxygen consumption. Subjects performed an incremental cycle exercise test (Lode Excalibur, Groningen, The Netherlands) to exhaustion, four times (study day 1, 16, 30, 45) throughout the study. The exercise test consisted of 1-min workload increments until exhaustion to determine maximal oxygen uptake (VO_{2peak}). Specifically, subjects expired breath was collected through a one-way valve connected to a pneumotach and mixing chamber and breathed normally during a 2-min resting period sitting quietly on the bike. After the rest period, subjects began pedaling at a self-selected cadence for a 4-min warm-up period of low intensity cycling (40-130 W) determined as 1.5 times body weight (kg). Following the 4-min warm-up, subjects started the maximal exercised test and continued cycling while workloads increased at 20 or 30 W every min until volitional fatigue. Selection of the workload increment was subjective based on sex and training status, with the goal of producing exhaustion within 9-12 min. Whole body oxygen consumption (VO_2) was measured with a mixing chamber (Parvomedics, Sandy, UT, USA). The peak test was stopped and a cool-down period (40-60 W) of 3-5 min was initiated once subject reached exhaustion. Criteria for ending the peak test include; respiratory exchange ratio of 1.10 or greater, age-predicted heart rate max (220 - age) was reached, pedaling cadence dropped below ~60 rpm, and perceived subjective exhaustion (rating of perceived exertion) on the Borg scale of 19-20 (Borg, 1970; Poole & Jones, 2017). The peak test was ended once 3 of 4 criteria were met or the subject could not continue pedaling. Additionally, subjects completed a supramaximal exercise test (110% work rate, 90-120 s) to verify peak O_2 consumption following the cool down (Poole &

Jones, 2017). Subjects continued to pedal and cool down for 3-5 min following the supramaximal exertion.

Exercise Training Intervention. This study utilized a combination of exercise approaches including continuous exercise (CE) of moderate intensity aerobic cycling for 1 h and high intensity interval training (HIIT) for 30 min. There were 21 exercise sessions (18 CE, 3 HIIT) through the 6-week exercise program with intensity prescription assessed every 7th exercise session. Continuous exercise has been shown to elicit a robust vasodilatory response during recovery from exercise that is reduced by ~80% via H_1 and H_2 histamine receptor blockade (Lockwood et al., 2005; Mccord & Halliwill, 2006; J.L. McCord et al., 2006). High intensity interval training was included to ensure increases in VO_{2peak} with training as some individuals will not show, or have blunted, adaptation to continuous exercise alone (Bacon et al., 2013).

Moderate Exercise Intensity. Continuous exercise consisted of a 5 min warm-up and cool-down at 30% VO_{2peak} separated by a workload that produced 60% VO_{2peak} for 50 min. Steady state continuous exercise VO_2 was confirmed to be 60% of peak within the first 2 exercise session after each assessment of VO_{2peak}. The workload was adjusted, if needed, to achieve a workload that produced steady-state 60% VO_{2peak}. This workload was used on each of the continuous exercise sessions until VO_{2peak} was re-assessed.

High Intensity Interval Training Intensity. High intensity interval training consisted of 5-min warm-up and cool-down periods at 30% VO_{2peak} separated by a 20-min period of 3:1 interval training at 90% and 30% VO_{2peak} for a total of five 3-min intervals at 90% VO_{2peak}.

Blood Volume.

Hemoglobin, hematocrit, and carbon monoxide bound to hemoglobin (carboxyhemoglobin (COHb)), were measured for the purpose of estimating blood volume and assessed with a carbon monoxide (CO) uptake method as previously described (Burge & Skinner, 1995). The CO uptake method was used for pre and post exercise training measurements (Day 2 and 46), while blood samples were collected to measure hematocrit and hemoglobin across all 4 data collection study days (2, 16, 30, and 46). The CO uptake blood volume technique is based on the dilution of a tracer, inhaled CO, which binds to hemoglobin. Once CO is bound to hemoglobin (HbCO) its color changes (Burge & Skinner, 1995; David Montero et al., 2017; Siebenmann et al., 2017) and can be detected spectrophotometrically with the diode-array spectrophotometer OSM3 hexoximeter (OSM3 hexoximeter, Radiometer, Copenhagen, Denmark). Hematocrit was measured with the microcapillary (75 mm) centrifuge method (Autocrit Ultra 3, Becton Dickson, USA) and spun for 10 min at 11,700 rpm. Hemoglobin was measured with the OSM3 hexoximeter (day 2 and 46) and hemocue (day 2, 16, 30, 46) (Wilburn Medical, Kernersville, NC, USA). All blood samples were collected in heparinized syringes or vacutainers and transferred to capillary tubes or microcuvettes when necessary. Subjects sat in a phlebotomy chair for at least 20 min in the same body position before every blood draw for the volume measurements.

Carbon Monoxide Uptake Method. The CO uptake method used a dosage of 1mL CO per kg body weight. This dosage is predicted to raise HbCO by ~6.5% to levels around 8% (Burge & Skinner, 1995). A closed rebreathing circuit was utilized for this measurement and consisted of a glass structure connected to 2 1 L non-diffusible

anesthesia bags and mouthpiece. The rebreathing circuit was pre-filled with 100% O_2 and equipped with a soda-lime CO_2 scrubber to prevent buildup of CO_2 in the circuit. Subjects were instructed to exhale most of their breath to the environment before going onto the rebreathing circuit mouthpiece, that was still open to the environment, and exhaling to residual volume. Once the subject had expired to residual volume, a glass syringe filled with a calibrated volume of pure CO was connected to an open port. Within 1 s of breathing down to residual volume, the subject inhaled maximally, and the CO was injected into the system. After reaching inspiratory capacity, the subject held their breath for 10 s before breathing normally for the remainder of the 2-min period, after which the subject breathed back down to residual volume and came off the mouthpiece. Blood samples were obtained before and 7 min after the CO was added to the rebreathing circuit in order to measure the change in HbCO% in accordance with previously described methods (Burge & Skinner, 1995).

Venous Blood Sampling. During each study day, either a butterfly needle (day 16 and 30) or venous catheter (day 2 and 46) was used to draw venous blood samples. A catheter was utilized for each CO uptake experiment to sample blood at specific times before and after CO administration. After disinfecting the antecubital region of the arm, an intravenous catheter was placed by inserting a 20- or 22-gauge needle into the vein. Once the catheter was in place, the needle was removed, and the catheter was connected to a saline locked extension set, held in place with a clear adhesive cover (Tagaderm, 3M, Maplewood, MN, USA). After each blood draw, the sampling line and catheter were cleared with non-lactated, non-dextrose saline solution (0.9%) and closed to both the

sampling line and the environment. Blood samples were obtained from the sampling line into a sterile heparinized syringe and immediately analyzed.

Skeletal Muscle Biopsy.

All skeletal muscle biopsies were performed on the University of Oregon campus in Pacific Hall by an expert muscle physiologist, Dr. Hans C. Dreyer. Prior to the procedure, subjects refrained from exercise for 24 h and arrived in lab fasted overnight. Biopsies were taken from the left vastus lateralis muscle under sterile technique. To start, the skin and underlying fascia were anesthetized using 1% lidocaine hydrochloride (Hospira Worldwide, Lake Forest, IL, USA) and subject perception of pain was verbally acquired to ensure the biopsy area was effectively anesthetized. Once verbal confirmation of local anesthetic was obtained, a small incision made in the skin and underlying fascia before introduction of a 5 mm Bergstrom biopsy needle to the vastus lateralis muscle. A 20-cc syringe and tubing were attached to the aspiration port on the Bergstrom needle in order to apply negative pressure to assist the biopsy. After the vastus lateralis muscle sample was collected, a series of Steri-Strips (3M, Maplewood, MN, USA) were placed over the incision and covered with a sterile transparent dressing (Tagaderm). The tissue sample was blotted with gauze, and any visible adipose tissue was removed before the muscle sample was mounted on cork, covered in medium (Optimal Cutting Temperature Compound (OCT)), immediately fresh-frozen in melting isopentane cooled with liquid nitrogen and stored at -80^{o}C until further analysis. The muscle sample mounted on cork and fresh frozen was used to access either the maximum velocity of the succinate

dehydrogenase reaction in single type-identified muscle fibers (SDH_{max}) or capillary density.

Capillary Density: The assessment of capillary density was assessed using a Hematoxylin and Eosin staining procedure as previously described ("Guidelines for Hematoxylin & Eosin Staining," 2001). Alternate 10 µm serial sections adjacent to those used for determination of SDH_{max} and fiber type, were placed on a cover slip and allowed to dry for 15 min to prevent lifting of the section. Samples were fixed in 10% buffered formalin solution for 20 s before being stained in hematoxylin (Gill's III, Millipore Sigma GHD332-1L, St. Louis, MO, USA) for 2 min. Staining was stopped by detaining slides in 1% acid alcohol (1ml HCL/100ml 70% EtOH) for 10 s then rinsed in tap water for 5 min. A bluing reagent (0.3% ammonia water) was applied for 1 min before the slides were rinsed in distilled water to color the hematoxylin stain. The Eosin stain was applied by serial washes in 70% then 95% ethanol for 60 s each before staining slides in eosin-Y (Polysciences Cat309859, Warrington, PA, USA) for 60 s. The slides were then dehydrated by serial washes in duplicate of 95% and 100% ethanol for 60 s before the slides were cleared in xylene for 5 min. Slides were imaged using a Motic slide scanner (Motic, Richmond, British Columbia, Canada) with a 20x aperture according to manufacturer specifications. The number of capillary contacts was manually counted for each type identified muscle fiber that could be matched across SDH_{max} and fiber type serial sections. Manual capillary counting was achieved through increasing the digital zoom on the Tiff file (Figure 3.5) for individual type and SDH_{max} identified muscle cells.

Muscle fiber types: Alternate 10 µm serial sections adjacent to those used for determination of SDH_{max} and H&E staining of capillaries, were placed on a cover slip

and allowed to dry for 15 min to prevent lifting of the section. A total of 5 sections were cut from each sample and mounted onto a single slide to assess all fiber types. Samples were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 5 min prior to starting immunofluorescence staining protocols. Slides were washed thoroughly in 0.1 M phosphate buffer before being incubated overnight at 4^{o}C with each section covered in a unique primary antibody.

A total of 4 primary antibodies were used to determine immunoreactivity of myosin heavy chain (MyHC) isoforms expressed by different muscle fiber types: (1) A specific skeletal muscle $MyHC_{Slow}$ antibody (Novus, NBP2-50299 Mouse IgG, Centennial, CO, USA) was used at a 1:10 dilution to identify slow type I muscle fibers. (2) A specific fast skeletal muscle MyHC 2A antibody (SC-71 Developmental Studies Hybridoma Bank, Mouse IgG, Iowa City, IA, USA) was used at a 1:1 dilution to identify fast type IIa muscle fibers. (3) A specific skeletal muscle $MyHC_{2X}$ antibody (Novus, NBP1-22811 Mouse IgG, Centennial, CO, USA) was used at a 1:10 dilution to identify fast type IIx muscle fibers. (4) A non-specific skeletal muscle $MyHC_{Fast}$ antibody (Vector VP-M665, Mouse IgG, Burlingame, CA, USA) was used at a 1:10 dilution to identify all fast type II muscle fibers and by exclusion any fibers expressing $MyHC_{2B}$ (type IIb muscle fibers for which there is no specific antibody). After the primary antibodies incubated overnight, the slides were washed thoroughly in 0.1 M phosphate buffer and then all sections were incubated for 2 h in secondary antibody at a 1:200 dilution (Cy3 donkey anti-mouse IgG, ThermoFisher Scientific, Waltham, MA, USA) to assist in detection during imaging. Finally, the samples were washed in 0.1M phosphate buffer and allowed to dry before confocal microscopy.

Transverse vastus lateralis muscle sections were imaged using a 20x oil-immersion objective (NA 1.0) on an Olympus FV2000 laser confocal microscope capable of simultaneous multilabel florescence imaging. Images were captured in a 1200 x 1200-pixel array, with similar acquisition parameters across preparations. Type-identified fibers were paired with the sections used to determine SDH_{max} and capillary density.

Peak Reactive Hyperemia. A measurement of vascular function termed, flow mediated dilation was collected 4 times throughout this study according to established methodology (Dick H.J. Thijssen et al., 2011) in order to examine well established increases in vascular function associated with aerobic exercise training and its role in oxygen delivery during exercise and improved exercise performance. However, technical issues associated with ultrasound video file size impacted the ability to observe peak dilation following arterial occlusion, which usually occurs about 30 s following release of the occlusion cuff. Due to these technical difficulties that would undermine validity of the measurement, our analysis of flow mediated dilation was excluded. However, we were able to consistently assess peak reactive hyperemia at the onset of occlusion cuff release. The measurement of peak reactive hyperemia can give some insight into microvascular adaptation with exercise training as increased reactive hyperemia can be indicative of more complex capillary networks allowing for increased bulk blood flow through the conduit artery.

Statistics.

The statistical analysis for this research was performed using Prism software (GraphPad Prism 8, CA, USA). All subject characteristics are reported as mean ± SD and

all additional data are reported as mean ± SEM. A Two-way ANOVA and Sidak *post hoc* test were used to compare groups and factor. There were repeated measurements over time with subject, condition, and sex analyzed. Significance was set at $P < 0.05$ for all analyses.

RESULTS

Subject Characteristics

Sixteen (6 men, 10 women) healthy, non-smoking individuals participated in this study. Demographic and anthropometric characteristics were obtained each study day and did not change throughout the course of the study with pre-study grouped values (age 25 ± 4 yrs, height 171.8 ± 9.3 cm, weight 73.8 ± 11.8 kg, body mass index 25 ± 3.2 m^2, mean ± SD).

Exercise Responses. The amount of oxygen consumed at peak exercise (VO_{2Peak}) throughout exercise training is shown in Figure 5.1. Pre-training VO_{2Peak} was not different between control (2.63 ± 0.27 L min^{-1}) and blockade (2.79 ± 0.23 L min^{-1}) conditions. There was a main effect of time as in both groups VO_{2Peak} increased with training ($P<0.001$); however no difference was observed between groups ($P=0.721$) or interaction of condition (histamine receptor blockade) and time (training) ($P=0.737$). The control group increased VO_{2Peak} above pre-training values at 2, 4, and 6 weeks ($P<0.05$), whereas the blockade group increased VO_{2Peak} above pre-training values only at 4 and 6 weeks ($P<0.05$) (Figure 5.1).

The maximal amount of work (Watts) performed during peak exercise throughout exercise training is shown in Figure 5.2. Pre-training work was not different

between control (227.5 ± 27.3 Watts) and blockade (227.5 ± 21.1 Watts) groups. There was a main effect of time (training) as maximal work performed increased in both groups ($P<0.001$); however no difference was observed between groups ($P>0.999$) nor was there an interaction of condition (histamine receptor blockade) and time (training) ($P>0.999$). Both groups increased work performed above pre-training values at 2, 4, and 6 weeks ($P<0.05$) (Figure 5.2).

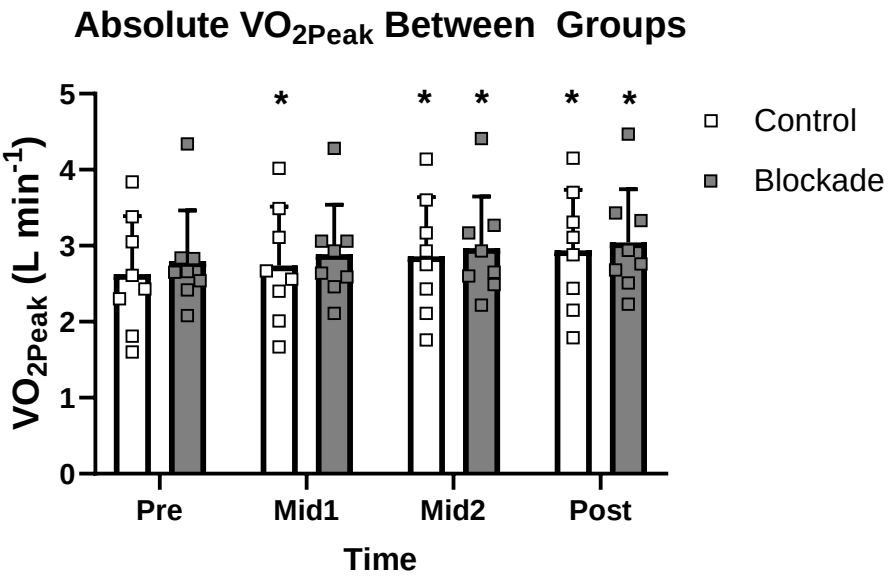


Figure 5.1. Absolute VO_{2Peak} attained at each time point throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * $P < 0.05$ vs Pre across both groups (main effect of time). There was no main effect of condition nor was there a condition x time interaction.

Blood Volume. Of the 16 individuals who participated in this study, only a subset of 12 completed all blood volume measurements. The demographic and anthropometric characteristics of these subjects are presented in Table 5.1. This information was obtained on the first study day and included age, height, weight, body mass index, Baecke sport index, Physical activity index, and their 60% cycling power output.

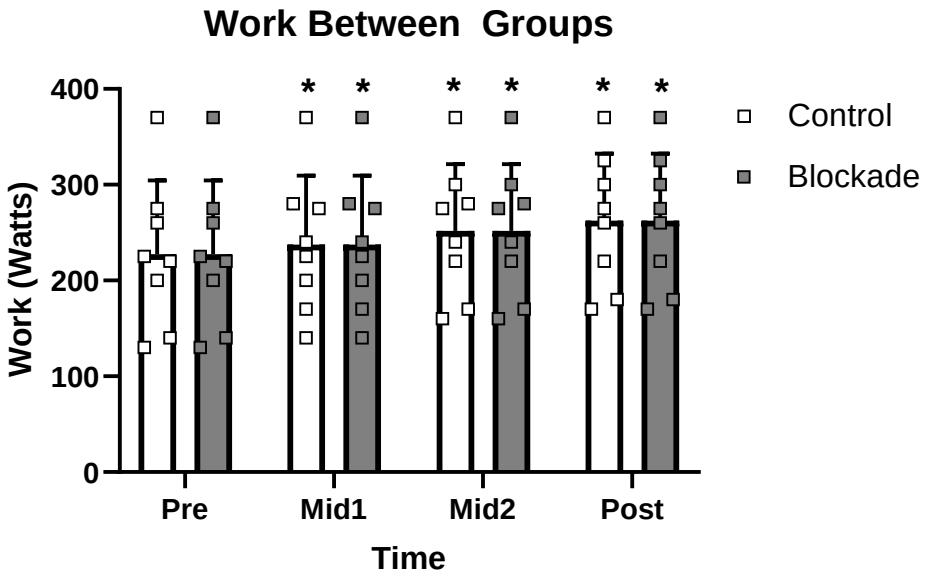


Figure 5.2. Maximal work attained at each time point throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * P < 0.05 vs Pre across both groups (main effect of time). There was no main effect of condition nor was there a condition x time interaction.

Table 5.1. Subject Characteristics

	Control	Blockade
N	6	6
Age (yrs)	24 ± 4	23 ± 3
Height (cm)	172.8 ± 6.0	170.8 ± 9.9
Weight (kg)	71.1 ± 12.5	77.1 ± 10.4
Body Mass Index (kg m^{-2})	23.8 ± 3.4	26.5 ± 3.5
Baecke sport index (arbitrary units)	2.8 ± 0.2	2.9 ± 0.4
Physical activity index (MET hr^{-1} $week^{-1}$)	23.8 ± 15.9	37.7 ± 12.3
60% power output (W)	130 ± 29	126 ± 41

Values are mean ± SD, MET, metabolic equivalents.

Blood volume was not different between groups and did not change with training. Pre-training blood volume was not different between control (5719 ± 529 mL) and histamine receptor blockade (6452 ± 431 mL) conditions. There was no main effect of time (training) in either group (P=0.434) or interaction of condition (histamine receptor blockade) and time (training) (P=0.329).

Plasma volume was not different between groups and did not change with training. Pre-training plasma volume was not different between control (3746 ± 373 mL) and blockade (4212 ± 237 mL) conditions. There was no main effect of time (training) in either group (P=0.477) or interaction of condition (histamine receptor blockade) and time (training) (P=0.327).

Red blood cell volume was not different between groups and did not change with training. Pre-training red blood cell volume was not different between control (1972 ± 173 mL) and histamine receptor blockade (2239 ± 245 mL) conditions. There was no main effect of time (training) in either group (P=0.451) or interaction of condition (histamine receptor blockade) and time (training) (P=0.606).

Packed cell volume or hematocrit throughout exercise training is shown in Figure 5.3. Pre-training hematocrit was not different between control (39.64 ± 0.82 %) and histamine receptor blockade (39.35 ± 1.34 %) conditions. There was a main effect of time (training) with an increase in hematocrit (P=0.002), however no difference was observed between groups interaction of condition (histamine receptor blockade) and time (training) (P=0.886). In the control group, hematocrit increased above pre-training values at 2 weeks (P=0.008) and 4 weeks (P=0.015), whereas in the histamine receptor blockade group, hematocrit tended to be higher at 2 weeks (P=0.079) and 4 weeks (P=0.056) after

exercise training. However, after 6 weeks of training, hematocrit levels in both groups were not different from pre-training values ($P > 0.05$) (Figure 5.3).

Hemoglobin levels, represented as grams of hemoglobin per deciliter of blood, throughout exercise training are shown in Figure 5.4. Pre-training hemoglobin levels were not different between control (13.24 ± 0.21 g dL^{-1}) and histamine receptor blockade (12.74 ± 0.45 g dL^{-1}) conditions.

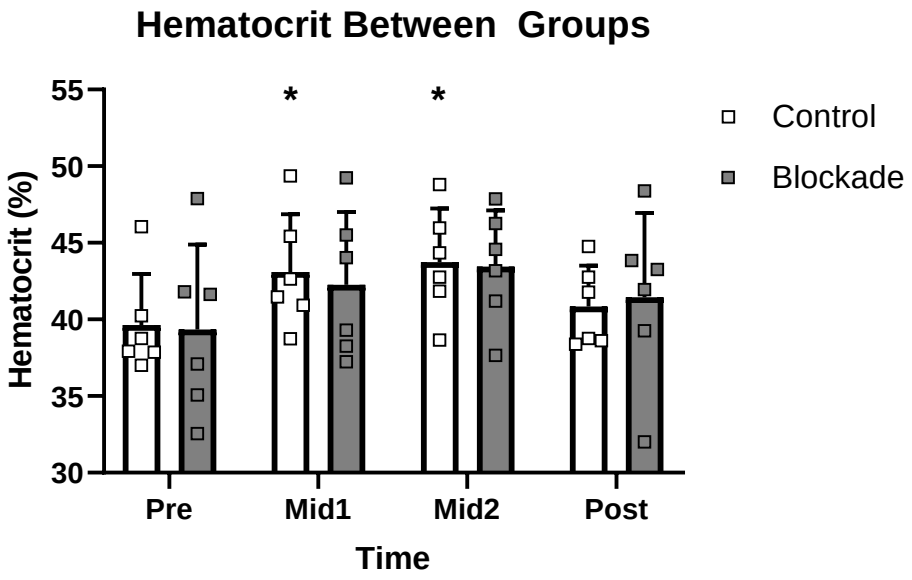


Figure 5.3. Hematocrit represented as a percentage at each time point throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * $P < 0.05$ vs Pre across both groups (main effect of time). There was no effect of condition nor was there a condition x time interaction.

There was a main effect of training as the control group, hemoglobin level increased ($P=0.004$); however no difference was observed between groups ($P=0.970$) or interaction of condition (histamine receptor blockade) and time (training) ($P=0.280$). In the control group, hemoglobin levels increased above pre-training values at 2 weeks ($P=0.046$), whereas in the blockade group, hemoglobin levels increased above pre-training values at 6 weeks ($P=0.043$). Additionally, in the histamine blockade group, hemoglobin levels

tended to be higher compared to pre-training levels at 2 weeks (P=0.053) and 4 weeks (P=0.051) after training.

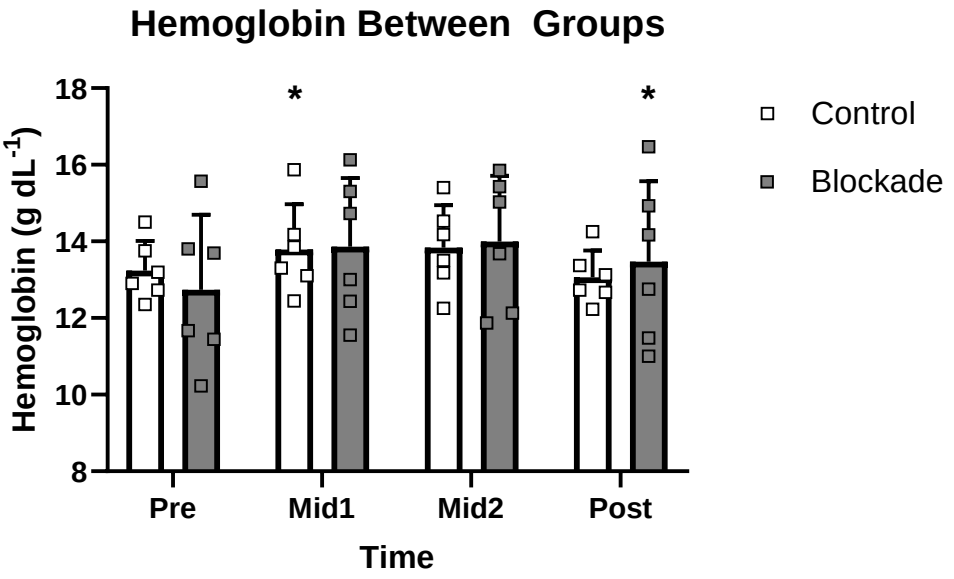


Figure 5.4. Hemoglobin represented as grams per deciliter of blood at each time point throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * $P < 0.05$ vs Pre across both groups (main effect of time). There was no effect of condition nor was there a condition x time interaction.

Muscle Responses. Of the 16 individuals who participated in this study, a subset of 10 completed biopsy procedures. The demographic and anthropometric characteristics of these subjects are presented in Table 6.1 in the next chapter that focuses more on the biopsy results. Vastus lateralis muscle samples were assessed for changes in fiber cross sectional area, the number of capillary contacts per fiber, and the number of capillary contacts relative to fiber cross sectional area. All measures were compared to pre-training values, separated by group, and assessed by muscle fiber type.

Muscle fiber cross sectional areas before and after exercise training are shown in Figure 5.5 through 5.7. Slow type I muscle fiber cross sectional area did not change from pre (3976 ± 521 μm^2) to post-exercise training (3847 ± 579 μm^2) in the control group

(P=0.929), nor from pre (3875 ± 610 μm^2) to post-exercise training (4129 ± 308 μm^2) in the histamine blockade group (P=0.828). Additionally, slow type I muscle fiber cross sectional area was not different between groups following training (P=0.905) (Figure 5.5).

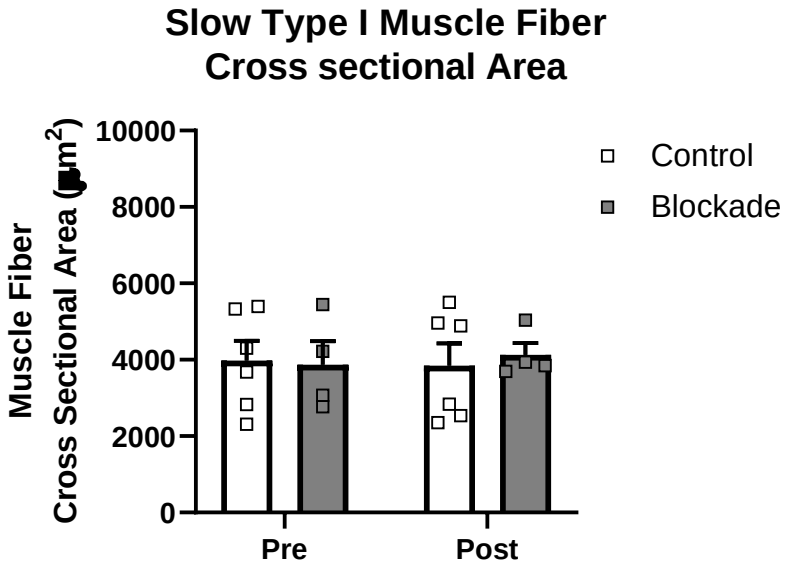


Figure 5.5. Slow type I muscle fiber cross section area represented as microns squared attained pre vs post throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. There was no main effect of time (training), no effect of condition, nor was there a condition x time interaction.

Fast type IIa muscle fiber cross sectional area did not change from pre- (4628 ± 473 μm^2) to post-exercise training (4787 ± 435 μm^2) in the control group (P=0.936), was not different from pre- (5035 ± 672 μm^2) to post-exercise training (5731 ± 273 μm^2) in the histamine blockade group (P=0.459). Additionally, fast type IIa muscle fiber cross sectional area was not different between groups following training (P=0.285) (Figure5.6). Fast type IIx muscle fiber cross sectional area did not change from pre- (6484 ± 502 μm^2) to post-exercise training (6948 ± 609 μm^2) in the control group (P=0.308) was not different from pre- (6725 ± 797 μm^2) to post-exercise training (6761 ± 736 μm^2) in the histamine blockade group (P=0.995). Additionally, fast type IIx muscle fiber cross

sectional area was not different between groups following exercise training (P=0.977) (Figure 5.7).

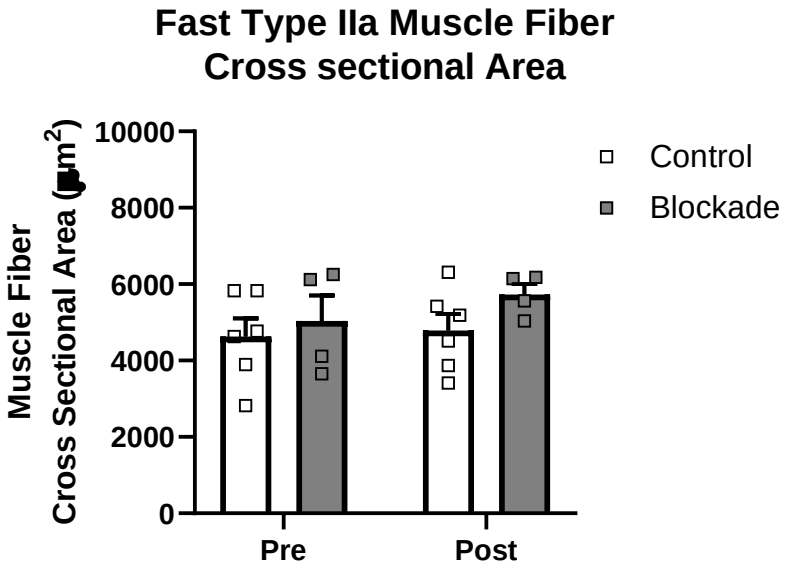


Figure 5.6. Fast type IIa muscle fiber cross section area represented as microns squared attained pre vs post throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. There was no main effect of time (training), no effect of condition, nor was there a condition x time interaction.

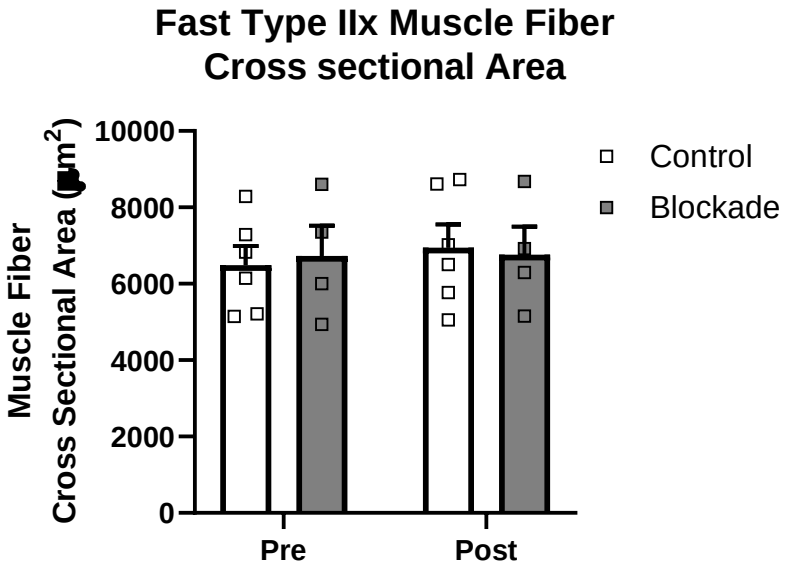


Figure 5.7. Fast type IIx muscle fiber cross section area represented as microns squared attained pre vs post throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. There was no main effect of time (training), no effect of condition, nor was there a condition x time interaction.

Capillary contacts per muscle fiber expressed in absolute number of capillaries before and after exercise training are shown in Figure 5.8 through 5.10. Slow type I muscle fiber capillary contacts were significantly increased from pre- (5.2 ± 0.2 capillary contacts) to post-exercise training (7.9 ± 0.2 capillary contacts) in the control group (P=0.0002), and from pre- (5.3 ± 0.5 capillary contacts) to post-exercise training (8.3 ± 0.2 capillary contacts) in the histamine blockade group (P=0.0005). The increase in the number of capillaries following exercise training was not different between groups in slow type I muscle fibers (P=0.477) (Figure 5.8).

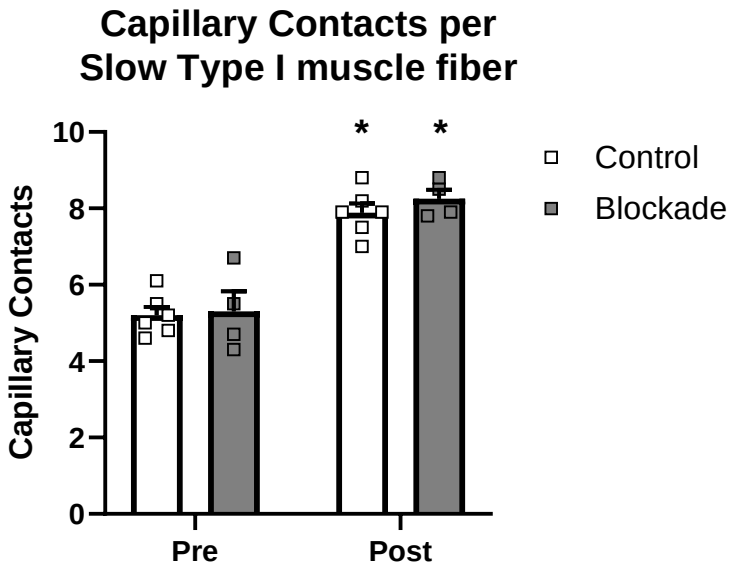


Figure 5.8. Capillary contacts per slow type I muscle fibers expressed in absolute number of capillaries before and after exercise training throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * P < 0.05 vs Pre (main effect of time). There was no effect of condition nor was there a condition x time interaction.

Fast type IIa muscle fiber capillary contacts were significantly increased from pre- (4.5 ± 0.2 capillary contacts) to post-exercise training (8.1 ± 0.6 capillary contacts) in the control group (P=0.0039), and from pre- (5.1 ± 0.5 capillary contacts) to post-exercise training (8.6 ± 0.5 capillary contacts) in the histamine blockade group

(P=0.0142). The post-training control group was not different from the histamine blockade group following exercise training in fast type IIa muscle fibers (P=0.667) (Figure 5.9). Fast type IIx muscle fiber capillary contacts were not significantly increased from pre- (4.2 ± 0.5 capillary contacts) to post-exercise training (4.9 ± 0.4 capillary contacts) in the control group (P=0.178), or from pre- (4.0 ± 0.2 capillary contacts) to post-exercise training (4.9 ± 0.2 capillary contacts) in the histamine blockade group (P=0.144). Following exercise training capillary contacts were not different between groups in fast type IIx muscle fibers (P=0.121) (Figure 5.10).

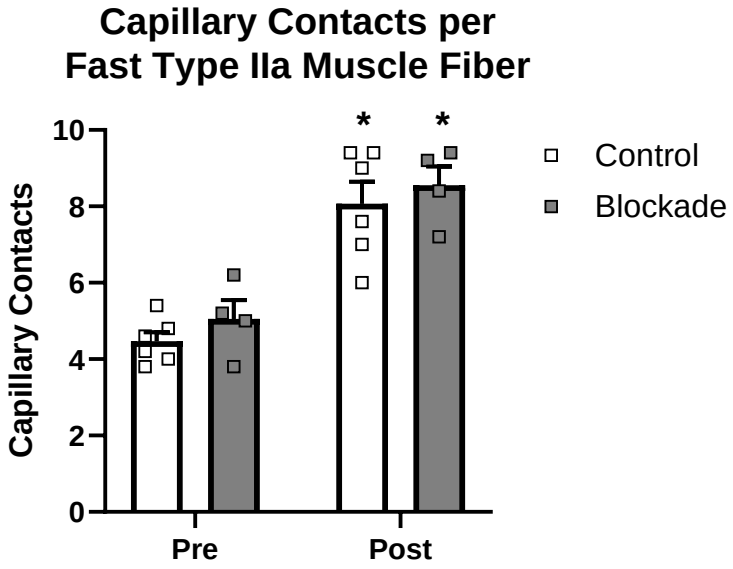


Figure 5.9. Capillary contacts per fast type IIa muscle fibers expressed in absolute number of capillaries before and after exercise training throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * P < 0.05 vs Pre (main effect of time). There was no effect of condition nor was there a condition x time interaction.

Capillary contacts per muscle fiber cross sectional area expressed in centimeters squared before and after exercise training is shown in Figure 5.11 through 5.13. Slow type I muscle fiber capillaries per fiber cross sectional area significantly increased from pre- (14.5 ± 1.5 cm^2) to post-exercise training (21.9 ± 3.1 cm^2) in the control group

(P=0.0175), but did not change from pre- (11.6 ± 2.6 cm^2) to post-exercise training (17.1 ± 2.9 cm^2) in the histamine blockade group (P=0.1395). The post-training control group was not different from the histamine blockade group following exercise training in slow type I muscle fibers (P=0.281) (Figure 5.11).

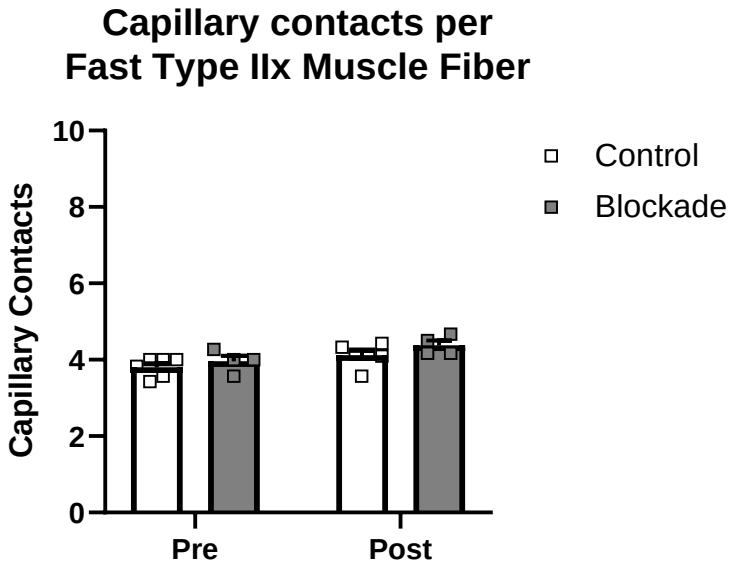


Figure 5.10. Capillary contacts per fast type IIa muscle fibers expressed in absolute number of capillaries before and after exercise training throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. There was no effect of time, condition, nor was there a condition x time interaction.

Fast type IIa muscle fiber capillaries per fiber cross sectional area were significantly different from pre- (10.0 ± 0.8 cm^2) to post-exercise training (16.1 ± 1.5 cm^2) in the control group (P=0.001), and from pre- (9.4 ± 1.2 cm^2) to post-exercise training (14.4 ± 1.8 cm^2) in the histamine blockade group (P=0.012). The post-training control group was not different from the histamine blockade group in fast type IIa muscle fibers (P=0.519) Figure 5.12). Fast type IIx muscle fiber capillaries per fiber cross sectional area were significantly different from pre- (7.0 ± 0.8 cm^2) to post-exercise training (9.1 ± 1.0 cm^2) in the control group (P=0.018), but not from pre (6.3 ± 0.8 cm^2) to post-exercise training

(7.2 ± 1.1 cm^2) in the histamine blockade group (P>0.459). The post training control group was not different from the histamine blockade group in fast type IIx muscle fibers (P=0.329) (Figure 5.13).

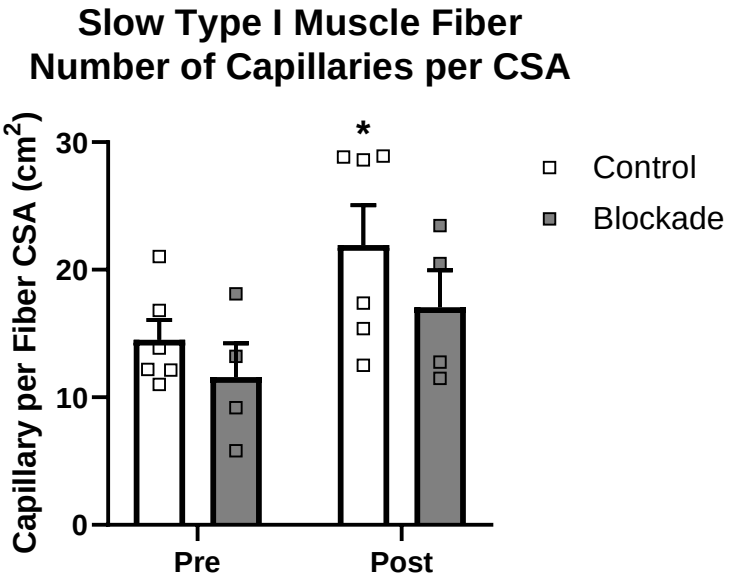


Figure 5.11. The number of capillaries per slow type I muscle fiber cross sectional area expressed in capillaries per centimeters squared before and after exercise training throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * P < 0.05 vs Pre (main effect of time). There was no effect of condition nor was there a condition x time interaction.

Femoral blood flow, shown in Figure 5.14, was measured following a 5 min vascular occlusion separated by experimental condition and expressed in millimeters min^{-1} over time. Pre-training peak femoral blood flow was not different between control (723± 73 mL min^{-1}) and histamine receptor blockade (902 ± 110 mL min^{-1}) conditions (P=0.407). There was a main effect of time (training) for both experimental groups (P<0.0001) as peak femoral blood flow was increased 24% following exercise training. The control group peak femoral blood flow were increased from pre- (723± 73 mL min^{-1}) to post-exercise training (1027 ± 97 mL min^{-1}) in the control group, and from pre- (902 ±

110 mL min^{-1}) to post-exercise training (1099 ± 119 mL min^{-1}) in the histamine blockade group (P<0.0001). As discussed in an earlier chapter, carotid artery lumen diameter increased following exercise training, but this increase was not different between groups (P=0.426) (Figure 4.5B).

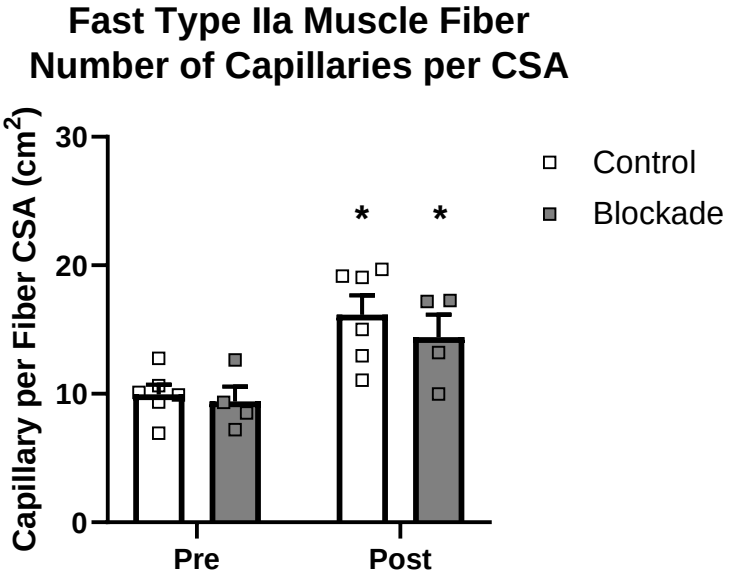


Figure 5.12. The number of capillaries per fast type IIa muscle fiber cross sectional area expressed in capillaries per centimeters squared before and after exercise training throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * P < 0.05 vs Pre (main effect of time). There was no effect of condition nor was there a condition x time interaction.

DISCUSSION

The purpose of this study was to determine the role of histamine receptor blockade on physiological adaptations induced by aerobic exercise training. We hypothesized that histamine receptor antagonists will blunt gains in aerobic capacity, by effecting the physiology behind adaptations to oxygen delivery. We focused on two of the early adaptations which support enhanced oxygen delivery during aerobic exercise: changes in blood volume and changes in capillarization of skeletal muscle. We did not

choose to explore changes in cardiac function, as the generally take longer to develop than the timeframe of our training protocol. The main finding of the present study was that combined blockade of histamine H_1 and H_2 receptors had no effect on the adaptations in oxygen delivery induced by 6-weeks of endurance exercise training as evidenced by changes in blood volume (not an evident adaptation during the time course of our protocol) and changes in capillarization of skeletal muscle (which did increase in response to training). These results suggest that chronically consuming antihistamines prior to exercise does not affect the adaptation that promotes a greater ability to deliver additional oxygen to working skeletal muscle and a greater aerobic capacity with training.

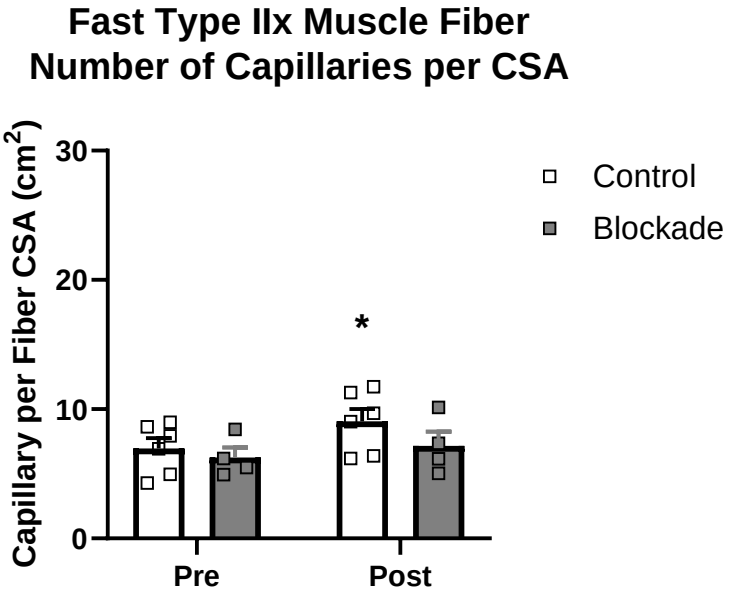


Figure 5.13. The number of capillaries per fast type IIa muscle fiber cross sectional area expressed in capillaries per centimeters squared before and after exercise training throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * $P < 0.05$ vs Pre (main effect of time). There was no effect of condition nor was there a condition x time interaction.

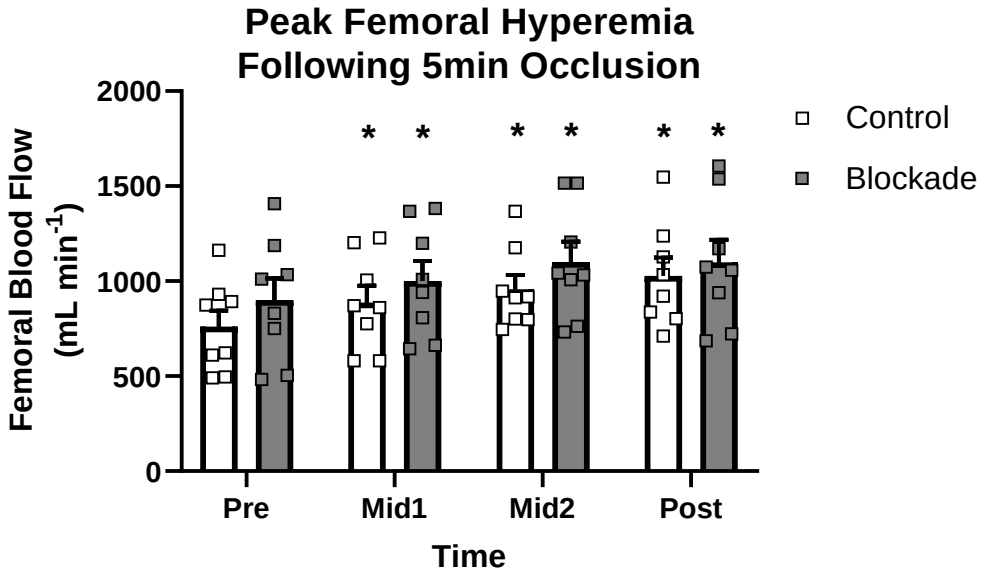


Figure 5.14. Peak femoral blood flow attained at each time point throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * $P < 0.05$ vs Pre (main effect of time). There was no main effect of condition nor was there a condition x time interaction.

Exercise Capacity

Exercise training resulted in increased aerobic capacity as evident by an increase in VO_{2Peak} and work performed following 6-weeks of combined continuous and high intensity exercise training, with no differences in these adaptations caused by histamine receptor blockade. On average there was about 11% improvement in VO_{2Peak} following the 6-week exercise training protocol, which is in line with what other comparable exercise training studies have found (Bonne et al., 2014; Hautala et al., 2009; D. H J Thijssen et al., 2007; Vollaard et al., 2009). The control group tended to demonstrate more improvement in VO_{2Peak} than the blockade group (13% vs 9% respectively), however with the high variability of improved aerobic capacity normally observed, the difference in VO_{2Peak} improvement is negligible and we conclude that the improvement in whole body oxygen consumption was not altered in a meaningful way between groups.

Adaptations to exercise that are oxygen delivery centric include blood volume expansion and angiogenesis. The current study demonstrated that changes in the ability to deliver oxygen through increased blood volume did not contribute to the increased aerobic capacity, however angiogenesis was increased following exercise training and likely played a critical role in the observed improvements in exercise capacity.

Blood Volume

Expansion of red blood cell and plasma volume with exercise training is a fundamental adaptation and primary driver of the enhanced ability to consume oxygen via increased oxygen delivery through increased cardiac preload and maximal cardiac output (David Montero et al., 2015). Improvements in oxygen consumption following exercise training are not solely due to passive increases in blood volume, and rely on the combined adaptations of vascular oxygen delivery mechanisms and blood volume expansion (Warburton et al., 2004).

Blood volume, plasma volume, and red blood cell volume did not change throughout exercise training in the present study. However, hematocrit was found to increase in the control group and hemoglobin levels were found to increase across both groups but at different time points. These results are not consistent with previous reports in the literature as increased red blood cell volume is thought to be a main determinant of cardiorespiratory fitness and is expected to increase with aerobic exercise training (Lundby et al., 2016). Evidence that blood volume should increase with exercise training comes from studies that show exercise training-derived increases in blood volume cause increased VO_{2Max} that is reversed when red blood cell volume is experimentally

manipulated to pre-exercise training levels through phlebotomy (Bonne et al., 2014). Additionally, sedentary individuals, who have no history of exercise training but higher than average VO_{2Max}, have enhanced red blood cell volume, demonstrating the importance of red blood cell volume and oxygen delivery in determining maximal oxygen consumption during exercise (Martino et al., 2002). Analysis of blood volume expansion with exercise training shows that when results are pooled across studies, red blood cell volume does not always increase with exercise training (D. Montero & Lundby, 2017; Michael N. Sawka et al., 2000). However, when data are stratified by age, young healthy individuals show a moderate, yet consistent increase in red blood cell volume in response to exercise training. Additionally, a positive correlation was found between the duration and intensity of training and the increase in red blood cell volume (D. Montero & Lundby, 2017). Based on this meta-analysis it is possible that the exercise training regimen used in the present study was not long or intense enough to observe increases in red cell volume that are normally associated with exercise training in similar populations.

The principle factor regulating red cell volume expansion is the hormone erythropoietin, which is stimulated by factors like hypoxia and reduced central venous pressure that occur during exercise (M.N. Sawka, 1999). Plasma erythropoietin levels increase approximately 30% following 60-min period of cycle exercise at 50-70% VO_{2Max} in untrained individuals. However, acute plasma erythropoietin responses to exercise decrease with continued exercise training (David Montero et al., 2017). These findings suggest sedentary individuals will have a robust erythropoietin response to exercise that will decrease with further exercise exposure at the same intensity and could

shine light on why there was no observed increase in red blood cell volume throughout the present study, with the majority of exercise sessions held at 60% of aerobic capacity.

Hematocrit is the ratio of the volume of red blood cells to the total volume of blood and usually slightly decreases with exercise training due to expansion of both red blood cells and larger increase in plasma volume (Michael N. Sawka et al., 2000). The present study found an increase in hematocrit after two and four weeks of exercise training. These data are difficult to interpret as there was considerable variability within and across subjects that could have led to the current results. Hematocrit returned to pre-training values by week 6 of training and there was no difference between groups.

Hemoglobin is the iron-containing oxygen-transport metalloprotein found in red blood cells and its levels are tightly correlated with changes in red blood cell volume. Hemoglobin increased in the control group following two weeks of exercise training but returned to pre-training values for the remainder of the study. The histamine receptor blockade group showed no change in hemoglobin levels for the first 4 weeks of training, but hemoglobin increased at week 6 of training. With no change in red cell volume it is difficult to justify increased hemoglobin at different time points throughout the study. When hemoglobin values from the carbon monoxide rebreathing technique are used, there is no main effect of time, condition, or interaction between the variables. If hemoglobin levels did increase slightly, it was not a large enough increase to justify the larger increase in oxygen consumption that was observed throughout the study.

Angiogenesis

Both sprouting and splitting angiogenesis have been shown to occur in response to increased blood flow which occurs during exercise (Egginton et al., 2001). Blood flow in the previously active skeletal muscle normally remains elevated following exercise and could be a continued stimulus for angiogenesis. However, blood flow in the previously active skeletal muscle following exercise is reduced with antihistamine consumption, which could limit the angiogenic stimulus (Barrett-O'Keefe et al., 2013; J.L. McCord & Halliwill, 2006). Additionally, acute aerobic exercise upregulates both pro- and anti-angiogenic growth factors within the previously active skeletal muscle such as vascular endothelial growth factor (VEGF), nitric oxide synthase, thrombospondin 1 and endostatin (Richardson et al., 1999; S A Romero et al., 2016). Importantly, histamine receptor blockade modulates the balance of pro- and anti-angiogenic factors following acute exercise by blunting both nitric oxide synthase and thrombospondin 1 messenger ribonucleic acid (S A Romero et al., 2016). The blunting of both a pro- and anti-angiogenic factor with histamine receptor blockade may influence vascular adaptations, like angiogenesis, with exercise training.

Angiogenesis occurred in response to 6-weeks of combined continuous and high intensity exercise training and was evident by an increase in the number of capillary contacts per fiber and the capillary density relative to fiber cross sectional area. All fiber types expressed increased numbers of capillaries per muscle fiber following training. Both slow type I and fast type IIa muscle fibers displayed an increase in angiogenesis, while fast type IIx fibers did not when the number of capillaries was normalized relative to fiber cross sectional area. Slow type I muscle fibers showed the greatest increase in

angiogenesis, which is appropriate for the intensity of exercise predominantly used throughout the training study. Exercise on a cycle ergometer at 60% VO_{2Max} for 50 min is accomplished through recruitment of slow type I and fatigue resistant fast type IIa muscle fibers; however fast type IIx fibers are not be recruited at this moderate intensity exercise (Egan & Zierath, 2013). Therefore, these angiogenesis results fall in line with what is expected as increased blood flow is the primary signal that drives angiogenesis and it is likely that only slow type I and fast type IIa muscle fibers were primarily recruited throughout exercise training.

Peak Femoral Hyperemia. Blood flow to any tissue is determined by the pressure gradient between the arterial and venous vasculature and the resistance to flow (Hautala et al., 2009). Vascular resistance, or conductance, is determined by the number and diameter of vessels within the tissue of interest. The femoral artery is a conduit vessel transporting blood to skeletal muscle along with every other tissue in the leg region. Peak femoral hyperemia increased, compared to pre-training measures, in both groups at all time points through the training study. This increase in peak femoral hyperemia is additional indirect evidence that there was microvascular adaptation in the skeletal muscle associated with exercise training and helps corroborate the increase in capillary density also found.

Methodological considerations

The use of haematoxylin and eosin for capillary density measurements needs some consideration in the current study due to the higher than normal capillary contacts per fiber reported. Haematoxylin and eosin is used commonly for histology and will non-

specifically stain nuclei and extracellular proteins. Therefore, it is possible that the high number of capillaries reported were inflated by the nonspecific staining of nuclei. This method has been verified in the past with additional immunohistochemical analysis of capillaries and found complete convergence of results (Proctor et al., 1995). Additional analysis by immunohistochemical analysis would help verify the high capillary counts in the current study.

Conclusion

Exercise training resulted in increased aerobic capacity evident by consuming more oxygen and preforming more work following 6-weeks of combined continuous and high intensity exercise training. Blood volume did not change throughout exercise training in this study; thus, it was not a necessary contributor to the observed aerobic capacity enhancements. However, angiogenesis increased with exercise training as evident in both histological measures of capillarization and functional tests of peak vasodilatory capacity. Thus, this well-known primary contributor to increased oxygen delivery to the working skeletal muscle and to enhanced aerobic capacity increased in our study but was not affected by histamine receptor blockade.

CHAPTER VI

Histamine and Cardiovascular Adaptation to Endurance Exercise – Oxygen Utilization

This study was made possible through the contributions of Brendan W. Kaiser, Joshua E. Mangum, Emily A. Larson, Michael A. Francisco, Sydney Kobak, Hans C. Dreyer, Matthew Fogarty, Gary C. Sieck, and John R. Halliwill. Brendan, Josh, Emily, and Sydney provided much needed assistance with data collection throughout the study. Hans and Matthew helped collect and experiment with human skeletal muscle samples. Gary gave me the opportunity to elevate the science around analyzing the muscle samples. John R. Halliwill assisted in the intellectual development of the project. I was involved with all aspects of this project including the development of the protocol, funding through fellowship applications, completed all data collection, compiled the data, analysis of the data, and developed the manuscript.

INTRODUCTION

Aerobic exercise capacity increases with repeated exercise stress and can be quantified by measuring oxygen consumption during graded exercise tests (VO_{2Max}). In young healthy sedentary individuals, VO_{2Max} is thought to be limited by cardiac output, or the ability to deliver oxygen to working skeletal muscle in addition to basal metabolic demand while maintaining homeostasis (González-Alonso & Calbet, 2003; Lundby et al., 2016; Saltin & Strange, 1992). However, the ability to utilize oxygen in skeletal muscle through oxidative metabolism can also be a limiting factor in some populations and must

be explored to understand the full picture of adaptation to exercise training (Joyner & Coyle, 2008; David Montero & Díaz-Cañestro, 2015). Adaptations to exercise training that are oxygen utilization centric are located within the working skeletal muscle and include increased mitochondrial volume density and oxidative function. Moderate intensity aerobic exercise training will demonstrate improvements in the ability to utilize oxygen during subsequent exercise and will continue to improve aerobic capacity with the duration of training (Egginton, 2009; Przyklenk et al., 2017).

Moderate intensity aerobic exercise causes increased histamine concentrations within the active skeletal muscle that is released from mast cell degranulation or through *de novo* formation through the enzyme histidine decarboxylase (Steven A. Romero et al., 2016). Histamine activates H_1 and H_2 receptors on vascular endothelial cells and vascular smooth muscle cells to acutely cause vasodilation and promote increased blood flow. Previously active skeletal muscle vasculature will remain dilated for hours following exercise and result in reduced arterial pressure during that time. The increased blood flow and reduced arterial pressure following exercise is attenuated by blocking histamine H_1 and H_2 receptors (Mccord & Halliwill, 2006; J.L. McCord et al., 2006; Steven A. Romero et al., 2016). Additionally, blocking histamine H_1 and H_2 receptors during exercise modulates the normal change in skeletal muscle mRNA expression related to endothelial and vascular function, metabolism, angiogenesis, cell maintenance, and inflammation (S A Romero et al., 2016).

It is unknown if chronically consuming antihistamines prior to exercise causes differences in the rate or scope to exercise-induced cardiovascular adaptation. The purpose of this study was to determine the role of histamine receptor activation in the

adaptations to aerobic exercise training. Specifically, following six weeks of aerobic cycling exercise training in young healthy inactive volunteers, we determined the effect of histamine receptor antagonists on improvements in aerobic capacity and skeletal muscle succinate dehydrogenase activity (SDH activity). The hypothesis of this study is that histamine receptor antagonists will blunt positive physiologic adaptation, or gains in aerobic capacity, by effecting the ability to utilize oxygen during subsequent exercise. Specifically, following six weeks of aerobic cycling exercise training in young healthy inactive volunteers, we hypothesized that histamine receptor antagonists will blunt exercise induced gains in aerobic capacity measured as VO_{2Peak} and maximal work, and blunt increases in skeletal muscle succinate dehydrogenase activity.

METHODS

Subjects

This study was approved by the Institutional Review Board at the University of Oregon. Written informed consent was obtained from all subjects prior to participation and the study conformed to the principles of the Declaration of Helsinki. Ten (4 men, 6 women) healthy, non-smoking individuals participated in this study and were considered recreationally active based on exercise habits over the previous 12 months (Baecke, Burema, & Frijters, 1982). Subjects were deemed healthy following a standard health screening. Sixteen (10 women) subjects completed the study while one subject withdrew from after the second study day due to scheduling conflicts. The data from this subject were not included in any analysis. All subjects were required to abstain from supplements, caffeine, and alcohol for at least 12 h, and exercise for at least 24 h prior to

all study days. No subjects were using any over the counter or prescription medications at the time of the study, with the exception of oral contraceptives. Women were studied irrespective of menstrual cycle phase and had a negative pregnancy test prior to all study days. All muscle biopsy samples were obtained between 7-8 am following an overnight fast. With the exception of the first study day, all data collection occurred between 24-48 h after the most recent exercise session.

Experimental Design

The research protocol consisted of a double-blind placebo-controlled exercise training study. Subjects underwent a 6-week exercise training intervention (exercising 3-4 times per week, totaling 21 sessions) with an experimental group receiving antihistamines (BLK) and a control group receiving a placebo (CTL) prior to each exercise session. There were 4 study days that included 2 study days for pre- and post-training measurements (muscle biopsy and VO_{2Peak}) and 1 study day after every 7 exercise sessions, or 2 weeks, depending on subject schedules (VO_{2Peak}). All experiments were conducted on the University of Oregon Campus with muscle biopsy procedures completed in Pacific Hall and exercise testing completed in Esslinger Hall. Prior to starting the first study day, subjects were block-randomly assigned to a control or blockade group.

Each exercise testing study day started with measurements of height and weight. The subject was then instrumented with a Polar chest-strap heart rate monitor and Hans-Rudolph two-way non-rebreathing valve with headgear and nose clip. Subjects sat quietly on the bike for 2 min before warming up for 5 min and starting the incremental exercise

test. Once peak oxygen consumption was reached, subjects cooled down for 3-4 min before completing a supra-maximal exercise effort to verify VO_{2Peak} had be attained.

Histamine Receptor Blockade and Placebo.

Common over-the-counter oral antihistamine drugs were used in the experimental arm of this study. Histamine H_1 and H_2 receptors were blocked using a dose of 540 mg fexofenadine and 300 mg ranitidine. Placebo pills were manufactured by a compounding pharmacy (Creative Compounds, Wilsonville, OR) and contained the inactive ingredients of the fexofenadine and ranitidine tablets. Subjects consumed the antihistamine medication, or placebo, with water 1 h prior to every exercise session, but not before data collection study days.

Exercise Testing and Training Protocol.

Peak oxygen consumption. Subjects performed an incremental cycle exercise test (Lode Excalibur, Groningen, The Netherlands) to exhaustion, four times (study day 1, 16, 30, 45) throughout the study. The exercise test consisted of 1-min workload increments until exhaustion to determine maximal oxygen uptake (VO_{2peak}). Specifically, subjects expired breath was collected through a one-way valve connected to a pneumotach and mixing chamber and breathed normally during a 2-min resting period sitting quietly on the bike. After the rest period, subjects began pedaling at a self-selected cadence for a 4-min warm-up period of low intensity cycling (40-130 W) determined as 1.5 times body weight (kg). Following the 4-min warm-up, subjects started the maximal exercised test and continued cycling while workloads increased at 20 or 30 W every min until volitional

fatigue. Selection of the workload increment was subjective based on sex and training status, with the goal of producing exhaustion within 9-12 min. Whole body oxygen consumption (VO_2) was measured with a mixing chamber (Parvomedics, Sandy, UT, USA). The peak test was stopped and a cool-down period (40-60 W) of 3-5 min was initiated once subject reached exhaustion. Criteria for ending the peak test include; respiratory exchange ratio of 1.10 or greater, age-predicted heart rate max (220-age) was reached, pedaling cadence dropped below ~60 rpm, and perceived subjective exhaustion (rating of perceived exertion) on the Borg scale of 19-20 (Borg, 1970; Poole & Jones, 2017). The peak test was ended once 3 of 4 criteria were met or the subject could not continue pedaling. Additionally, subjects completed a supramaximal exercise test (110% work rate, 90-120 s) to verify peak O_2 consumption following the cool down (Poole & Jones, 2017). Subjects continued to pedal and cool down for 3-5 min following the supramaximal exertion.

Exercise Training Intervention. This study utilized a combination of exercise approaches including continuous exercise of moderate intensity aerobic cycling for 1 h and high intensity interval training for 30 min. There were 21 exercise sessions (18 continuous exercise and 3 high intensity exercise training) through the 6-week exercise program with intensity prescription assessed every 7 exercise sessions. Continuous exercise has been shown to elicit a robust vasodilatory response during recovery from exercise that is reduced by ~80% via H_1 and H_2 histamine receptor blockade (Lockwood et al., 2005; Mccord & Halliwill, 2006; J.L. McCord et al., 2006). High intensity interval training was included to ensure increases in VO_{2peak} with training as some individuals

will not show, or have blunted, adaptation to continuous exercise alone (Bacon et al., 2013).

Moderate Exercise Intensity. Continuous exercise consisted of 5-min warm-up and cool-down periods at 30% VO_{2peak} separated by a workload that produced 60% VO_{2peak} for 50 min. Steady state continuous exercise VO_2 was confirmed to be 60% of peak within the first 2 exercise sessions after each assessment of VO_{2peak}. The workload was adjusted, if needed, to achieve a workload that produced steady-state 60% VO_{2peak}. This workload was used on each of the continuous exercise sessions until VO_{2peak} was re-assessed.

High Intensity Interval Training Intensity. High intensity interval training consisted of 5-min warm-up and cool-down periods at 30% VO_{2peak} separated by a 20-min period of 3:1 interval training at 90% and 30% VO_{2peak} for a total of five 3-min intervals at 90% VO_{2peak}.

Skeletal Muscle Biopsy.

All skeletal muscle biopsies were performed on the University of Oregon campus in Pacific Hall by an expert muscle physiologist, Dr. Hans C. Dreyer. Prior to the procedure, subjects refrained from exercise for 24 h and arrived in lab fasted overnight. Biopsies were taken from the left vastus lateralis muscle under sterile technique. To start, the skin and underlying fascia were anesthetized using 1% lidocaine hydrochloride (Hospira Worldwide, Lake Forest, IL, USA) and subject perception of pain was verbally acquired to ensure the biopsy area was effectively anesthetized. Once verbal confirmation of local anesthetic was obtained, a small incision made in the skin and underlying fascia

before introduction of a 5 mm Bergstrom biopsy needle to the vastus lateralis muscle. A 20-cc syringe and tubing were attached to the aspiration port on the Bergstrom needle in order to apply negative pressure to assist the biopsy. After the vastus lateralis muscle sample was collected, a series of Steri-Strips (3M, Maplewood, MN, USA) were placed over the incision and covered with a sterile transparent dressing (Tagaderm). The tissue sample was blotted with gauze, and any visible adipose tissue was removed before the muscle sample was mounted on cork, covered in medium (Optimal Cutting Temperature Compound (OCT)), immediately fresh-frozen in melting isopentane cooled with liquid nitrogen and stored at -80^{o}C until further analysis. The muscle sample mounted on cork and fresh frozen was used to access either the maximum velocity of the succinate dehydrogenase reaction in single type-identified muscle fibers (SDH_{max}) or capillary density.

Succinate dehydrogenase activity: Vastus lateralis muscle biopsy samples were cut in transverse serial sections at 6 µm thickness using a cryostat (Reichert Jung Frigocut 2800 Cryostat, Reichert Microscope Services, Depew, NY, USA), maintained at a temperature of -30^{o}C. Sections were used for a quantitative assessment of the maximum velocity of succinate dehydrogenase reaction using a previously described quantitative histochemical procedure (Blanco et al., 1988; Enad et al., 1989; Fogarty et al., 2020; Proctor et al., 1995). Sections were cut and placed on a cover slip where they were immediately visualized to determine cross-section orientation of cells, maintained at 5^{o}C with a control solution covering the section on the slide. Once the orientation of the section was confirmed, serial sections were cut from the sample at 6 µm for SDH_{max} measurements and at 10 µm thickness to identify capillaries (haematoxylin and eosin,

H&E stain) and fiber type (immunoreactivity to myosin heavy chain, MyHC, antibodies), as described below in detail.

For SDH_{max} determinations, a baseline image was acquired (Olympus IX71, Olympus America, Melville, NY) with a camera and image capture system running software (Elements C-ER, Nikon, Tokyo, Japan) for image processing. Images were acquired using a 20x objective (1.0 NA) and captured in a 1200 x 1200-pixel array, with similar acquisition parameters across samples. An interference filter (570 nm) was used to limit the spectral range of the light source to the optimal absorbance wavelength for nitroblue tetrazolium diformazan (the SDH reaction indicator). Once the baseline image was acquired, the control solution was removed via suction and a solution containing 80 μM succinate (to maximize substrate availability for the SDH reaction) was added. Thereafter, images were acquired every 15 s for a 10-min period with no changes to the environmental conditions or image processing system throughout the experiment. Both control (no succinate) and succinate (80 nM) solutions contained 1.5 mM nitro blue tetrazolium (NTB – reaction indicator), 5 mM EDTA, 0.2 mM PMS, and 0.1 mM azide in 0.1 M phosphate buffer (pH = 7.6). The maximum concentration of succinate for the succinate dehydrogenase activity reaction was previously determined (Blanco et al., 1988; Enad et al., 1989) and validated for human muscle fibers (Proctor et al., 1995). In the quantitative histochemical procedure, the progressive precipitation of diformazan from the reduction of nitro blue tetrazolium is used as the reaction indicator (Blanco et al., 1988; Proctor et al., 1995). The linearity of the accumulation of NBT diformazan in individual vastus lateralis muscle fibers was confirmed across a 20-min period. Based on

the linearity of the SDH reaction, a final end-point time of 10 min was selected to avoid any saturation of the imaging system.

Muscle fiber types: Alternate 10 µm serial sections adjacent to those used for determination of SDH_{max} and H&E staining of capillaries, were placed on a cover slip and allowed to dry for 15 min to prevent lifting of the section. A total of 5 sections were cut from each sample and mounted onto a single slide to assess all fiber types. Samples were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer for 5 min prior to starting immunofluorescence staining protocols. Slides were washed thoroughly in 0.1 M phosphate buffer before being incubated overnight at $4^{o}C$ with each section covered in a unique primary antibody.

A total of 4 primary antibodies were used to determine immunoreactivity of myosin heavy chain (MyHC) isoforms expressed by different muscle fiber types: (1) A specific skeletal muscle $MyHC_{Slow}$ antibody (Novus, NBP2-50299 Mouse IgG, Centennial, CO, USA) was used at a 1:10 dilution to identify slow type I muscle fibers. (2) A specific fast skeletal muscle MyHC 2A antibody (SC-71 Developmental Studies Hybridoma Bank, Mouse IgG, Iowa City, IA, USA) was used at a 1:1 dilution to identify fast type IIa muscle fibers. (3) A specific skeletal muscle $MyHC_{2X}$ antibody (Novus, NBP1-22811 Mouse IgG, Centennial, CO, USA) was used at a 1:10 dilution to identify fast type IIx muscle fibers. (4) A non-specific skeletal muscle $MyHC_{Fast}$ antibody (Vector VP-M665, Mouse IgG, Burlingame, CA, USA) was used at a 1:10 dilution to identify all fast type II muscle fibers and by exclusion any fibers expressing $MyHC_{2B}$ (type IIb muscle fibers for which there is no specific antibody). After the primary antibodies incubated overnight, the slides were washed thoroughly in 0.1 M phosphate buffer and

then all sections were incubated for 2 h in secondary antibody at a 1:200 dilution (Cy3 donkey anti-mouse IgG, ThermoFisher Scientific, Waltham, MA, USA) to assist in detection during imaging. Finally, the samples were washed in 0.1M phosphate buffer and allowed to dry before confocal microscopy.

Transverse vastus lateralis muscle sections were imaged using a 20x oil-immersion objective (NA 1.0) on an Olympus FV2000 laser confocal microscope capable of simultaneous multilabel florescence imaging. Images were captured in a 1200 x 1200-pixel array, with similar acquisition parameters across preparations. Type-identified fibers were paired with the sections used to determine SDH_{max} and capillary density.

Statistics.

The statistical analysis for this research was performed using Prism software (GraphPad Prism 8, CA, USA). All subject characteristics are reported as mean ± SD and all additional data are reported as mean ± SEM. A Two-way ANOVA and Sidak *post hoc* test were used to compare groups and factor. There were repeated measurements over time with subject, drug, and training effect analyzed. Significance was set at $P<0.05$ for all analyses.

RESULTS

Subject Characteristics

Ten (4 men, 6 women) healthy, non-smoking individuals participated in this study. The demographic and anthropometric characteristics of subjects obtained on their first study day included: age, height, weight, body mass index, Baecke sport index,

Physical activity index, and their 60% cycling power output (Table 6.1). Similar demographic and anthropometric information was obtained each study day and did not change throughout the course of the study with pre-study grouped values (age 24 ± 5 yrs, height 171.8 ± 9.6 cm, weight 74.3 ± 11.3 kg, body mass index 25 ± 3.5 m^2, mean ± SD).

Exercise Responses. The amount of oxygen consumed at peak exercise (VO_{2Peak}) throughout exercise training is shown in Figure 6.1. Pre-training VO_{2Peak} was not different between control (2.36 ± 0.32 L min^{-1}) and blockade (2.60 ± 0.18 L min^{-1}) conditions. There was a main effect of time (training) as VO_{2Peak} in both groups increased with training (P=0.001); however no difference was observed between groups (P=0.732) nor was there an interaction of condition (histamine receptor blockade) and time (training) (P=0.807). The control and blockade group increased VO_{2Peak} above pre-training values (P=0.01; P=0.019, respectively) (Figure 6.1).

Table 6.1. Subject Characteristics

	Control	Blockade
n	6	4
Age (yrs)	24 ± 6	24 ± 4
Height (cm)	173.9 ± 9.5	168.8 ± 10.2
Weight (kg)	71.6 ± 12.1	78.4 ± 9.9
Body Mass Index (kg m^{-2})	23.6 ± 2.0	27.7 ± 4.0
Baecke sport index (arbitrary units)	2.2 ± 0.5	2.8 ± 0.6
Physical activity index (MET hr^{-1} $week^{-1}$)	19.4 ± 9.3	29.7 ± 7.0
60% power output (W)	120 ± 33	128 ± 27

Values are mean ± SD, MET, metabolic equivalents.

The maximal amount of work (Watts) performed during peak exercise throughout exercise training is shown in Figure 6.2. Pre-training work was not different between control (208.0 ± 24.7 Watts) and blockade (232.5 ± 17.9 Watts) groups. There was a main effect of time (training) as maximal work performed increased in both groups ($P<0.001$), however no difference was observed between groups ($P>0.523$) nor was there an interaction of condition (histamine receptor blockade) and time (training) ($P>0.696$). Both control and blockade groups performed increased work during exercise after 6 weeks of training as compared to pre-training values ($P=0.0002$; $P=0.0018$, respectively).

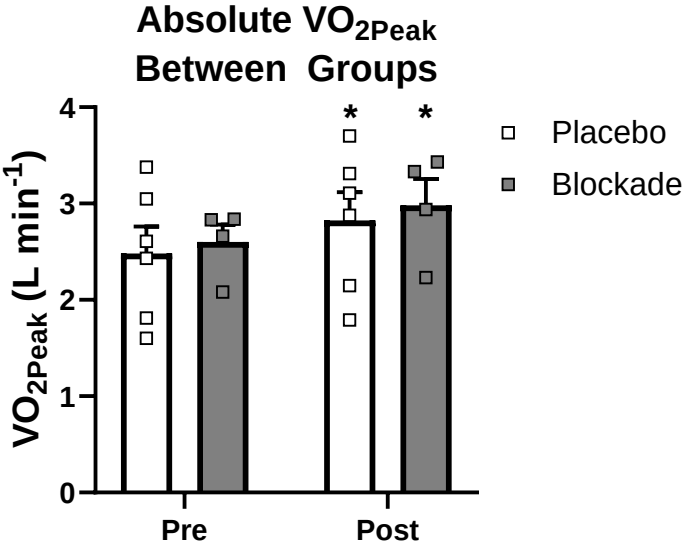


Figure 6.1. Absolute VO_{2Peak} attained pre vs post throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * $P < 0.05$ vs Pre across both groups (main effect of time). There was no main effect of condition nor was there a condition x time interaction.

Muscle Responses. Vastus lateralis muscle samples were assessed for changes the maximum velocity of the succinate dehydrogenase reaction (SDH_{max}). All measures were compared to pre-training values, separated by group, and assessed by muscle fiber type.

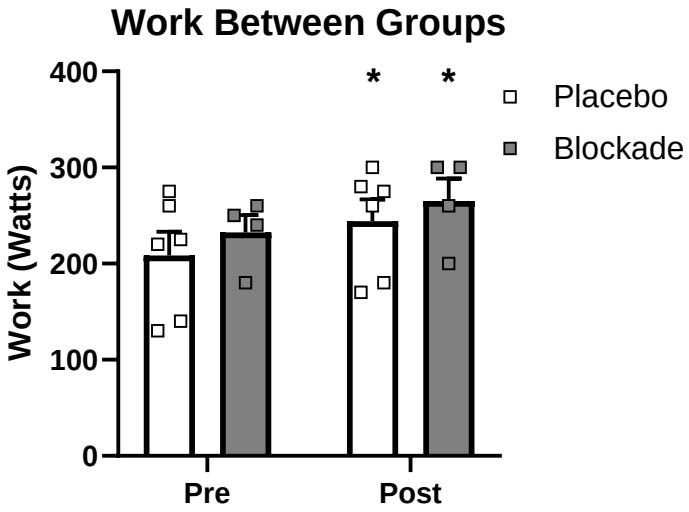


Figure 6.2. Maximal work attained pre vs post throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * $P < 0.05$ vs Pre (main effect of time). There was no effect of condition or condition x time interaction.

Changes in SDH activity for slow type I muscle fibers, as reflected by the maximal velocity of the succinate dehydrogenase reaction, are shown throughout exercise training in Figure 6.3. Slow type I muscle fiber maximal velocity of the succinate dehydrogenase reaction was significantly different from pre- (1.4 ± 0.1 mmol fumerate L^{-1} min^{-1}) to post-exercise training (3.4 ± 0.4 mmol fumerate L^{-1} min^{-1}) in the control group (P=0.0002), and from pre- (2.0 ± 0.5 mmol fumerate L^{-1} min^{-1}) to post-exercise training (2.5 ± 0.6 mmol fumerate L^{-1} min^{-1}) in the histamine blockade group (P=0.039). The post-training control group was not different from the histamine blockade group (P=0.707).

Changes in SDH activity for fast type IIa muscle fiber maximal velocity of the succinate dehydrogenase reaction was significantly different from pre- (1.2 ± 0.2 mmol fumerate L^{-1} min^{-1}) to post-exercise training (2.7 ± 0.3 mmol fumerate L^{-1} min^{-1}) in the control group (P<0.0001), and from pre- (1.6 ± 0.3 mmol fumerate L^{-1} min^{-1}) to post-

exercise training (2.6 ± 0.4 mmol fumerate L^{-1} min^{-1}) in the histamine blockade group (P=0.008). The post-training control group was not different than the histamine blockade group in fast type IIa muscle fibers (P=0.718) (Figure 6.4).

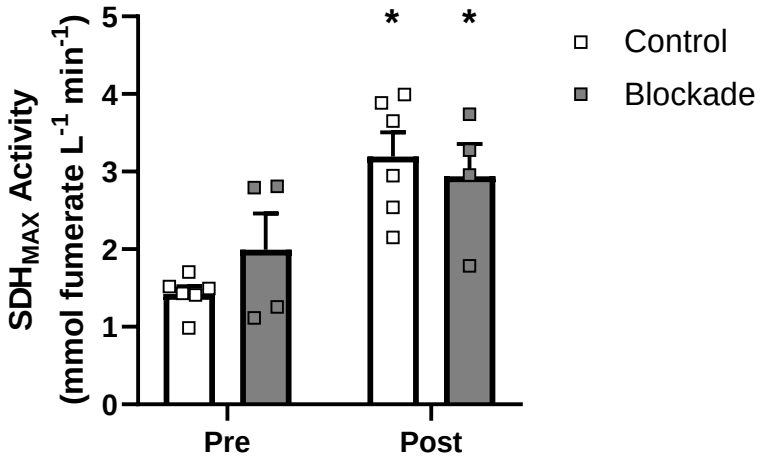


Figure 6.3. Slow type 1 SDH activity represented as maximal succinate dehydrogenase activity before and after exercise training throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * P < 0.05 vs Pre (main effect of time). There was no effect of condition nor was there a condition x time interaction.

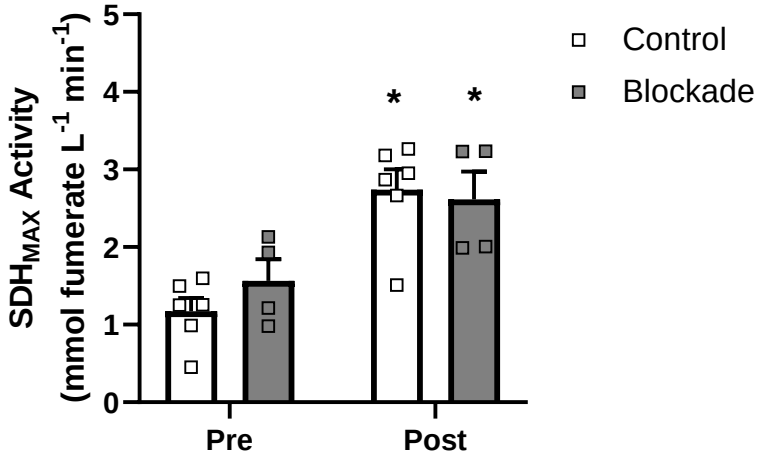


Figure 6.4. Fast type IIa SDH activity represented as maximal succinate dehydrogenase activity before and after exercise training throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * P < 0.05 vs Pre (main effect of time). There was no effect of condition nor was there a condition x time interaction.

Fast type IIx muscle fiber maximal velocity of the succinate dehydrogenase reaction was significantly different from pre- (0.8 ± 0.2 mmol fumerate L^{-1} min^{-1}) to post-exercise training (1.7 ± 0.3 mmol fumerate L^{-1} min^{-1}) in the control group (P=0.004), but not different from pre- (1.0 ± 0.2 mmol fumerate L^{-1} min^{-1}) to post-exercise training (1.4 ± 0.3 mmol fumerate L^{-1} min^{-1}) in the histamine blockade group (P=0.252). Additionally, the post-training control group was not different than the histamine blockade group in fast type IIx muscle fibers (P=0.956) (Figure 6.5).

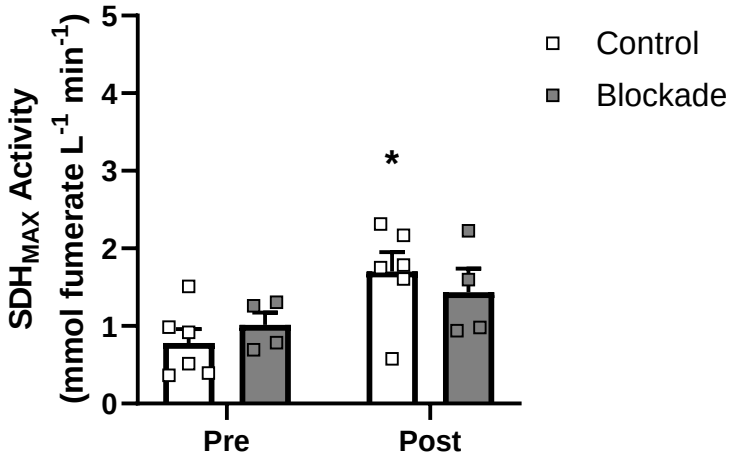


Figure 6.5. Fast type IIx SDH activity represented as maximal succinate dehydrogenase activity before and after exercise training throughout the training study. White bars = Control; Gray bars = Blockade. Values are means ± SEM. * P < 0.05 vs Pre (main effect of time). There was no effect of condition nor was there a condition x time interaction.

DISCUSSION

The purpose of this study was to determine the role of histamine receptor blockade on physiological adaptations induced by aerobic exercise training. We hypothesized that histamine receptor antagonists will blunt gains in aerobic capacity, by effecting the ability to utilize oxygen. The main finding of the present study was that combined blockade of histamine H_1 and H_2 receptors did not blunt adaptations in oxygen

utilization induced by 6-weeks of endurance exercise training as determined by either whole body VO_{2Peak} or direct measures of the capacity of a representative oxidative enzyme. These results suggest that chronically consuming antihistamines prior to exercise does not affect the adaptive ability to utilize additional oxygen within working skeletal muscle, an adaptation that promotes greater aerobic function during subsequent exercise bouts.

Exercise Capacity

Exercise training resulted in increased aerobic capacity as evident by an increase in VO_{2Peak} and work performed following 6-weeks of combined continuous and high intensity exercise training, with no differences in these adaptations caused by histamine receptor blockade. For the subjects who underwent biopsy measures, on average there was about 14% improvement in VO_{2Peak} following the 6-week exercise training protocol, which is in line with what other comparable exercise training studies have found (Bonne et al., 2014; Hautala et al., 2009; D. H J Thijssen et al., 2007; Vollaard et al., 2009). The control group demonstrated slightly more improvement in VO_{2Peak} than the blockade group (15% vs 14% respectively), however with the high variability of improved aerobic capacity normally observed, the difference in VO_{2Peak} improvement is negligible and we conclude that the improvement in whole body oxygen consumption was not altered in a meaningful way between groups. We note that the changes in this set of subjects is comparable to what was reported in the preceding chapters which were based on a larger set of subjects. Changes in the ability to utilize oxygen through increased maximum velocity of the succinate dehydrogenase reaction are consistent with the increased aerobic

capacity observed and likely a direct contributor. That said, our hypothesis that histamine receptor blockade would blunt the increase in oxygen utilization is not supported by these results.

SDH Activity

Mitochondrial content will increase up to 100% and remain at that elevated steady state following 6-weeks of exercise training depending on intensity and duration of exercise (Hoppeler, 1986). Moderate improvements in VO_{2Max} (around 5-20%) with 6-weeks of endurance exercise training can be explained by the large increases (50-100%) in mitochondrial content and ability to utilize oxygen. Interestingly, moderate improvements in VO_{2Max} fail to explain the large increases in exercise performance within the same time frame (Levine, 2008; Vollaard et al., 2009). The stimulus for mitochondrial biogenesis is muscle contraction; thus, mitochondrial biogenesis is fiber type specific (Hood, 2001). Muscle fibers that are not recruited during exercise training will display limited or no mitochondrial adaptations. With moderate intensity endurance training, mitochondrial biogenesis will be greatest in slow type I muscle fibers due to recruitment patterns during exercise. Moderate intensity endurance training will also drive modest changes in fast type IIa fibers, but limited changes in mitochondrial biogenesis of fast type IIx skeletal muscle fibers (Howald, 1985).

Mitochondrial density is not heterogeneous throughout skeletal muscle and is different across muscle fiber types, which are separated into slow (type I) and fast (type IIa and IIx). Muscle fiber type is dependent on contractile protein (myosin heavy chain isoform) expression, which determine the mechanical and energetic properties of muscle

fibers (Widrick, Romatowski, Karhanek, & Fitts, 1997). Requirements for motor unit recruitment for force production determine the activation patterns of different muscle fiber types. Slow motor units comprising type I muscle fibers are recruited first, followed by fast fatigue resistant motor units comprising type IIa fibers and finally more fatigable fast motor units comprising type IIx fibers (Bawa, 2002; Mantilla, Seven, Zhan, & Sieck, 2011; Seven, Mantilla, & Sieck, 2014). Slow type I and fast type IIa muscle fibers have the highest mitochondrial volume densities and oxidative capacity followed while fast type IIx muscle fibers have the lowest mitochondrial volume densities and oxidative capacities. Due to the high oxidative capacity of slow type I and fast type IIa muscle fibers, they also require the greatest amount of oxygen delivery and thus have the greatest number of surrounding capillaries (Anderson, 1975; Mantilla, Seven, & Sieck, 2014). Slow type I and fast type IIa muscle fibers are the first to be recruited during exercise and are the most fatigue resistant due to their relative size and high mitochondrial volume density; however they produce the least force per cross-sectional area among the different fiber types (Widrick et al., 1997).

During exercise, motor units are recruited depending on intensity and duration of work required. Therefore, metabolic pathways will also differ through different modalities, intensities, and durations of exercise. For example, exercise on a cycle ergometer at 60% VO_{2Max} for 50 min will recruit all slow type I and most fast type IIa muscle fibers, however fast type IIx fibers will not be recruited (Egan & Zierath, 2013). Due to the high proportion of slow type I and fast type IIa muscle fiber types involved with this intensity and duration of exercise; ATP will be produced primarily through oxidative metabolism. During high intensity exercise at 90% VO_{2Max} for 3 min, it is likely

that recruitment of all muscle fiber types will be required to achieve the desired force production. Due to all muscle fiber types being recruited and the short duration of high intensity exercise, energy production will be a mix of aerobic and anaerobic metabolism to achieve adequate ATP production for force generation (Egan & Zierath, 2013). Additionally, cycling exercise is predominantly completed by knee extensor muscles, specifically the vastus medialis and vastus lateralis, with approximately 80% of total muscle activation, measured by electromyogram, coming from the two muscles equally (Duggan, Donne, & Fleming, 2017). Therefore, increases in whole-body oxygen consumption during cycling exercise will primarily come from oxidative phosphorylation of the slow type I and fast type IIa muscle fibers found within the vastus medialis and vastus lateralis muscles.

SDH activity increased in response to 6-weeks of combined continuous and high intensity exercise training and was quantified by an increase in the maximum velocity of the succinate dehydrogenase reaction. SDH activity increased across all three muscle fiber types and showed the largest increases in slow type I fibers with less substantial increases in fast type IIa muscle fibers and the smallest increase in fast type IIx muscle fibers. The maximum velocity of the succinate dehydrogenase reaction increased by about 35% in slow type I muscle fibers, which is similar in scale to what others have reported with similar duration exercise training (Hoppeler, Howald, & Weibel, 1985). These results agree with what was expected as slow type I and fast type IIa muscle fibers were likely to be recruited most frequently throughout exercise training. Histamine receptor blockade did not blunt the gains in SDH activity.

Methodological Considerations

Increases in mitochondrial biogenesis can be assessed using multiple methods including direct assessment of organelle volume in relation to total cellular volume (mitochondrial volume density), total count of mitochondrial DNA copy number, or expression of inducible coactivators like peroxisome proliferator-activated receptor gamma coactivator 1-alpha that regulate the expression of mitochondrial proteins (Hood, 2001). Additionally, mitochondrial content can be assessed by the maximal velocity of reactions of marker enzymes like citrate synthase or succinate dehydrogenase under supraphysiological substrate concentration conditions in vitro (Henriksson & Reitman, 1977; Proctor et al., 1995). Changes in mitochondrial volumes estimated morphometrically are highly correlated with changes in enzyme maximum velocity values, which allows functional measurements of potential oxygen utilization (Reichmann et al., 1985).

It is well established that the mitochondrial enzyme citrate synthase increases with aerobic exercise training (Tonkonogi, Harris, & Sahlin, 1997). Citrate synthase is the initial enzyme of the citric acid cycle and a marker of mitochondrial matrix 1. Citrate synthase is qualitatively assessed through colorimetric assay in which the intensity of light absorbance is proportional to increased citrate synthase activity levels. The enzyme succinate dehydrogenase is unique in that it is the only enzyme that participates in both the citric acid cycle and the electron transport chain. In the citric acid cycle, succinate dehydrogenase catalyzes the oxidation of succinate to fumarate, a necessary step as the cycle will not continue without the presence of fumarate. Additionally, following succinate oxidation, the enzyme transfers electrons directly to the quinone pool, which

makes up complex II of the electron transport chain and serves as a vital connection between the two aerobic energy systems (Cecchini, 2003). The critical role of succinate dehydrogenase in both the citric acid cycle and electron transport chain makes the maximum velocity of the succinate dehydrogenase reaction a great marker of oxidative capacity (Fogarty et al., 2020). Additionally, the maximum velocity of the succinate dehydrogenase reaction has been validated in skeletal muscle and gives a quantitative assessment of mitochondrial function (Blanco et al., 1988). A limitation of the maximum velocity of the succinate dehydrogenase reaction is that it does not indicate if existing mitochondria have improved function or additional mitochondrial biogenesis has occurred, which would also exhibit increased SDH activity. These issues can be answered by quantifying mitochondrial DNA copy number, which was completed in muscle samples obtained for this study. However, this data was not included as fiber type proportion could not be quantified, which is necessary to determine if mitochondrial volume, mitochondrial function, or both contributed to changes in mitochondrial DNA copy number. Therefore, we know the maximum velocity of the succinate dehydrogenase reaction was increased with exercise training, but don't know if that increase came from increased function of previously existing mitochondria, or newly formed mitochondria.

Conclusion

Exercise training resulted in increased aerobic capacity evident by consuming more oxygen and preforming more work following 6-weeks of combined continuous and high intensity exercise training. SDH activity increased with exercise training and is known to be a primary contributor to the observed aerobic capacity enhancements when

inactive individuals first begin exercise training. Histamine receptor blockade did not blunt improvements in SDH activity associated with aerobic exercise training nor did the negatively impact on whole body measures of aerobic capacity.

CHAPTER VII

CONCLUSIONS AND FUTURE DIRECTIONS

Main Findings

It has been well established by the previous work done in this lab that histamine receptor activation is intricately involved with the response to exercise. We know that histamine concentrations increase within skeletal muscle during moderate intensity aerobic exercise. Histamine is released locally from working skeletal muscle by mast cell degranulation and *de novo* formation via the enzyme histidine decarboxylase. Local skeletal muscle histamine receptor activation plays a large role in the sustained post-exercise vasodilation as well as systemic responses following exercise such as post-exercise hypotension. Histamine receptor activation appears to either drive or modulate the differential regulation of hundreds of protein coding genes in skeletal muscle that are linked to endothelial and vascular function, metabolism, angiogenesis, cell maintenance, and inflammation. Due to the large effect that histamine receptor activation has on post-exercise responses to acute exercise, it presented a unique opportunity to explore how histamine receptor activation could have the potential to modulate physiological adaptions to repeated exercise stress.

The overall purpose of this dissertation was to determine the role of histamine receptor activation in adaptations to aerobic exercise training. We hypothesized that histamine receptor antagonists will blunt positive physiologic adaptation, such as gains in aerobic capacity, affecting the ability to deliver and utilize oxygen during subsequent exercise. Exercise training resulted in increased aerobic capacity evident by both a greater ability to consume oxygen and to preform work at a higher rate following 6-

weeks of combined continuous and high intensity exercise training, as highlighted in Chapter IV. Chapter IV also presented the response to exercise training for measurements of vascular health that are commonly improved with habitual exercise. Vascular health measurements such as arterial compliance, pulse wave velocity, and resting hemodynamics were unchanged following exercise training. The lack of effectiveness of exercise to change these measures of vascular health could indicate the subject population was not starting from a deficit, or not sedentary enough, to be able to observe changes in these variables. Subjects were only included if they were deemed healthy, and they were generally young, which leaves little room for improvement in these vascular health measures. Another factor that likely played a role in in unchanged measures of vascular health and resting hemodynamics was the moderate intensity continuous exercise modality used in this study and the relatively short duration of the intervention. Adaptations to exercise training are largely duration and intensity dependent, so increased exercise intensity would have been more likely to demonstrate changes in these measurements. Further, it is well-known that some of the adaptations to aerobic exercise, such as cardiac restructuring, take longer to develop than the time frame of our exercise intervention. In contrast, femoral arterial lumen diameter increased with exercise training, indicating that some conduit vascular adaptation had occurred in response to the level of stimuli provided by this exercise program. Thus, while improved aerobic capacity following exercise training was not accompanied by changes in vascular health measurements, it was associated with more directly related vascular adaptations that promote greater blood flow to active muscle. However, these adaptations did not appear to be dependent on activation of histamine receptors during exercise, as blockade of these

receptors did not influence pattern of vascular and functional changes to exercise training.

Chapter V investigated mechanisms of oxygen delivery and how they played a role in the increased aerobic capacity observed following exercise training. Blood volume and red blood cell volume did not change throughout exercise training. Blood volume expansion has been reported as a primary driver involved with increases in aerobic capacity associated with exercise training, and blood volume expansion involves both an increase in plasma volume and an increase in red blood cell mass through erythropoiesis. With these expectations in place, it was unexpected that we did not observe a change in blood volume, although there were some transient and inconsistent changes in hemoglobin and hematocrit observed at intermediate timepoints during the project. It is possible that a longer duration exercise training program or one involving higher intensity exercise would be more likely to demonstrate changes in blood volume. It is unfortunate that the stimuli for a change in blood volume was insufficient, as histamine receptor activation has direct links to red blood cell production and an effect in response to exercise training could have become apparent under conditions in which blood volume expansion had occurred. In contrast to the lackluster blood volume response, angiogenesis as measured by the number of capillary contacts per muscle fiber and capillary density (the number of capillaries relative to fiber cross sectional area) increased with exercise training in slow type I and fast type IIa muscle fiber types. This adaptation to exercise training was also evident in the peak vasodilatory capacity as assessed by reactive hyperemia in the femoral artery. However, despite previous work in this lab which has shown that histamine receptor activation increases pro-angiogenic factors

following acute exercise, histamine receptor blockade did not blunt the angiogenic gains associated with exercise training. The result of the current study demonstrate that histamine receptor blockade does not blunt pro-angiogenic factors enough to cause differences in physiologic adaptation, or that other redundant signals are available which can drive angiogenesis in the absence of a histaminergic signal. These combined results give evidence that the physiological adaptations that support greater oxygen delivery after exercise training are not dependent on histamine receptor activation during or immediately after exercise to be fully expressed. Either the activation of histamine receptors does not contribute to these adaptations, or the systems for adaptation are resilient and other mechanisms are equally capable of driving adaptations such as angiogenesis.

Chapter VI investigated mechanisms of oxygen utilization and how they play a role in the increased aerobic capacity observed following exercise training. Muscle fiber aerobic capacity as measured by the maximum velocity of the succinate dehydrogenase reaction increased with exercise training in all fiber types. The changes in SDH activity were type dependent with greater adaptation occurring in slow type I and fast type IIa muscle fibers, which were recruited at greater proportions during most of the exercise completed. When interpreted in conjunction with the improvements in whole-body peak oxygen consumption, they support the dogma that early adaptations to exercise training that improve aerobic fitness are largely peripheral adaptation within the active skeletal muscle that support higher energy production via oxidative energy systems. Despite prior evidence from our lab that histamine receptor activation modulates adaptation in these energy systems following a single bout of exercise, results from this project were not able

to show any evidence that these adaptations within skeletal muscle are affected by histamine receptor blockade. Thus, the many measures in this dissertation project suggest that histamine receptor activation is not necessary to drive an increase in oxidative capacity. Either the activation of histamine receptors does not contribute to these adaptations, or the systems for adaptation are resilient and other mechanisms are equally capable of driving adaptations such as mitogenesis.

To summarize, the results of my thesis indicate that the ability to deliver oxygen through increased blood volume via hematopoiesis did not contribute to increased aerobic capacity and was not affected by histamine receptor blockade. However, the ability to deliver oxygen through increased angiogenesis (assessed via capillary density and corroborated by peak vasodilatory capacity) and utilize oxygen through increased SDH_{Max} and corroborated by VO_{2peak} contribute to increased exercise capacity but that these adaptations occur either independent of the effects of histamine receptor activation or that adaptation is generated by redundant mechanisms in a highly resilient manner.

Significance

Activation of the histamine H_1 and H_2 receptor pathway appears responsible for much of the robust post-exercise hemodynamic response and either drives or modulates many changes in gene expression that occur following acute aerobic exercise. However, many of these acute effects linked to histamine do not appear to translate into the longer-term adaptations to chronic exercise. The research objectives outlined in this dissertation represent an advance in the basic scientific and mechanistic literature of adaptation to aerobic exercise training. The results of this thesis research reject the hypothesis that

activation of the histamine H_1 and H_2 receptor pathway is necessary for the full expression of the adaptations to aerobic exercise in young sedentary individuals. The mechanisms by which repeated exercise stress are translated into beneficial physiological adaptation are yet to be fully explained, although molecular transducers are being cataloged by many research teams. Activation of the histamine H_1 and H_2 receptor pathway during exercise is intricately involved with acute exercise responses, however the robust mechanisms responsible for physiological adaptation to exercise training are not clearly affected due to redundant systems in place to achieve hormesis. Thus, a fundamental question remains: why and to what affect is histamine released from skeletal muscle in response to exercise?

Future Directions

The Halliwill Lab is known for studying cardiovascular regulation in response to exercise and how histamine receptor activation plays a vital role in driving these physiological consequences of aerobic exercise stress. Histamine is produced and released from skeletal muscle during exercise and plays a vital role in multiple physiological pathways associated with acute aerobic exercise responses. The current study provides new information about the interaction between histamine receptor activation and exercise training, but raises more questions than it answers, and results in what can be considered a “histamine paradox”. Why do acute exercise studies show such clear evidence for a role of histamine in adaptation to exercise when a chronic exercise training study shows no evidence of a role. How does histamine blockade paradoxically leave a broad footprint on the exercise transcriptome, yet no imprint on training adaptions

that are inherently dependent on transcriptional changes for key outcomes such as the ability to deliver and utilize oxygen through increases in angiogenesis and SDH activity?

Based on previous research from the Halliwill Lab and the current results, this line of research would benefit from an exercise training program of greater intensity and duration. Increasing the intensity and duration of exercise training would widen any potential differences that could be observed in capillary density and/or SDH activity. The current study utilized exercise intensity and duration that had been used in previous research in the Halliwill Lab. This intensity and duration of exercise was designed, in part, to be achievable by young healthy sedentary individuals, which it was successful in doing as exercise adherence was close to 100%. Using that knowledge to move forward, exercise intensity should be increased by 15-20% of VO_{2Peak} with additional exercise days included each week. One possibility would be to move the high intensity interval training sessions between the continuous exercise training sessions with fewer recovery days resulting in increased exercise stress. Additionally, the subject population of young healthy sedentary individuals would be improved by utilizing a clinical population that is starting at more of an aerobic deficit. Subject populations such as aged individuals, peripheral artery disease, or spinal cord injury (with different exercise modalities) would be more likely to demonstrate differences between groups due to their aerobic deficit and increased ability to improve or may have fewer or less robust redundancies to drive adaptation to chronic exercise.

Additional research is also needed to discern whether observed increases in SDH activity are due to improvements of existing mitochondrial oxidative capacity or due to mitochondrial biogenesis. This could be accomplished with the same skeletal muscle

biopsy technique and the addition of mitochondrial DNA copy number measurements extrapolated by fiber type proportions in the biopsy samples. Skeletal muscle fiber type shifts associated with exercise training would also illuminate the issue of mitochondrial volume vs function.

Future exercise training research should focus on the interaction between improvements in capillary density and SDH activity and if they adapt at the same rate or if one variable drives the other. This question would be difficult to answer in human research model and would benefit from animal or tissue work where the stimulus for either variable could be isolated. For example, an animal model could be used to induce muscle contraction (a stimulus for mitochondrial biogenesis) while clamping blood flow (a stimulus of angiogenesis) through the muscle.

APPENDIX A

INFORMED CONSENT DOCUMENT

INFORMED CONSENT FORM

TITLE: Histamine and Cardiovascular Adaptation to Endurance Exercise

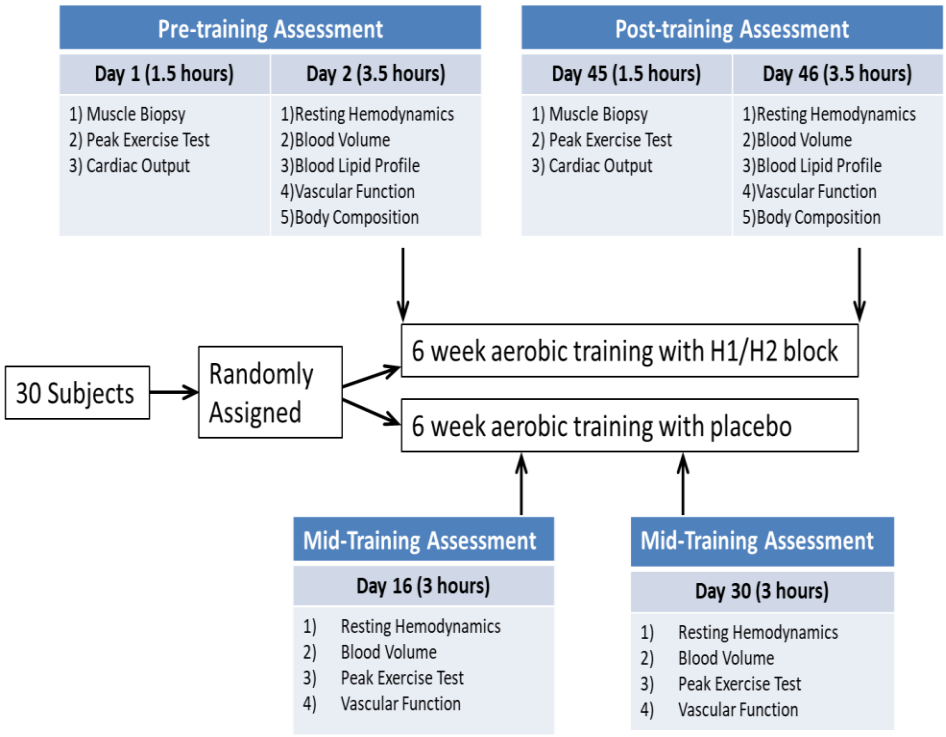
INVESTIGATOR: Dr. J. R. Halliwill and Colleagues

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign the form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this research study being done? When humans exercise, histamine is released by exercising muscle and has the potential to either trigger or modulate the recovery response to exercise. The long-term goal of this research protocol is to better understand the link between chronic aerobic exercise, histamine, and adaptation in the context of exercise training and physical recovery from exercise. This topic is both clinically and scientifically important. You have been asked to participate in this study because you are a healthy individual who does not participate in routine aerobic or cardiovascular exercise training.

What will happen in the study?

This schematic summarizes the research measurements that are made throughout this experiment. This experiment will span 46 consecutive days, which will include all experimental testing and exercise sessions. To participate in this research, you will need to be able to travel to the research area, located on the University of Oregon campus, multiple days of the week, including weekends. The exercise training is designed to be difficult and will be challenging to complete. You are expected to consistently participate and communicate with the researchers throughout the experimental period.



Initial Visit

Prescreen. You will arrive at Dr. Halliwill's laboratory in Esslinger Hall at the University of Oregon for an initial visit. This initial visit will take approximately 1 hour. You will meet with one of the investigators of the study to discuss the project, to see the laboratory, and to read this form. Your height and weight and resting blood pressure will be measured, and you will be asked questions about your health history. You will be given a physical activity questionnaire, which will allow investigators to determine your activity level.

Exercise Training Sessions (~21 hours over 6 weeks)

Aerobic exercise. The exercise training protocol will span 6 weeks. You will conduct 21 exercise sessions on a stationary bicycle with a rest day between every exercise session. All exercise will be completed in the Exercise and Environmental Physiology Lab in Esslinger Hall at the University of Oregon. You are advised to wear a t-shirt with shorts and tennis shoes for all exercise sessions. One hour prior to arrival, you will consume medication we have previously provided.

A member of the research team will be present during all exercise sessions. Upon arrival, a research assistant will instrument you with a heart rate monitor that straps around your chest. Subjects will not be provided with food or beverages (other than water) during the exercise sessions. Upon completion of each exercise session, subjects will be given a snack (Granola bar) and fluids (18oz Gatorade) to eat and drink.

There will be two different types (moderate/hard) of stationary bicycle exercise completed during this study. The majority of exercise (16 out of 21 sessions) will consist of moderate intensity exercise, which will be difficult, but at a level where you could keep

up a conversation while exercising. You will also complete hard intensity exercise, (5 out of 21 sessions) which will be very difficult and at a level where you could not keep up a conversation while exercising. Hard intensity exercise will have a short amount of easy exercise before and after, and the hard exercise will last 3 minutes at a time.

If you are a woman who can still become pregnant, you will be asked to undergo a pregnancy test during each exercise session. You will be asked to provide a sample of urine in the women's restroom near the physiology lab. If the test is "positive," indicating that you are pregnant, you will not be allowed to participate and will be advised to see your physician or the University of Oregon Health Center.

Antihistamines. A custom pillbox organizer containing 2 weeks of either an antihistamine or placebo will be provided. You will consume the pills provided one hour before each exercise session. Once you have consumed the pills you will contact (text/e-mail) research personnel with a picture of the labeled pillbox organizer. Your exercise session should start one hour after this information has been sent. The antihistamines are Allegra and Zantac, common over-the-counter medications. The placebo tablets contain the inactive found in Allegra and Zantac, but none of the active ingredients. You will not know which pills you are taking throughout the study.

Pre and post-exercise testing.

Measurements made before (Pre-) and after (Post-) the exercise training program will take two consecutive days to complete. You will report to the Exercise and Environmental Physiology Lab in Esslinger Hall at the University of Oregon. Day 1 will include a skeletal muscle biopsy, a maximal exercise test (VO_{2peak}) and measures of cardiac output. Day 2 will include resting hemodynamic testing, blood profiling and volume measurements, vascular function tests, and body composition analysis. All measurements included are detailed below.

Pre and post-exercise training study day 1 (~3 hours).

1. Muscle biopsies. You will arrive at Dr. Dreyer's laboratory in the Center for Medical Education and Research at the University of Oregon to participate in the experimental protocol. The testing will take approximately 1-2 hours. A total of 2 biopsies will be taken from your right leg throughout the whole experiment (one before and one after all your training sessions are completed). This biopsy will be performed through a single incision and involves taking a small piece of muscle from your leg. First, a small patch of hair is removed (if needed) from your thigh and the skin is cleaned and sterilized. Next, the skin and tissue below where the biopsy will be obtained will be numbed with a local anesthetic (lidocaine). After your leg is numb we will make a small incision about the size of this dash "____" at approximately mid-thigh. Through this incision a needle about the size of the letter "O" will be advanced into the muscle. A single piece of thigh muscle will then be removed with the needle, the skin closed with a single stitch and light dressing will be applied. This will be done once in one leg before exercise training, and once in the same leg after you have completed the exercise training.
2. Peak exercise testing. You will cycle on a stationary cycle ergometer while wearing a mouth piece, nose clip, and electrocardiogram electrodes (heart rhythm monitor). After 5 minutes of spinning at a comfortable speed, a constant

speed will then be used, while the resistance of the ergometer is increased until you reach exhaustion. This is to measure your overall aerobic fitness level. It normally takes 10 to 15 minutes for people to reach their maximum effort. This test will establish your maximal exercise tolerance and will be used to select an appropriate workload for exercise during the study visits.

3. Cardiac Output. Cardiac output will be estimated before and during the peak exercise test using an open-circuit acetylene uptake method. You will breathe a gas mixture containing 0.6% acetylene, 9.0% helium, 20.9% oxygen, and balance nitrogen for 8 - 10 breaths. The acetylene and helium are physiologically inert and used for routine pulmonary function testing.

Pre and post-exercise training day2 (~7 hours).

1. Resting Heart rate and blood pressure. We will connect you to a 3-lead electrocardiogram that involves placing pads on your skin on both of your chest and stomach. Blood pressure will be measured using an inflation cuff on the right upper arm.

2. Blood volume assessment. We will estimate your total blood volume using a technique where you breathe in a small amount of the gas, carbon monoxide (CO), and measure how much CO makes it into your blood. In the CO uptake test, first, we will place a small flexible needle (these are called “intravenous catheters”, and are smaller than the lead of a pencil) into a vein near your elbow. The skin will be cleaned thoroughly before this procedure with alcohol swabs. The catheter placement will be performed by a member of the lab highly-trained in this technique. After the intravenous catheter has been placed, we will ask you to sit quietly for 30 minutes before the procedure begins. Throughout the test, we will take two small blood samples of ~5mL each (about 1 teaspoon), for a total of ~10mL on each day (~20mL total for the entire study), to measure the amount of the gas, CO, in your blood. The vials in which we collect the blood will be coded such that the investigators can determine all samples came from the same subject and the time the sample was taken. No one will be able to determine your identity from the sample.

   During the blood volume assessment, you will breathe on a closed rebreathing system for several minutes. This means you will inhale and exhale into a bag. Your nose will be closed with a nose clip. The air in this rebreathing system is pre-filled with 100% oxygen. We will inject a dose of the gas CO into the breathing system and you will inhale deeply to make sure you inhale as much CO as possible. CO is a gas that quickly moves from the air in your lungs to your blood. To further help this you will hold your breath for ~10 seconds after you deeply inhale. You will then continue to rebreathe on the system for an additional minute and 50 seconds (2 minutes total). At the end of the rebreathing period, you will exhale all of the air out that you possibly can into the bag. When you cannot get any more air out, you will come off of the rebreathing system and breathe normal room air. We will measure the amount of CO present in your lungs by breathing into a small tube connected to a device that can measure CO, and take a small blood sample to measure the amount of CO in your blood before and after you breathe on the rebreathing system. After the last blood sample, we will remove the catheter from your arm and place a sterile bandage over the site.

It is important for you to realize that you may stop when you wish because of feelings of nausea, light- headedness or discomfort.

3. Blood sampling for lipid, glucose, and lactate profiling. We will collect a venous blood sample of less than 10 ml for subsequent testing for lipid, glucose, and lactate profiling. This blood sample will be collected before mearurments of blood volume are conducted.

4. Arterial compliance. A small probe (ultrasound Doppler probe) will be held over an area of skin over multiple areas of your body including your groin, neck, and arm. We will use this probe over your groin-hip intersection to image your femoral artery. We will use this probe over your neck to image your carotid artery. We will use this probe over your arm to image your brachial artery. The ultrasound Doppler probe uses ultrasound waves to measure blood flow in these arteries. A pencil-type probe that measures changes in pressure will be used over the same areas. All measurements will take approximately 2 min per site, and you will be asked to be calm and quiet during these measurements. This is a non-invasive procedure and should not be uncomfortable, other than some minor pressure at the measurement sites.

5. Flow mediated dilation. A small probe (ultrasound Doppler probe) will be held over an area of skin over multiple areas of your body including your groin and arm. We will use this probe over your groin-hip intersection to image your femoral artery and over your arm to image your brachial artery. We will use the ultrasound probe to make a 2 minute measurement at baseline and a 3 minute measurements following a 5 minute occlusion caused by the inflation of a cuff around your lower arm or leg.

6. Body composition alaysis. Measurements will be made using calipers to measure skinfold thickness at various sites on the body. The sum of the skinfold thickness can be used with height and weight measurements to calculate percent body fat. We will measure skinfold thickness at 7 sites around your body including your chest, arm, stomach, back, and leg.

Mid-Training Assessments (~6 hours).

The two mid-training assessment days will be identical and a number of measurements that have already been detailed above will be repeated. These tests include measuring resting heart rate and blood pressure, blood volume, peak exercise test, arterial compliance, and flow mediated dilation. All measurements will be the same as outlined above except for the blood volume measurement, which will switch methodology to use a small blood draw to estimate blood volume and takes much less time.

Food intake log and other restrictions. Subjects will be asked to refrain from consuming caffeine or medications for 12 hours and abstain from exercise or alcohol for 24 hours prior to all data collection visits. Subjects will be asked to refrain from food consumption for 2 hours before each exercise visit. Subjects will be asked to complete a Food Intake Log of their food consumption 24 hours prior and during experiment data collection visits. Subjects will be given a copy of their food logs and will be asked to match their food intake to the best of their ability, as indicated in the log, on the subsequent data collection visits.

Post-biopsy care

After the biopsy procedures, you will go over things that you must do to help minimize the risk of infection. We will also provide a hardcopy of these instructions for you to take home. The handout is titled “Things to Remember After Muscle Biopsy,” and must be taken seriously. We will also periodically contact you to see how the biopsy site is doing.

We ask that the day after each biopsy, if you are not scheduled for a Follow-Up Visit, you email a close-up picture of each biopsy site and send it to us for us to ensure everything looks healthy. If anything appears to need further inspection, you will be asked to come in to the lab for a closer look. We ask that you return to the lab 7 days after the experiment to have your sutures removed and the biopsy site inspected. This will take about 20 minutes.

If you are unable to return for the 7-day post biopsy visit, you must have a qualified medical professional familiar with suture removal take out the two sutures. You will be asked to send the researchers images of each site before and after the sutures are removed, so that the researchers can confirm the sites are healthy and healing as expected. If after viewing the images, the researchers decide that they require further inspection and/or medical treatment, you will be advised to see your doctor as soon as possible or visit urgent care or the emergency room. You will be asked to follow up with us and let us know the outcome of the doctor’s visit.

Should you opt for the alternative path, any costs incurred by the medical professional are the responsibility of the subject as well as any costs incurred as a result of further needed medical attention for the site. If after viewing the images, if the researchers decide that they require further inspection and/or medical treatment, the subject will be advised to see their doctor as soon as possible or visit urgent care or the emergency room. You will be asked to follow up with the researchers and let us know the outcome of the doctor’s visit.

If you would prefer to have a qualified medical professional outside of the research team perform the suture removal, please initial here: _____ date:_____

How long will I be in the study?

You will be in the study for a total of 46 days consisting of 6 data collection days and 21 exercise sessions. Your total involvement would be about 36 hours.

What are the risks of the study?

Muscle biopsies. The muscle biopsy carries the potential risk of pain, bleeding, bruising, infection, and scar formation at the site of the biopsy. Careful sterile technique should reduce the likelihood of any of these complications. The risk of bleeding is about 0.2%; the risk of bruising or a blue-and-black mark is 1.4%; and infection is so small that the precise number is unknown. After the study, you have a 50% chance of experiencing soreness at the site of biopsy for 24 to 48 hours. Additionally, you may experience numbness around the area of the biopsy site (2x2 inches), which will likely go away with time (sensation returns) but in very rare instances may never return. The risk that you experience numbness is less than 0.5% and the risk that the numbness never goes away is much less. The scar will be approximately as long as the dash “____”.

Lidocaine. The risk associated with lidocaine is similar in character to those observed with other local anesthetics. Some of the more common adverse reactions include

nervousness, dizziness, blurred vision, tremor, drowsiness, tinnitus, numbness, disorientation, hypotension, nausea and vomiting.

Exercise testing. There is some minor discomfort associated with exercise testing and training, including temporary fatigue, shortness of breath, and muscle soreness. These sensations resolve within minutes after the testing or training sessions are completed. There is a high possibility of residual muscle soreness in the few days following the exercise test and the higher intensity training sessions. This muscle soreness will be greater at the beginning of the experimental period because you will have completed exercise that you are unaccustomed to performing. Muscle soreness will usually begin around 1 day after you finish an exercise bout, and last for 1-2 days. Heart rate and blood pressure will be constantly monitored during exercise. The exercise session will be terminated if you feel light-headed, nauseated, or experience any other adverse signs or symptoms. There is also the risk of a heart attack or death during an exercise test. The risk of a complication requiring hospitalization is about 1 incident in 1000. The risk of a heart attack during or immediately after an exercise test is less than 1 incident in 2500. The risk of death during or immediately after an exercise test is less than 1 incident in 10,000. In the unlikely case of a life-threatening heart rhythm, the laboratory is equipped with an Automatic Electronic Defibrillator that is in the same room where the study is taking place. In the event of an emergency, 911 will be called and we will direct an ambulance to the correct location.

Blood Volume Assessment. Healthy nonsmoking city dwellers typically have CO level of 1.5-2.0% (the percentage of hemoglobin in the blood that is bound by CO).. This test will increase this level by roughly 6%. Although you do not want to increase your level of CO, the levels used for this test are low and present minimal risks. The side effects associated with excessive CO typically occur at levels above 8% and may include headache, fatigue, shortness of breath, nausea, cherry-red colored lips, dizziness, and death, the last of which rarely occurs below 15% CO in normal individuals. This method has been in use in research for measuring blood volume for over 100 years without notable complications. Over time the CO bound to hemoglobin in your blood will be removed. This process will occur naturally over the several hours following the test. For every 5 to 6 hours that passes after the test, half of the CO will leave your blood. Thus, after 15-18 hours you would have less than 1% CO in your blood.

If, for any reason, your post-administration CO rises above 10% or you develop side-effects associated with excess CO, we will initiate O_2 therapy to accelerate CO clearance from your system. O2 will be given using a nasal administration similar to what someone may wear while in the hospital. Administering O_2 reduces the half-life of CO (speeds up the process of getting rid of it) from ~5 hours to ~80 minutes. We will measure CO throughout the O_2 administration and periodically afterwards. We will stop treatment if CO is < 8% or the symptoms go away. If symptoms progress or do not improve, 911 will be called and we will direct an ambulance to the correct location. In the unlikely case of an adverse cardiovascular event, the laboratory is equipped with an Automatic Electronic Defibrillator.

Venous Blood sampling. In total, about 80 ml of blood will be withdrawn over the course of the study, which is less than that associated with standard blood donation programs, where 450-500ml of blood (half a quart) is routinely withdrawn. You should not donate blood or volunteer for another research study where blood will be drawn for 8 weeks

following completion of the study. The 8-week period is recommended to allow your body to reproduce the blood that was taken during the study days.

Oral fexofenadine and ranitidine. There are minimal risks associated with the oral administration of fexofenadine (brand name Allegra) or ranitidine (brand name Zantac), which include nausea, diarrhea, and headache. You will be asked to consume 540 mg of fexofenadine and 300 mg of ranitidine, these amounts are within the upper limits of single dosage recommended by the manufactures. Fexofenadine in one time dosages up to 800 mg and 28 days of 690 mg every 12 hours have been given without adverse effects. Similarly, ranitidine has been given in dosages of 400 mg for 28 days without any major side effects. The 540 mg dose of fexofenadine hydrochloride is greater than the 180 mg over-the-counter recommended. This amount of fexofenadine is still safe for humans as single doses up to 1980 mg and 690 mg two times per day for a month showed no ill outcomes. The 300 mg dose of ranitidine hydrochloride is in-line with the manufacturers recommended dose. These medications are some of the most widely used over the counter (OTC) medications and are effective for treatment of seasonal allergies and excessive stomach acid secretion. There are no know risks associated with the placebo tablets, which were manufactured by a compounding pharmacy (Creative Compounds, Wilsonville, OR) and contain the inert ingredients found in the Allegra and Zantac antihistamine tablets without the active ingredients. You will not know which pills you are taking throughout the study.

Occlusion by blood pressure cuff. The inflation of the blood pressure cuff to stop blood flow may cause a slight tingling sensation and may cause slight bruising. The sensations with prolonged blood flow occlusion greater than 10 minutes are similar to those when a limb has “fallen asleep.” During certain surgical procedures, blood flow is often stopped for 2 hours without any significant risk to the patient. If, at any time, you experience any discomfort you may request that the blood pressure cuff be either loosened or removed.

Tracking of Taxable Income. Please note, compensation from participation in Human Subjects Research studies is taxable income. If your compensation totals $600 or more in a calendar year, the University is required to report the income to the IRS. The University requires its departments to track participant compensation and may contact you to complete a Form W-9 for tax reporting purposes. Because of the federal and University tracking requirements, your name will be associated with participation in research. Department and University administrators will have access to this information, but will not have access to research data.

The study may include risks that are unknown at this time.

Are there benefits to taking part in this study?

This study will not make your health better. Measurements are not being conducted for diagnostic purposes. The results will not be reviewed by a physician. The purpose of this study is to provide more information on how humans respond to exercise. Our hope is that by better understanding the physiology of how the human body responds to exercise, we will be better able to use exercise in the prevention and treatment of diseases such as hypertension (high blood pressure) and other forms of cardiovascular disease.

What other choices do I have if I don't take part in this study?

This study is only being done to gather information. You may choose not to take part in this study.

What are the costs of tests and procedures?

You will not pay for any tests or procedures that are done just for this research study. You will get up to $200 for participating in this study, including $170 for completing all the research testing and $30 for completing at least 90% of the aerobic exercise training sessions. This money is for the inconvenience and time you spent in this study. If you start the study but stop before the study has ended, the amount of money you receive will be pro-rated at a rate of $10 per research testing hour that you complete.

Who is funding this research study?

This study is being funded by The Eugene & Clarissa Evonuk Memorial Graduate Fellowship in Environmental or Stress Physiology

Who can answer my questions?

You may talk to Dr. John Halliwill at any time about any question you have on this study. You may contact Dr. Halliwill by calling the Department of Human Physiology at (541) 600-4337.

What are my rights if I take part in this study?

Taking part in this research study is your decision. You do not have to take part in this study, but if you do, you can stop at any time. Your decision whether or not to participate will not affect your relationship with the University of Oregon.

You do not waive any liability rights for personal injury by signing this form. Biomedical research involves some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study.

The investigators may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped. For example, if the investigators determine the study is overly stressful for you (as indicated by body language and an elevated heart rate and/or blood pressure) they may stop you from participating in the study. Or if you express pain or discomfort beyond what is normally expected, the investigators stop you from participating in the study.

If you are physically injured because of the project, you and your insurance company will have to pay your doctor bills. If you are a UO student or employee and are covered by a UO medical plan, that plan might have terms that apply to your injury.

If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel	Research Compliance Services
Office of the President	5237 University of Oregon
1226 University of Oregon	Eugene, OR 97403-5237

Eugene, OR 97403-1226 (541) 346-2510

(541) 346-3082

A law called the Oregon Tort Claims Act may limit the amount of money you can receive from the State of Oregon if you are harmed.

What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Subject identities will be kept confidential by assigning you a “subject identification number”. The names associated with each subject identification number will be kept in a locked file cabinet in Dr. Halliwill’s office area.

Research Compliance Services and/or authorized representatives of the Food and Drug Administration (FDA) or National Institute of Health (NIH) may need to review records of individual subjects. As a result, they may see your name, but they are bound by rules of confidentiality not to reveal your identity to others.

The list of names will be destroyed when study results are published or 24 months after your participation, whichever comes first. All blood and muscle samples will be destroyed when study results are published or 5 years after your participation, whichever comes first. Other information may be stored by the researchers indefinitely.

You will be asked to complete a Medical History Form on the study day which will list personal identifying information so that in the unlikely event of a medical emergency in which we would activate the emergency medical system, we would be able to provide this information to emergency healthcare providers. This document will be retained in a locked file cabinet in the Exercise and Environmental Physiology Lab until you complete the study, after which it will be placed in a locked confidential documents disposal bin which is emptied by a secure shredding service.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this study.

If you have questions regarding your rights as a research subject, contact Research Compliance Services, 5219 University of Oregon, Eugene, OR 97403, 541/346-2510.

Your signature indicates that you have read and understand the information provided above, that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty, that you will receive a copy of this form, and that you are not waiving any legal claims, rights or remedies.

_____

(Signature of Participant) (Date)

_____

(Signature of Individual Obtaining Consent) (Date)

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