HISTAMINE AND THE EXERCISE RESPONSOME

by

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DISSERTATION ABSTRACT

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Title: Histamine and the Exercise Responsome

Histamine, a biogenic amine, commonly associated with the immune and inflammatory response is produced and released within skeletal muscle during exercise. The exact role(s) of histamine in the exercise response is largely unknown and understudied. Histamine's function in physiological contexts outside of exercise, such as its role in the modulation of tissue blood flow and inflammation, has guided the formation and interpretation of the studies included in this dissertation. Specifically, this research was divided into three separate studies intended to expand upon previous research on endurance capacity, exercise/functional hyperemia, and exercise-induced inflammation. In summary, the results of the studies suggest that skeletal muscle histamine may have a duration and intensity dependent function during exercise. Systemically blocking histamine's interaction with H_1 and H_2 receptors had no effect on low- to moderate-intensity exercise, but compromised the ability of individuals to perform exercise of long durations and high intensities. The reduced exercise capacity may relate to histamine's influence on the microvasculature. In immune and inflammatory responses, histamine dilates arterioles and increases capillary permeability to facilitate blood delivery and nutrient, immune cell, and metabolic byproduct transfer between the tissue and circulation. Blocking histamine H_1/H_2 receptors did not reduce,

but in fact increased leg blood flow during exercise. The increased blood flow may have been a consequence of alterations within the muscle milieu increasing the hydrogen ion concentration, thereby increasing the stimulus for blood flow. The increased blood flow and reduced pH suggest a role of histamine in mediating capillary nutrient transfer during exercise. Finally, blocking histamine H_1/H_2 receptors did not affect the systemic immune response (circulating leukocytes and cytokines) resulting from muscle-damaging exercise, but this study did not address responses within the skeletal muscle tissue where histamine is released/produced. Future research should focus on specific functions of histamine within the skeletal muscle, as these studies did not detect alterations in systemic function when histaminergic signaling was blocked. The findings of these studies suggest that histamine plays vital roles in many physiological pathways associated with the stress of exercise within the muscle. Future research expanding upon these findings will be important as very little is known about autocrine, paracrine, and endocrine role of histamine as part of the exercise responsome.

This dissertation includes previously published co-authored material.

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CHAPTER I

INTRODUCTION

Statement of the Problem

Histamine is a biogenic amine commonly associated with immune and inflammatory reactions, but is also produced and released within skeletal muscle during exercise (255). While the role of histamine in the exercise response is largely unknown, *in vivo* analysis of intramuscular fluid during exercise revealed that histamine concentrations increase by mast cell degranulation and through *de novo* synthesis (255) which occurred secondary to increased translation, transcription, and activation of the histidine decarboxylase (HDC) enzyme (212, 328). Histamine release by mast cell degranulation commonly occurs by antigen-immunogloblin interaction during allergic reactions, but can also occur with non-immunologic stimuli associated with exercise such as hypoxia, hyperosmolality, oxidative stress, chemokines, increased temperature, and vibration (97, 213, 279, 294). The HDC enzyme continually synthesizes minute quantities of histamine in resting muscle, and increases in HDC histamine formation occurs through positive allosteric modulation from hypoxia, temperature elevations, decreases in pH, and with increases in interleukin-1 concentrations, all of which may occur singularly or in combination with repeated muscle contractions (267, 268, 271). Therefore, multiple exercise related stimuli are potentially responsible for the increased histamine concentrations, which implies that histamine has an important function in the exercise response.

The importance of histamine in the exercise response can be gleaned from investigations in which histaminergic signaling has been disrupted. In humans, blocking

histamine interactions with H_1 or H_2 receptors had no effect on isokinetic muscle strength or endurance, oxygen consumption measures during steady state submaximal exercise or maximal aerobic exercise (VO_{2peak}), or the ability to complete high intensity intermittent sprint exercises (194, 195). In murine species, blocking histamine's actions on the H_1 and H_2 receptors diminished the speed and duration components of multi-hour endurance gnawing and walking tasks (212, 328). Similarly, reduced endurance walking capacity was noted in mice bred without either HDC, mast cells, or H_1 receptors (212, 328). Two major differences between the results of these studies are the species tested and the duration of these tests. First, murine species are often used as surrogates for understanding human physiology, but genomic differences often result in divergent findings between the species (278). Second, the longest duration of human testing was approximately 30 minutes using high-intensity sprint intervals alternated with rest periods. The exercise-rest interval study design does not reveal the effect of blocking histamine's actions on sustained endurance performance in humans as tested in the mice. A possible physiological explanation for the differing impact of blocking histamine's actions between the short-duration and long-duration tests may be related to the histamine forming capacity of HDC. HDC activity is positively correlated to exercise duration as the mRNA for the formation of new HDC enzymes increase after 60 minutes of exercise and the histamine formation activity of HDC is elevated following 120 minutes of exercise (13, 14, 320). Therefore, given the variations in study designs, it is unknown if the differing outcomes between the human and murine studies are simply due to differences in histamine's actions between species or indicate that histamine's importance in the exercise response is related to the intensity and/or duration of the exercise tests.

The duration of the exercise may be the essential variable to elucidate histamine's role in the exercise response. Histamine is a primary mediator of blood flow in the microcirculation during inflammatory and immune responses (18, 270, 272, 273) and has also been associated with increased blood flow during reactive hyperemia (10, 72), increased skin blood flow during heat stress (325), increased blood flow in tumor formation and growth (83, 226), and with sustained post-exercise vasodilation within previously active muscles (168, 187, 188). It is unknown if exercise-induced elevations in histamine concentrations have a similar vasodilatory influence to facilitate an increased blood flow to the contracting skeletal muscle (exercise hyperemia). If exercise-induced increases in histamine aid in increasing blood flow during exercise, blocking this action would decrease nutrient and oxygen delivery as well as carbon dioxide and metabolite removal. The impact of this mismatch of perfusion and metabolism would have a larger negative influence on longer-duration exercise compared to short-duration exercise, and could be an explanation for the decreased endurance task performances observed in mice (82, 84, 212, 328) but not in short-duration exercise studies in humans (194–196, 236).

The increased skeletal muscle histamine concentrations during exercise may contribute to the exercise response beyond facilitating elevations in blood flow. Histamine also has many associations with the acute inflammatory response. Histamine concentrations are increased locally at sites of tissue damage (203, 291) to promote diapedesis of leukocytes by acting directly and indirectly in the production of chemokines, cytokines, endothelial adhesion molecules (289), and increasing capillary permeability (176, 272). Additionally, i*n vitro*, histamine has been shown to alter the expression of a number of cytokines involved with the macrophage M_1 ("pro-

inflammatory") and M_2 ("anti-inflammatory") phenotype conversion (76, 83). Endurance exercise itself initiates an acute systemic inflammatory profile (i.e. leukocytosis and elevated chemokines) (229–231) and blocking histamine H_1/H_2 receptors during exercise depresses the mRNA expression of chemokines and cytokines related to inflammation within skeletal muscle (254). Therefore, exercise associated elevations in intramuscular histamine may be a primary signal to initiate the acute systemic inflammatory response following endurance exercise in order to repair damaged tissue from exercise.

The studies detailed in this dissertation were designed to gain insight into the role of histamine during and following exercise. The proposed research is divided into three separate studies intended to expand upon previous research on endurance exercise capacity, skeletal muscle blood flow during exercise, and exercise induced inflammation. In Chapter IV, the association of histamine to endurance exercise capacity was tested. In Chapter V, histaminergic control of blood flow during exercise was examined. In Chapter VI, the association between exercise induced histamine release/production and immune response following muscle-damaging exercise was investigated.

PURPOSE and HYPOTHOSES

This dissertation involved three separate studies, detailed in Chapters IV – VI, with the following purposes and hypotheses:

1. Chapter IV: The purpose of this study was to gain insight on histamine's role in endurance exercise in humans and potentially reconcile the contrasting results between human and animal studies. Specifically, on multiple days human subjects performed short-duration high-intensity exercise performance tests following a

time matched period of rest or endurance exercise. Additionally, subjects performed the tests when histamine's interactions with H_1 and H_2 receptors were blocked and in a placebo condition. It was hypothesized that blocking histamine's interactions with H_1 and H_2 receptors would decrease the time-to-completion of a fixed-distance time-trial in comparison to a placebo and the effect would be greater following an endurance exercise bout.

2. Chapter V: The purpose of this study was to compare leg blood flow during exercise at set increments of exercise intensity before and after a prolonged exercise bout, in addition to examining histamine's influence on blood flow by antagonizing histamine H_1 and H_2 receptors. It was hypothesized that 1) in a placebo condition skeletal muscle blood flow would increase with increasing exercise intensities, 2) in the placebo condition the rise in skeletal muscle blood flow would be greater following a prolonged exercise bout, and 3) blocking histamine's actions on H_1 and H_2 receptors would attenuate the rise in skeletal muscle blood flow with increasing exercise intensities and the attenuation would be larger following a prolonged exercise bout.

3. Chapter VI: The purpose of this study was to examine the effect of blocking histamine's actions on H_1 and H_2 receptors on the inflammatory response following exercise-induced muscle damage. It was hypothesized that 1) exercise-induced muscle damage would elevate pro-inflammation chemokine, cytokine, and immune cell concentrations within the blood, and 2) blocking histamine's

actions on H_1 and H_2 receptors would attenuate the rise in pro-inflammatory chemokine, cytokine, and immune cell concentrations within the blood.

SIGNIFICANCE

The beneficial effects of exercise are well known but the mechanisms by which exercise improves health and prevents disease is largely unknown. Specifically, the molecular profiles in cells and tissues in response to exercise, the "Exercise Responsome", remains understudied (205). For example, approximately 50% of protection afforded by exercise to cardiometabolic health remains unexplained (144). The studies in this dissertation provide novel insight that histamine may be a contributing factor in the "Exercise Responsome" acting as an autocrine and/or paracrine molecular transducer of exercise within the skeletal muscle. Specifically, histamine's involvement in endurance exercise capacity, exercise blood flow, and exercise-associated inflammation.

The findings of these studies broadly address important issues identified by The National Institutes of Health (NIH) on the interaction of physical activity, inflammation, and immune function (205). These studies also address the issue of pharmacotherapy, in the interaction between commonly used medications ("antihistamines") and the exercise-induced immune response (205). Given the widespread use of common antihistamine medications, these studies provide insight into potential interactions between common drugs and exercise. These results are of interest to individuals new to exercise, those who regularly exercise, as well as competitive athletes in relation to exercise capacity and recovery from exercise.

CHAPTER II

REVIEW OF THE LITERATURE

Part I: *The Exercise Responsome*

The “Exercise Responsome” refers to the molecular profiles within cells and tissues in response to physical exercise (205). Understanding these molecular profiles is important as very little is known about autocrine, paracrine, and endocrine factors operating to facilitate cross talk within and between tissues as part of the exercise response (256). Exercise is known to be beneficial to health by reducing morbidity and delaying mortality, but the mechanisms contributing to these positive outcomes is largely unknown. For example, the sum of all known cardiometabolic health related factors can only approximate 50% of the protection afforded by exercise (144). The cellular and tissue cross talk likely initiate changes aiding exercise and the healthy adaptions that are associated with exercise.

Skeletal muscle appears to be an integral component of exercise responsome. Skeletal muscle, once classified as only the contractile tissue needed for movement, functions similar to an “endocrine organ” by producing and releasing molecules that influence the functions of other bodily tissues both during and after exercise (43, 185, 232). For example, the myokine interleukin-6 is produced within the muscle during contractions and once it is released into circulation causes an increase in glucogenesis within the liver and lipolysis of adipose tissue (185, 232). The circulating glucose and fatty acids are in turn metabolized by skeletal muscle for energy during exercise (43, 232). Therefore, skeletal muscle may mediate some of the beneficial systemic health effects, including cardiovascular adaptions, from exercise (185).

Along these lines, histamine which is commonly associated with immune and inflammatory responses is produced and released within the skeletal muscle during endurance exercise (255) but it's role in contributing to the exercise response is largely unknown. Histamine, in immune and inflammatory responses, acts directly and indirectly to increase blood flow, increase tissue permeability, attract immune cells (chemotaxis), and in the alteration of local cytokine expression. Therefore, skeletal muscle histamine production and release during exercise may participate in the cellular cross talk and contribute to one or more pathways to facilitate exercise and improve health.

Evidence that histamine is innately involved in the exercise responsome comes from studies investigating the origin and causes of post-exercise hypotension. These studies showed that histamine formed within skeletal muscle during exercise activated histamine H_1 and H_2 receptors on the vascular endothelium and smooth muscle, respectively, to induce an arterial vasodilation. The vasodilation increased local blood flow and reduced systemic mean arterial pressure which lasted multiple hours (187, 188, 255). The increased blood flow and post-exercise hypotension could be attenuated by blocking histamine H_1 and H_2 receptors (187, 188). Not only did blocking histamine interaction with H_1 and H_2 receptors reduce post-exercise blood flow and hypotension but also reduced the expression of skeletal muscle protein-coding genes related to inflammation, endothelial and vascular function, metabolism, and cell maintenance (254). Furthermore, the blockade of histamine H_1 and H_2 receptors during muscle damaging exercise reduced long-lasting perceptions of muscle soreness and pain (78) which may be related to down regulation of two neurotrophic factors (nerve growth factor, glial derived neurotrophic factor) within the muscle that are associated with regulation, growth, and

survival of neurons (78, 254). Evidently, histamine appears to be a major molecular transducer of exercise but the exact, or likely multiple, functions of histamine during exercise are only beginning to be understood.

The following chapter provides a brief overview of the scientific study of histamine starting with its identification, formation, and degradation. Thereafter, the chapter's specific focus is on the biological processes and mechanisms of histamine within human and animal physiology. The studies presented in this dissertation attempt to build upon the pathways presented in the review of literature (Chapter II) to determine if histamine production and release is an integral pathway during exercise (Chapter IV), if histamine facilitates an increased skeletal muscle blood flow during exercise (Chapter V), and/or if histamine is associated with the inflammatory response resulting from muscle damaging exercise (Chapter VI).

Part II: *Histamine*

Historical Perspective

In 1910-11 Dale and Laidlaw characterized the effects of a systemically infused extract from fungus, β-aminoethylimidazole, on the human vasculature. They documented that β-aminoethylimidazole increased vascular permeability, increased limb volume, and decreased arterial pressure (56, 57). In 1927 β-aminoethylimidazole was identified within liver and lung samples of humans and was subsequently renamed to histamine, a derivative of the Greek word *histos* as it was determined to be a natural constituent of tissue (28). Since these early observations, histamine has been linked to variety of other physiological actions including the secretion of gastric fluid, cell growth,

cell differentiation, wound healing, and in some brain regions histamine is a neurotransmitter regulating circadian rhythms (stimulant) and memory formation (131, 301, 311).

Specifically, histamine is identified as a biogenic amine (aka nitrogenous compound, $C_5H_9N_3$) with a molecular mass of 111 Dalton. (83, 136). Histamine is present in single cell and multiple cell organisms that have not undergone radical evolution in the last 700 million to 1 billion years. In these organisms histamine has multiple functions including promoting phagocytosis, cell growth, glucose metabolism, and is involved with chemotaxis (52). These findings indicate that the functions of histamine in the single cell and multicellular organisms are similar to those in humans (172).

Histamine Formation

Histamine is formed by the actions of a single enzyme, histidine decarboxylase (HDC). This enzyme removes carbon dioxide (decarboxylation) from the amino acid histidine (Figure 1). Histidine is present in all animal and plant protein, contains an α-amine group ($-NH_3^+$), a carboxylic acid group ($-COO^-$), an imidazole side chain ($C_3N_2H_4$), and has a positive charge at a physiological pH. HDC is specific in that it only converts the L configuration of histamine (L-histidine) and not the D configuration (D-histidine)(L=left versus right orientation of the amine group on the carbon chain of the amino acid)(267, 268).

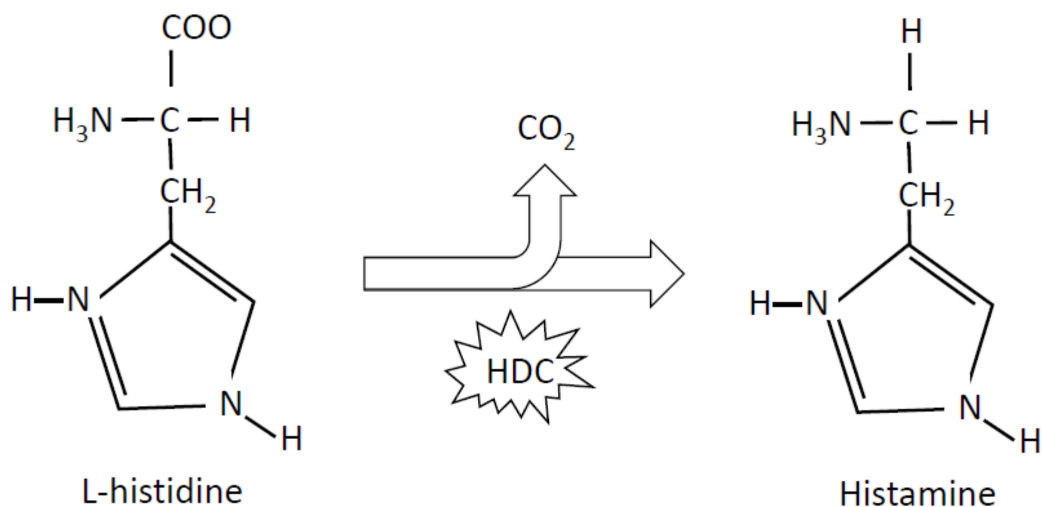

Figure 1. Histamine formation from L-histidine via the histidine decarboxylase (HDC) enzyme.

Histidine decarboxylase has been identified in all organ tissue examined including the kidney, heart, lungs, intestines, brain, uterus, stomach, endothelial cells, and skeletal muscle (72, 125, 270, 272, 273, 312). Within the skeletal muscle, as evidenced by HDC staining of mouse muscle, HDC is concentrated within mast cells, endothelial cells that line the circulatory system, and skeletal muscle monocytes (270, 312). In resting skeletal muscle, HDC activity is very low as it continually synthesizes minute quantities of histamine (82, 320). Exercise increases the mRNA transcription (138, 254, 328), protein abundance (13), and activity of the skeletal muscle HDC (13, 81, 82, 320). The HDC activity extends several hours beyond the cessation of exercise with the length of the prolonged activity positively correlated to the duration of exercise (14, 212).

The increased presence and activity of HDC within the muscle may be due to changes in temperature, pH, oxygen content, shear stress on endothelial cells, or the production of enzymatic modulators formed during exercise. For example, a rise in temperature leads to an immediate increase in HDC histamine synthesis with an optimal

operation temperature near 56˚C (268), a temperature conscious humans are unlikely to attain. But a temperature change within a physiological range of human muscle temperatures (~37 to 42˚C) elevates HDC histamine production approximately two fold (267, 268, 271). Similarly, exercise can decrease the pH of skeletal muscle from approximately 7.0 at rest to 6.5 at maximal exercise intensities (119). HDC activity is increased with a decreasing pH and functions optimally between a pH of 6.6 to 7.0 (Michaelis-Menton constant of HDC is 0.19mM at a pH of 6.8) (267, 268). Heavy exercise results in a cellular hypoxic stress which activates hypoxia inducible factor (HIF1) which is another factor that increases HDC activity (138). During exercise there is an increased and prolonged shear stress on endothelial cells from elevated blood flows which increases HDC activity (61, 124, 125). Additionally, another positive allosteric modulator of HDC that increases within skeletal muscle during exercise is interleukin-1 (IL-1)(13, 81, 82, 320). Interestingly, exercise training may diminish the elevated activity of HDC (82) as it was shown "gnawing training" in mice reduced HDC activity compared to "non-trained" mice (12). The reduction of HDC activity with training may be due to intramuscular adaptations that may buffer reductions in pH and hypoxic stress at similar absolute work rates pre- to post-training (12).

Other positive allosteric modulators of HDC activity, not necessarily associated with exercise, include glucocorticoids, intracellular cAMP, calcium ions, protein kinase C (PKC), rapidly accelerated fibrosacoma (RAF-), granulocyte-marcophage colony stimulating factor (GM-CSF), interleukin -3, -12 and -18 (IL-3, IL-12, IL-18), and tumor necrosis factor alpha (TNFa) (206, 274).

Histamine Formation, Storage, and Release - Mast Cells

Mast cells, first identified and described in 1877 by Paul Erlich (211), are derived from undifferentiated hematopoietic precursor cells (CD34+)(294) and mature within tissues (213). Mast cells form histamine via intracellular HDC that does not appear to be inducible, i.e. the enzyme activity does not increase with known positive allosteric influences (270, 272, 273). This histamine is stored within cytoplasmic granules with other vasoactive substances and inflammatory mediators including prostaglandins, leukotrienes, heparin, cytokines, tumor necrosis factor, and proteases (tryptase, chymase, carboxypeptidase A) (211, 315).

Mast cell release of histamine, by way of degranulation, commonly occurs from antigen-immunoglobulin interaction during allergic reactions. Mast cell degranulation has also been shown to occur with non-immunologic stimuli associated with exercise such as mechanical disruption of the mast cell membrane, hypoxia, hyperosmolaility, superoxides, chemokines, increased temperature, and vibration (97, 190, 213, 279, 294). Other factors, that can lead to mast cell degranulation, not necessarily associated with exercise are substance P, bradykinin, and nerve growth factor (NGF) (141, 186).

Mast cells are predominantly located in close proximity to afferent nerves, lymph vessels, and blood vessels in the microcirculation of skeletal muscle (211, 213, 328). Due to their location, mast cells have been described as part of the vascular control network responsible for maintaining microcirculatory homeostasis (213) as degranulation leads to smooth muscle relaxation and increased blood flow, increased capillary leak, and edema formation (279, 315). The histamine release in combination with chemokines and cytokines initiate an inflammatory response through leukocyte adhesion to endothelial

cells and extravasation from the circulation into the tissue along chemokine gradients (279). Mast cells also control key elements of wound healing designed to limit damage, initiate revascularization of the damaged tissue, increase epithelization, aid in the deposition of temporary connective tissue, and remodeling of the extracellular matrix (213).

Histamine Breakdown, Inactivation, and Reabsorption

Histamine, within tissues or in circulation, has a half-life of approximately 100 s (130). Histamine is either broken down by diamine oxidase (DAO), inactivated by histamine-N-methyltransferase (HNMT), or absorbed into mast cells and basophiles (22). DAO is present in high concentrations in the vascular endothelium, blood, white blood cells, liver, and the kidney. DAO degrades histamine by removing the amino group and forming imidazole acetaldehyde. HNMT is highly concentrated in the liver and monocytes where it adds a methyl group (methylenation) to histamine creating N-methlylimidazole acetic acid (22, 204). HNMT has a higher affinity for histamine (Michaelis-Menton constant 6-12 umol) than DAO (Michaelis-Menton Constant 20 umol/L)(175). The optimal activity of DAO occurs at a pH of 7.2 while HNMT functions best from a pH of 7.5 to 9.0 (175). Histamine is also reabsorbed into mast cells and basophils by transport through two membranes (plasma and vesicular) via a bidirectional organic cation transporter 3 and vesicular monoamine transporter 2 (as reviewed by Ohtsu 2010 (221)).

Histamine Receptors

Histamine acts through four different G-protein coupled receptors:

H_1: H_1 receptors are mainly found on the smooth muscle of intestines, bronchi, and blood vessels as well as on the endothelium lining blood vessels. H_1 receptors are G-protein coupled receptors (G_q) with 7 transmembrane domains containing amino terminal glycosylation sites which stimulate the formation of inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) resulting in increased intracellular calcium (203). The H_1 receptor is known to be involved with inflammatory responses, vascular smooth muscle relaxation by stimulating nitric oxide (NO) production in endothelial cells (221), and sensory nerve stimulation (167).

H_2: H_2 receptors are found in high concentrations on the gastric mucosa, uterus, brain, and vascular smooth muscle. This G-protein coupled receptor (G_s) increases intracellular concentrations of cAMP and decreases intracellular calcium concentrations (203, 221).

H_3: H_3 receptors are found in high concentrations in brain and spinal cord on pre-synapic nerve terminals. The H_3 receptor is an autocrine regulator of histamine synthesis and release (as well as other neurotransmitters) from histaminergic neurons (206, 221). The H_3 receptor is a G-protein coupled receptor (G_i) which inhibits the adenylate cyclase pathway (203). The H_3 receptor is likely involved with the central nervous system regulation of circadian rhythms and spontaneous

ambulatory activity (261). Histamine H_3 receptor antagonists are currently used for narcolepsy treatment (206).

H_4: H_4 receptors are found mainly on peripheral blood mononuclear cells, neutrophils, eosinophils, mast cells, and CD4+ T lymphocytes and centrally on dendritic cells (206, 220, 274). Little is known about the H_4 receptor due to its recent discovery in the early 2000s (220) but it may play a role in the chemotaxis of mast cells (123) and eosinophils (167) in addition to the expression of adhesion molecules on eosinophils (167). The H_4 receptor is a G-protein coupled receptor ($G_{i/o}$) that leads to an increase of intracellular calcium (203, 221).

Note: The studies in this dissertation used histamine H_1 and H_2 receptor antagonists in an effort to target the histamine H_1 and H_2 receptors which are present in high concentrations on the luminal side of endothelial cells near endothelial junctions and the vascular smooth muscle of blood vessels within the skeletal muscle, respectively (118). However, it is acknowledged that the systemic blockade of these receptors may have effects on other organs and tissues during rest and exercise.

Exercise-Induced Elevation of Histamine within Skeletal Muscle

Skeletal muscle interstitial histamine concentrations are approximately 12 ng/ml at rest (255). Histamine concentrations increase with 60 minutes of moderate intensity aerobic knee-extension exercise to ~30 ng/ml and remain elevated (~20 ng/ml) for at least 60 minutes post-exercise while in a rested state (255). The rise of intramuscular histamine

concentrations are specific to the exercised skeletal muscle as concentrations in non-exercised muscle do not rise above resting levels (255). Due to current technology limitations, adequate sample volumes for histamine quantification in intramuscular fluid by microdilaysis can only be obtained once every ~30 minutes. Therefore, the rate of rise of intramuscular histamine concentration after the initiation of exercise are relatively unknown. It is possible that histamine concentrations are elevated within minutes of exercise onset.

The increased histamine concentrations are a result of mast cell degranulation and *de novo* production by the HDC enzyme (98, 212, 255). Evidence that the elevated skeletal muscle histamine concentrations were from mast cells were made by simultaneous detection of tryptase, a proteolytic enzyme co-released with histamine from mast cell granules within muscle dialysate (255). The remaining rise in intramuscular histamine is likely from increased activity of HDC, as the infusion of α - flouromethylhistidine hydrochloride (αFMH), an inhibitor of HDC activity, reduced rise of interstitial histamine concentrations by approximately 60% (255).

The exercise signal causing histamine release and production is unknown but as noted above, exercise associated increases in muscle temperature, hypoxia, shear stress, cytokine release, decreases in pH etc. (61, 110, 124, 125) are all associated with degranulation of mast cells and increased HDC activity. Only one known study has attempted to identify the factor within muscle that may be the primary stimulus for the increase histamine concentrations (253). In this study, Romero et al. systemically infused high doses of N-acetylcysteine, a potent anti-oxidant, to reduce exercise-associated oxidative stress. This method did not blunt histaminergic post-exercise vasodilation (253)

indicating that oxidative stress is not a major contributor leading to elevated skeletal muscle histamine concentrations.

Blood Histamine Concentrations

Resting plasma histamine concentrations in humans is approximately 0.20 ng/ml (73, 130, 251, 255). The source producing this baseline concentration is unknown, but is likely continuously produced or released as its breakdown is rapid and occurs on the endothelial lining of all blood vessels. Whether blood histamine concentrations rise during or after exercise is unknown as some investigations have noted either no change (15, 113, 168, 187, 188) or elevations (9, 40, 45, 67, 71, 92, 112, 113, 184, 246) in blood histamine concentrations (Table 1).

The intensity, duration, and/or muscle mass exercised appear to be the difference between the studies that document an increase in blood histamine concentrations and those that do not. On average, studies that used whole-body high-intensity and/or long-duration aerobic exercise sessions document elevations in venous plasma histamine concentrations in the 4-6 ng/ml range. Studies that do not detect a change in blood histamine concentrations exercised single muscle groups and/or employed light to moderate exercise intensities (Table 1). For skeletal muscle histamine to contribute to a rise in venous blood histamine concentrations, the rate of histamine release or production within the skeletal muscle must exceed its removal and breakdown. Additionally, the skeletal muscle histamine must diffuse or be transported out of the muscle. It is possible that a large amount of muscle mass in combination with high-intensity or long duration

exercise increased production of histamine beyond the rate of degradation and removal to result in a rise in circulating histamine concentrations.

Whether exercise induced elevations within skeletal muscle histamine contribute to an increased blood histamine concentration is unknown, but a few studies indicate it is a possibility. In humans, skeletal muscle interstitial histamine concentrations increase to ~30 ng/ml during 60 min of moderate-intensity exercise (60% peak power)(255). This rise of intramuscular histamine is approximately 30X greater than venous blood concentrations providing the concentration gradient necessary for simple diffusion from the skeletal muscle to the circulation. Next, in isolated canine skeletal muscle, the arterial blood supplying the muscle and the venous blood leaving the muscle contain similar histamine concentrations during resting conditions but when the muscle was electrical stimulated to contract the venous histamine concentrations increased 3-4 fold while arterial concentrations did not change (9). Similarly in humans, restriction of arterial blood flow to exercising limbs elevated venous histamine concentrations (9, 10).

Table 1. Change in blood histamine concentrations with exercise.

Author	Year	Exercise				Histamine Concentration		
		Type	Intensity	Duration	Blood/Serum/Plasma	Baseline	Exercise	Change
McCord et al.	2006a	Cycle	60% VO_2	60 min	Whole Blood	4.6 ± 0.6 ng/ml	4.9 ± 0.5 ng/ml	↔
					Plasma	0.4 ± 0.1 ng/ml	0.2 ± 0.1 ng/ml	↔
		Cycle	60% VO_2	60 min	Whole Blood	2.9 ± 0.7 ng/ml	3.5 ± 0.9 ng/ml	↔
					Plasma	0.3 ± 0.2 ng/ml	0.5 ± 0.2 ng/ml	↔
McCord et al.	2006b	Cycle	60% VO_2	60 min	Whole Blood	4.6 ± 0.6 ng/ml	5.5 ± 0.7 ng/ml	↔
					Plasma	0.4 ± 0.1 ng/ml	0.4 ± 0.2 ng/ml	↔
Morgan et al.	1983	Treadmill	Unknown-Moderate	6 min	Plasma	0.22 ± 0.09 ng/ml	0.33 ± 0.16 ng/ml	↔
Barnes et al.	1981	Treadmill	Unknown-Moderate	6 min	Plasma	~0.38 ± 0.07ng/ml	1.6 ± 0.2 ng/ml	↔
						0.38 ± 0.07 ng/ml	~0.53 ± 0.02 ng/ml	↔
Hartley et al.	1981	Cycle	150 bpm HR	8 min	Plasma	0.67 ± 0.11 ng/ml	1.0 ± 0.11 ng/ml	↑↔
Garden et al.	1966	Treadmill + Heat Stress	3.5 mph	120 min	Plasma	5.5 ± 0.5 ng/ml	6.6 ± 1.1 ng/ml	↑
Matthews et al.	1970	Stair Running	Unknown - Hard	12 min	Plasma	4 ± 1 ng/ml	6 ± 1 ng/ml	↑

Table 1. Continued: Change in blood histamine concentrations with exercise.

Author	Year	Type	Intensity	Duration	Blood/Serum/Plasma	Baseline	Exercise	Change
Doh et al.	2016	Cycle	60% VO_2	12 min	Plasma	1.83 ± 0.14 ng/ml	2.33 ± 0.23 ng/ml	↑
Campos et al.	1999	Treadmill	Up to Max	~9 min	Whole Blood	~23 ± 1 ng/ml (?)	~35 ± 2 ng/ml (?)	↑
Harries et al.	1979	Outdoor Running	Max	6 min	Plasma		Δ 1.1 ± 1.56 ng/ml	↑
					Whole Blood		Δ 31.25 ± 10.4 ng/ml	↑
Anrep et al.	1944	Forearm Contractions	Max	2 min	Plasma	~12 ng/ml	~70 ng/ml	↑
Duner et al.	1958	Cycle	Max	18-24 min	Whole Blood	~4.2 ± 0.7 ng/ml	~6.7 ± 0.7 ng/ml	↑
					Serum	~1.6 ± 0.3 ng/ml	~2.9 ± 0.7 ng/ml	↑
Anselme et al.	1994	Cycle	Up to Max	12 min	Plasma	1.2 ± 0.17 nM	4.66 ± 1.03 nM	↑
					Plasma	1.3 ± 0.23 nM	5.98 ± 0.86 nM	↑
Charles et al.	1979	Cycle	Moderate (HR>140 bpm)	8 min	Plasma	~0.73 ng/ml	~1.04 ng/ml	↑
Prefaut et al.	1997	Cycle	Max Test	~10 min	Plasma	1.2 ± 0.16 ng/ml	6.02 ± 1.03 ng/ml	↑

~ = Units were converted, * = Values were estimated from figures, ↑ = Increase, ↓ = Decrease, ↔ = No Change

If blood histamine concentrations rise with exercise, the physiological effect of this histamine is unknown. It is worth noting that infusion of histamine in the 4-6 ng/ml range into circulation of canines results in arteriolar vasodilation of resting muscle arteries (9). A similar infusion of 4-6 ng/ml of histamine in humans would result in a decreased diastolic blood pressure, increased heart rate, and increased skin temperature (130, 175) (Table 2). For reference, Table 2 provides a description of systemic physiological effects with increased plasma histamine concentrations. The approximate range of plasma histamine concentrations during light and heavy exercise have been added for comparison based off the changes in plasma histamine concentrations during exercise (Table 1),

Table 2. Clinical effects according to plasma histamine concentrations

Histamine (ng/ml)	Clinical Effect
0-1	normal resting
1-2	increased gastric acid secretion, increased heart rate
3-5	tachycardia, headache, flush, urticarial, pruritus
6-8	decreased arterial pressure
7-12	bronchospasm
~100	cardiac arrest
~0.3 - 0.5	light exercise (<60% max)
~2 - 6	heavy exercise (>60% max)

Table Modified from Maintz 2007 (175)

Histamine and Exercise Capacity/Performance

It is clear that histamine is released and produced within exercising skeletal muscle but its role in the exercise response it unknown. Previous findings in murine species suggest that histamine performs an important role in facilitating endurance exercise. In murine species, administration of H_1 receptor antagonists decreased gnawing

activity (328) in addition to walking speed and duration in a forced multi-hour walking task (212). A similar but non-significant reduction in endurance walking duration was documented with the administration of H_2 receptor antagonists in mice (212, 328). Additionally, mice bred to be deficient in HDC (HDC knockout models) or given aFMH (an HDC inhibitor) also displayed reduced gnawing activity (328). The reduced endurance is likely associated with H_1 and H_2 receptors as blocking histamine's actions on the H_3 and H_4 receptors had no effect on endurance walking (212)(Table 3).

In contrast to the murine studies, only short-duration high-intensity measures of performance have been made while blocking histamine H_1 and H_2 receptors in humans. In humans H_1 receptor antagonists had no effect on of skeletal muscle strength, endurance (194), VO_2 obtained in a cycling peak test, or the total distance covered during 30 min of high intensity intervals (195) (Table 3). These results suggest that histamine's role in exercise may differ between the murine and human species or that histamine has an important function that is exercise intensity and/or duration dependent. This theory is examined in Chapter IV.

Table 3. The Effect of Histamine Receptor Antagonists on Exercise Performance tests.

Author	Year	H_1 Antagonist	H_2 Antagonist	Species	Exercise Type	Exercise Duration	Exercise Intensity	Outcome
Farzin et al.	2002	Diphenhydramine (40 mg/kg)		Mouse	Prolonged Walking Rota Rod	40 min	light/moderate	↓
			Ranitidine (100 µg)	Mouse	Prolonged Walking Rota Rod	40 min	light/moderate	↓
Niijima-Yaoita et al.	2012	Fexofenadine (20 mg/kg)		Mouse	Prolonged Walking Rota Rod	up to 5 hrs	light/moderate	↓
		Fexofenadine (40 mg/kg)		Mouse	Prolonged Walking Rota Rod	up to 5 hrs	light/moderate	↓↓
		Pyrilamine (6 mg/kg)		Mouse	Prolonged Walking Rota Rod	up to 5 hrs	light/moderate	↓
		Pyrilamine (24 mg/kg)		Mouse	Prolonged Walking Rota Rod	up to 5 hrs	light/moderate	↓↓
Yoneda et al.	2013	Fexofenadine (40 mg/kg)		Mouse	Prolonged Gnawing	up to 4 hrs	light/moderate	↓↓
		Pyrilamine (25 mg/kg)		Mouse	Prolonged Gnawing	up to 4 hrs	light/moderate	↓↔
			Ranitidine (15 mg/kg)	Mouse	Prolonged Gnawing	up to 4 hrs	light/moderate	↔

Table. 3 Continued: The effect of Histamine Receptor Antagonists on Exercise Performance tests.

Author	Year	H_1 Antagonist	H_2 Antagonist	Species	Exercise Type	Exercise Duration	Exercise Intensity	Outcome
Ely et al.	2018	Fexofenadine (540 mg)	Ranitidine (300 mg)	Human	Isometric Contractions	5 sec	Max	↔
Saltissi et al.	1981		Cimetidine (400 mg)	Human	VO_2 max (Treadmill)	~12 min	Max	↔
Montgomery et al.	1991	Diphenhydramine (50 mg)		Human	Isokinetic Strength	<1 min	Max	↔
		Diphenhydramine (50 mg)		Human	Velocity Spectrum test	<1 min	Max	↔
		Diphenhydramine (50 mg)		Human	Muscle endurance test	45 sec	Max	↔
			Terfenadine (60 mg)	Human	Isokinetic Strength	<1 min	Max	↔
			Terfenadine (60 mg)	Human	Isokinetic Strength	<1 min	Max	↔
			Terfenadine (60 mg)	Human	Velocity Spectrum test	45 sec	Max	↔
Montgomery et al.	1992	Diphenhydramine (50 mg)		Human	Treadmill VO_{2max}	~12 min	Max	↔
		Diphenhydramine (50 mg)		Human	Treadmill Steady State (55% VO_{2max})	30 min	Moderate	↔

Table. 3 Continued: The effect of Histamine Receptor Antagonists on Exercise Performance tests.

Author	Year	H1 Antagonist	H2 Antagonist	Species	Exercise Type	Exercise Duration	Exercise Intensity	Outcome
Montgomery et al.	1992	Diphenhydramine (50 mg)		Human	High intensity intervals (30 sec on/off) to fatigue	~30 min	Max	↔
			Terfenadine (60 mg)	Human	Treadmill VO_{2max})	~12 min	Max	↔
			Terfenadine (60 mg)	Human	Treadmill Steady State (55% VO_{2max})	30 min	Moderate	↔
			Terfenadine (60 mg)	Human	High intensity intervals (30 sec on/off) to fatigue	~30 min	Max	↔

↓ = Decrease, ↓↓ = Large Decrease, ↔ = No Change

Part III: Histamine Actions

The following sections start by proposing questions concerning histamine's potential function within skeletal muscle during exercise. The focus is on skeletal muscle because tissue responses to histamine are location and concentration dependent (252). The questions are followed by scientific findings on histamine's function during health and disease within different body systems and organs. Specific attention is given to histamine's function in humans while at rest and when possible during exercise. These sections are meant to provide a road map for the investigation of histaminergic signaling during exercise.

Does Histamine Contribute to Elevated Skeletal Muscle Blood Flow During Exercise?

It is possible that histamine mediates the increase in skeletal muscle blood flow during exercise (exercise hyperemia). There are multiple lines of research indicating vasodilatory properties of histamine ranging from direct injection/infusion, to reactive hyperemia responses, sustained post-exercise vasodilation and post-exercise hypotension, increased skin blood flow, and the dilation of capillaries. In each of these situations application or infusion of H_1 and/or H_2 histamine receptor antagonists blunt the normal hyperemia responses. A summary of each topic is presented below.

Histamine Infusion

Multiple studies have demonstrated that injection or infusion of histamine into the arterial blood (not venous) decreased arteriole resistance (74, 104, 252, 273, 316, 321) and increased limb blood flow in a

dose response manner (49, 59, 69, 70, 74, 80, 104, 266). This histamine induced increase in limb blood flow could be attenuated with the pre-infusion or co-infusion of histamine H_1 and/or H_2 receptor antagonists (49, 58, 70, 115, 266, 321). One study concluded that the initial rise in blood flow involved activation of H_1 receptors while H_2 receptors were mediating an extended rise in blood flow once histamine infusion was halted (49).

The dilation of resistance vessels may be an indirect effect of a histamine induced increase in nitric oxide and/or a direct action on vascular smooth muscle. The arterial intraluminal histamine likely has a direct interaction with endothelial cell expressed H_1 receptors. The endothelial H_1 receptors increase nitric oxide synthase (NOS) activity leading to production of nitric oxide. The nitric oxide diffuses to the surrounding smooth muscle to cause a relaxation of vascular smooth muscle and a decrease in resistance to blood flow (164, 252). The histaminergic increase in nitric oxide synthase activity occurs in a dose and time dependent manner (164). The histamine-nitric oxide pathway may be active during exercise as 1) blockade of nitric oxide production reduces histamine induced dilation of arteries (252), and 2) mice treated with an H_1 receptor antagonist display reduced intramuscular production of nitric oxide (specifically nitric oxide metabolites) following endurance exercise (212). Histamine may also diffuse out of circulation and directly interact with H_2 receptors on the vascular smooth muscle to induce a

relaxed state. Both pathways may be active as “antihistamines”, regardless of structure, when applied topically to microcirculation preparations constrict smooth muscle cells (273).

Reactive Hyperemia

Reactive hyperemia refers to the transient elevation in limb blood flow following a period of occlusion. Early examinations of venous blood exiting the previously occluded limb found elevated plasma histamine concentrations (10). It was speculated by Lewis (1927) that the elevated histamine concentrations were related to the subsequent vasodilation and increased blood flow upon ischemic release (As reviewed by Duner 1960 and Duff 1964 (70, 72)). This speculation may have been proven true as it was later demonstrated that infusion of H_1 “antihistamine substances” into the occluded limb blunted the normal reactive hyperemic response (69).

Sustained Post-Exercise Vasodilation

Similar to reactive hyperemia, there is an increase in skeletal muscle blood flow following exercise upon returning to a rested state. The increased blood flow is due to a drop in vascular resistance below pre-exercise levels (108, 277). This post-exercise increase in blood flow remains elevated for an extended length of time dependent on the type, duration, and intensity of exercise (108, 197). The hyperemia can be

divided into two phases, an initial short-term phase and a longer phase (162).

The initial phase of elevated blood flow is seconds to minutes in duration and involves factors that likely contribute to exercise hyperemia. The short-phase factors include increased body temperature, nitric oxide, prostanoids, adenosine, oxygen deficits, mechanical compression, and release of sympathetically mediated vasoconstriction (108, 162).

The second phase of increased blood flow has been termed sustained post-exercise vasodilation (SPEV) and can last several hours into recovery (109). There is a neural (~30%) and vascular (~70%) component to SPEV. Neurally, there is a resetting of the barroreflex, a reduced sympathetic influence mediated by an internalization of neurokinin-1 receptors in the nucleus tractus solitarii (46), and a reduced capacity of the smooth muscle to contract to sympathetic stimulation (108, 109). The vascular component involves a dilation (decreased peripheral vascular resistance) that is mediated by activation of histamine H_1 and H_2 receptors (187, 188). SPEV is consistently observed following moderate intensity (50-60% $VO_{2\ peak}$) and prolonged duration (≥30 minutes) exercise. The vascular component can be isolated with small muscle mass exercise where neural adjustments do not occur (109).

The SPEV is 80% blunted with H_1 and H_2 receptor antagonism (187, 188, 197). Both H_1 and H_2 receptors appear to independently contribute to the SPEV as blockade of just H_1 receptors diminishes the

first 30 min of SPEV while blockade of the H_2 receptors diminishes SPEV from 30-90 minutes post-exercise (187, 188). The differential modulation of SPEV initially by H_1 and a prolonged dilation by H_2 receptors is similar to earlier observations of decreased vascular resistance and increased limb blood flow responses to co-infusion of histamine with H_1 or H_2 histamine receptor antagonists (49). Chipman et al. noted that histamine infusion increased limb blood rapidly and the dilation extended beyond the infusion cessation. The initial rapid increase in blood flow could be attenuated with H_1 antagonists and the prolonged dilation could be blunted with H_2 antagonists(49). SPEV is also blunted by infusion of high doses of vitamin C which has the ability to directly breakdown histamine (253), and by infusion of α-FMH to inhibit histidine decarboxylase activity (255).

The dilator actions of histamine through infusion and in SPEV not only decreases resistance to flow but can also, secondarily, reduce systemic blood pressure. Blood pressure, which is a function of blood flow and the resistance to flow (Arterial Pressure = Cardiac Output X Total Peripheral Resistance) is reduced by SPEV. This reduction in systemic pressure from SPEV is termed post-exercise hypotension (72).

Post-Exercise Hypotension

Post-exercise hypotension (PEH) is primarily driven by reductions in vascular resistance (increase vascular conductance) in the previously active muscle (277). The hypotension following whole body exercise is

primarily the result of a skeletal muscle histamine mediated dilation of resistance arterioles (109, 162). In normotensive individuals, hypotension of 5-10 mmHg occurs after 30-60 min of moderate intensity exercise (50-60% VO_{2peak}) and lasts up to 2 hours. In hypertensive individuals, hypotension can be up to 20 mmHg and extend beyond 12 hours (108).

Skin Blood Flow

Human skin blood vessels contain H_1 and H_2 receptors (99, 100). Blocking histamine receptors at rest has no discernable effect on blood flow (323, 325). Infusion of either histamine or H_1 and H_2 receptor agonists result in an almost immediate increase in cutaneous vascular conductance (99, 100, 102, 143). The rise in cutaneous vascular conductance is blunted with either H_1 or H_2 receptor antagonists (100, 143, 323). Physiologically, damage to the skin results in a degranulation of histamine from mast cells leading to an increase in blood flow to the surrounding area (99). Heat stress may also increase histamine concentrations in the skin as H_1 or H_2 receptor antagonists blunt skin blood flow during local (102) and whole body heating (326).

Capillary Dilation and Permeability

Capillary permeability refers to the ease of blood plasma and proteins to cross from the circulation through the capillary walls into the surrounding tissue. Capillary permeability increases through an increase in

hydrostatic pressure secondary to an increase in microcirculatory blood flow (decreased vascular resistance) and/or by an increase in the size of fenestrations (gaps) between the endothelial and pericyte cells lining the capillaries. Regulation of the microcirculatory blood flow and permeability of the capillaries is important as this area supplies oxygen and nutrients to cells and removes metabolic byproducts.

Following endurance exercise, the permeability of the capillaries is increased for multiple hours in only in the active limbs (114). How endurance exercise increases permeability is unknown, but it is possible that exercise-associated elevations in histamine concentrations will increase both microcirculatory blood flow and the size of intercellular gaps contributing to the increase in capillary permeability.

Histamine is presumed to be the major controller of blood flow to microcirculation as minute quantities of histamine are formed at rest provide a continuous dilator influence (270–273). During exercise, an increased production of histamine may control the vascular permeability by increasing blood flow, as there is an inverse relationship between peripheral resistance and permeability measures (capillary filtration constants) (154).

Exercise-induced elevations in histamine may also increase permeability by widening intracellular gaps. Studies have shown that histamine infusion into muscle (cremaster, abdominal) widens intercellular gaps between endothelial and pericyte cells (0.1-0.8 to 7-8 micra)(176).

The majority of plasma and protein extravasation occurs on the post capillary venules where histamine receptors are highly expressed and large gaps form (42, 118, 177, 240, 300). The infused histamine causes a transient contraction of the endothelial and pericyte cells in a way that they gain a globular shape and form projections into the lumen of the vessels (178, 191) and produces a partial detachment between endothelial and pericyte cells by phosphorylation of adherin proteins (VE-cadherin) (177, 240).

During exercise the increased mechanical shear stress upon endothelial cells may result in an elevated endothelial HDC activity and histamine production (61, 124, 125). The endothelial histamine may act as the coupling signal between elevated shear stress and increased capillary permeability (124).

Lymph flow

Histamine infusion increases lymph production and flow (105, 300). The increased lymph production may not be a direct action of histamine and may be due to the increased capillary transmural pressure and filtration of plasma fluids and proteins.

If histamine is contributing to a skeletal muscle arterial dilation during exercise, it may also influence the maintenance and regulation of systemic blood pressures and cardiac function. Direct infusion of histamine into isolated animal limbs results in a

decrease of diastolic pressure (59, 148) along with an elevation of heart rate (59). In humans, direct infusion of histamine lowers vascular resistance, as well as systolic, diastolic, and mean arterial pressures while elevating heart rate (317). The decreased arterial pressure with histamine infusion can be attenuated with H_1 and H_2 receptor antagonism (148). Outside of the reduced blood pressure from infusion of supra-physiologic doses of histamine, a reduction in arterial pressure is caused by endogenously produced histamine after an endurance exercise (PEH), as noted above (109, 110, 172).

Therefore, blocking histamine's actions may have a blood pressure elevating effect during exercise by preventing the normally occurring histamine mediated dilation of skeletal muscle. Studies that examined systemic hemodynamic changes during exercise with histamine receptor blockade are presented in Table 4. There does not appear to be a clear consensus between the studies, as most show no changes hemodynamic variables and others showing varied increases. A well regulated blood pressure is not surprising as it is constantly monitored and adjusted through aortic and carotid baroreceptor neural pathways and the interaction of many systems and tissues (258, 259). These neural pathways reflexively control heart rate, myocyte contractility, and peripheral resistance through autonomic control and the influence of a singular dilator in skeletal muscle likely has little influence on systemic regulation.

Table 4. The effect of histamine receptor antagonists on cardiac and blood pressure measures during exercise.

		Antagonist		Cardiac /Blood Pressure Variable							Exercise		
Author	Year	H_1	H_2	HR	SV	SBP	DBP	MAP	TPR	CO	Type	Duration	Intensity
Peterlin et al.	1998	Chlorphiramine Maleate (4 mg)		↔		↔	↔	↔	↔	↔	Cycle	20 min	50% VO_2
Lockwood et al.	2005	Fexofenadine (540mg)		↔				↔			Cycle	60 min	60% VO_2
Warburton et al.	1979		Cimetidine (200mg)	↓↔		↓↔		↓↔			Treadmill	10 min	Sub-Max
Hughes et al.	1989		Cimetidine (300 mg)	↓↔		↔	↔	↔			Treadmill	~12 min	25, 50, 75, 100% VO_2
			Ranitidine (150 mg)	↓↔		↔	↔	↔				~12 min	25, 50, 75, 100% VO_2
McCord et al.	2006		Ranitidine (300 mg)	↔				↔			Cycle	60 min	60% VO_2
Saltissi et al.	1981		Cimetidine (400 mg)	↔		↔					Treadmill	~11min	100%

Table 4. Continued: The effect of histamine receptor antagonists on cardiac and blood pressure variables during exercise.

		Antagonist		Cardiac /Blood Pressure Variable							Exercise		
Author	Year	H_1	H_2	HR	SV	SBP	DBP	MAP	TPR	CO	Type	Duration	Intensity
Doh et al.	2016		Ranitidine (300 mg)	↔	↔	↑	↔	↑	↑		Cycle	12 min	60% VO_2
McCord et al.	2006	Fexofenadine (540mg)	Ranitidine (300 mg)	↔				↔			Cycle	60 min	60% VO_2
Emhoff et al.	2011	Fexofenadine (540mg)	Ranitidine (300 mg)	↓				↑			Cycle	60 min	60% VO_2
Pellinger et al.	2013	Fexofenadine (540mg)	Ranitidine (300 mg)	↔				↔			Cycle	60 min	60% VO_2
Romero et al	2015	Fexofenadine (540mg)	Ranitidine (300 mg)	↔				↔			DKE	60 min	60% peak power

↑ = Increased, ↓ = Decreased, ↔ = No Change, HR = Heart Rate, SV = Stroke Volume, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, MAP = Mean Arterial Pressure, TPR = Total Peripheral Resistance, CO = Cardiac Output, DKE = Dynamic Kicking Exercise

Does Histamine Contribute to Skeletal Muscle Glucose Delivery and Uptake During Exercise?

Histamine H_1 receptor blockade in mice reduced endurance walking duration which was associated with a significantly lower muscle glycogen content compared to control mice (212). It is plausible that blocking the histamine mediated dilator actions in the arteries and capillaries during exercise reduced blood flow and therefore glucose delivery. The reduced glucose delivery from circulation into the interstitial fluid surrounding contracting myocytes would place a greater reliance upon intracellular glycogen for energy. Alternatively, histamine may have blunted glucose uptake into the myocytes. In either scenario, there would be a greater reliance on intracellular glycogen as delivery/uptake of extra-myocellular glucose would be limited. The increased reliance on intracellular glycogen would decrease the time to fatigue onset as intramuscular glycogen levels diminished.

There is evidence that glucose delivery and insulin sensitivity are reduced following exercise. Following 60 min of endurance exercise blood flow is reduced to the previously exercised muscle with histamine H_1 and H_2 receptor antagonists and this in turn reduced interstitial glucose concentrations in the vastus lateralis muscles (79, 234). In humans, glucose uptake during exercise is not dependent upon insulin but it is worth noting that histamine may activate a currently unknown pathway to increase glucose uptake within the skeletal muscle myocytes. As evidence 1) histamine application causes an increase in glucose uptake in single cell organisms (156) and 2) histamine application to human cardiac endothelial cells *in vitro* increases glucose uptake that is attenuated with H_2 receptor antagonism (306).

In the previously exercised human, histamine H_1 and H_2 receptor antagonism blunted insulin sensitivity by ~25% during a glucose tolerance test (233). The reduced sensitivity to insulin following exercise may be specific to H_1 and H_2 receptors as mice selectively bred without H_3 receptors display reduced insulin sensitivity at all times (301).

Is Histamine Involved in an Exercise-Induced Inflammatory/Immune Response?

Inflammatory or immune responses are initiated to protect tissue and maintain function following injury (279). These responses are initiated within the injured tissue and are comprised of complex and coordinated reactions involving multiple cell types, molecules, and signals (107, 279). The progression of the responses are similar regardless of the affected tissue or cause of injury (e.g. bacteria, virus, chemical, heat, or trauma), but the intensity of the reaction is proportional to the severity of the injury (279). The acute phase of the inflammatory/immune response is characterized by changes in the microvasculature, including the vasodilation of blood vessels, increased permeability, clotting or leaking of proteins through the capillaries, and migration of leukocytes into the tissue (240).

The first leukocyte responders to the tissue injury are resident macrophages. Next, circulating leukocytes increase in number (leukocytosis) and are recruited to the damaged tissue through margination, diapedesis, and chemotaxis. Approximately an hour after the injury, circulating neutrophils traverse into the tissue. In the following hours to days blood monocytes follow the same route and mature into macrophages under the influence of chemical signals from T-helper cells (107, 279). The neutrophils and macrophages

protect the tissue by removing invading organisms or damaged tissue and initiate the formation of new healthy tissue. Histamine potently influences the initiation and modulation of the these inflammatory and immune responses by regulating leukocyte recruitment, leukocyte maturation, and cytokine expression (22, 146, 224).

Leukocyte recruitment; margination, diapedesis, and chemotaxis

In order for leukocytes to pass from the circulation to the area of injury, openings need to form in between the cells lining the blood vessels. The widening of the gaps between these endothelial and pericyte cells is referred to as margination. The gaps widen due to conformational changes in the internal cellular structure to form projections into the lumen of the vessels and partial detachments with surrounding cells (178, 191).

Local histamine production may have a significant role in the margination process. The endothelial and pericyte cells on the venule side of the capillaries contain high concentrations of histamine receptors (42, 118, 177, 240, 300). When these receptors are stimulated by histamine, the intercellular gaps increase in size (176). The widened gaps are necessary for leukocyte diapedesis into the tissue.

The leukocytes are traveling with the blood and need a way to attach to the blood vessel lining in order to pass through the newly formed intercellular gaps. Normally, leukocytes are prevented from attaching to endothelial cells due to a lack of binding sites (receptors) and the expression of heparin on the cell surface. During inflammation, endothelial cells in close proximity to the injury have

increased expression of adhesion molecules (i.e. selectins, ICAM, VCAM) that attach to leukocytes, specifically neutrophils and macrophages (203, 240, 291).

The presence of histamine may also have a significant role in the process of leukocyte adhesion. *In vivo* experiments involving histamine infusion into the capillaries have shown an increase in the number of leukocytes attached to the endothelium (74). In fact, histamine may increase the expression of adhesion molecules on endothelial cells as *in vitro* models indicate histamine increases the intracellular molecule, NF-kB, that is associated with the translocation of adhesion molecules (ICAM) to from the cytoplasm to the cell membrane (169, 203). Additionally, leukocytes exposed to histamine *in vitro* increase CD11/CD18 integrins, transmembrane receptors involved with cell adhesion (169). The histamine induced expression of ICAM is reduced with H_1 receptor antagonists (166, 203).

Once the leukocytes have entered the intercellular space, they move along a chemical gradient toward the site of injury. Here, histamine may again play an important role. Histamine appears to be chemotactic to neutrophils (22, 24). Histamine can also initiate other cells (T-cells) to produce leukocyte chemoattractants (e.g. IL-5) (22, 241). The expression of interleukin chemoattractants has been attenuated with H_1 receptor antagonism (319).

Monocyte maturation into macrophages; TH_1 and TH_2, response

Monocytes, at the site of tissue injury mature into macrophages under the influence of chemical signals from T-helper cells (107) and nerve growth factor (186). The T-helper cells can be divided into subsets based on their cell surface receptors, production of cytokines, and function. The subsets play distinctive roles in the development, initiation, and regulation of the immune response (224). The influence of the T-helper subsets are not predetermined but are influenced by signals that drive the cells toward either subset (147). Associated with a TH_1 response is an increased IL-2, INF-y, IL-4, IL5, IL-12, IL-13, and TNFB concentrations (206). The TH_2 response is associated with elevated IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 production (310).

Similar to the Th_1 and Th_2 responses, mature macrophages are identified using similar nomenclature, M_1 and M_2 macrophages (201). Broadly categorized, the TH_1/M_1 response is pro-inflammatory and includes phagocytosis or removal of dead/dying cells or cellular debris. A greater TH_1/M_1 compared to TH_2/M_2 response is associated with the start of an inflammatory response. A greater TH_2/M_2 compared to TH_1/M_1 response is considered anti-inflammatory, follows the upregulated TH_1/M_1 response, and is associated with tissue repair/wound healing (107, 201).

Histamine can influence the subpopulation of cell concentrations (22, 83, 224) and alter the TH_1 and TH_2 response (147). Specifically histamine, via the H_2 receptor on monocytes, inhibits the production Interleukin-12 (IL-12) and enhances Interleukin-10 (IL-10). These interleukins remove the stimulus for TH_1

and increases the stimulus for TH_2 (75, 221, 310). There also appears to be H_1 receptors on monocyte to directly stimulate the TH_2 response (206). In isolated murine TH_2 cells treated with histamine there is an increase production of IL-13 via activation of H_1 and H_2 receptors (76). This shift in TH_1/TH_2 balance is blocked via H_1 and H_2 receptor antagonists (203, 223). These responses may be reversed within the damaged tissue as the predominant expression of histamine H_2 receptors can shift to H_1 when monocytes mature into macrophages (274, 302).

Exercise associated leukocytosis

Following exercise, there is an increase in the number of circulating white blood cells (leukocytosis) (39, 51, 210, 230, 280). The origin of the additional circulating white blood cells are unknown but it is theorized that an increase in shear forces and blood pressure during exercise cause a flushing of "marginal pools" into the circulation, possibly from the spleen, bone marrow, liver and/or lungs (280). In some reports, the rise in leukocyte count produced by vigorous aerobic exercise is followed by a decrease in cell count to levels below pre-exercise (111, 208). This drop in white blood cell count had been interpreted as an immune suppression, but it is more likely that these cells were redistributed into tissues (39). For example; radio-isotope labeled white blood cells were infused into the circulation of individuals prior to performing 300 eccentric one-legged knee-extension contractions (173). In the initial hours after exercise , the labeled cells showed up in greater number in the exercise quadriceps than the non-exercised quadriceps (173).

The pathway leading to skeletal muscle uptake of white blood cells could be due to concomitant increases in monocyte chemoattractant protein-1 (MCP-1) and selectin adhesion molecules (51). MCP, produced by endothelial as well as skeletal muscle myocytes during contraction, is involved with selective monocyte recruitment into tissues (51). Additionally, E-selectin, an inducible adhesion molecule that recruits endothelial progenitor cells to ischemic tissue, is also increased after strenuous exercise (160). If exercise associated increases in histamine influence margination, diapedesis, and chemotaxis of circulating white blood cells into the skeletal muscle, then blocking histamine's actions may delay or prevent the fall in circulating white blood cells in the hours after exercise by impeding their entry into the previously active musculature.

Studies indicate that the leukocytosis after exercise is not limited to one cell type as increases were noted in neutrophils, lymphocytes (CD19+), monocytes (CD14+ & CD16+), mononuclear, T-cells (CD3+), T-helper cells (CD4+), and cytotoxic t-cells (CD8+)(209, 232). All cells might not increase in the same proportion as there have been decreased overall percentages of lymphocytes and natural killer cells after 60 min of cycling at 65% of VO_{2peak} (122). In addition, it was shown that type I monocyte percentages decreased while type II monocyte percentages increased. The change in the predominant cell types indicate a shift in toward the proangiogenic over the phagocytotic type monocytes (160). As the number of circulating leukocytes represent only 1-10% of the total cell population, small increases/decreases are of clinical significance (280).

Exercise associated changes in cytokines/myokines

Immediately after acute intense exercise, there is an increase in a number of circulating cytokines such as IL-6, TNFa, CRP, IL-1B, MCP-1, IL-8, IL-1ra, and IL-10 (47, 85, 210, 232, 291). A number of these cytokines may originate from skeletal muscle myocytes or the surrounding tissue (232). The increase in these muscle-derived cytokines (myokines) are, at least in part, due to increases mRNA transcription (IL-6, IL-8, IL-15) (170). The cytokines that are upregulated have strong ties to metabolism, inflammation, tissue growth and development, and cell death (43).

Exercise-associated increases in skeletal muscle histamine may be an initial trigger for short and long-term alterations in myokine expression. It was recently shown that blocking histamine H_1 and H_2 receptors during exercise reduced the expression of myokine genes associated with inflammation, endothelial and vascular function, metabolism, and cell maintenance (e.g. IL-6, TNFa, IL-10 and MCP-1) within the skeletal muscle (254). This is important as very little is known about the cross talk between muscle and other organs that could underlie the effects of exercise on systemic health (185, 205). In the short term, increases in myokines (e.g. IL-6) appear to mediate substrate utilization and availability. Long term, the myokines may influence metabolic adaptions resulting from exercise training (232) and may be involved with mediating the health benefits of exercise including cardiovascular adaptions (185).

Wound healing/tissue damage

Tissue damage activates an acute inflammatory response (291). At the site of the damage, there is a high concentration of histamine (177) likely liberated from resident mast cells (213) and increased histidine decarboxylase activity (214). This histamine appears to play a critical role in the healing response especially in areas of rapidly growing tissue (166, 214). For example, mice that are bred without histidine decarboxylase, and those treated with H_1 receptor antagonists have extended skin wound healing times (221). Associated with the extended healing time was a reduced macrophage infiltration and formation of blood vessesls (angiogenises) at the wound edge (221). Conversely, mice treated with histamine, or the mast cell degranulator (48/80), at the site of damage have accelerated cutaneous wound healing times (54, 55, 221). The faster healing times were associated with increased collagen formation (54) and tensile strength of skin surface (55, 329). Therefore, exercise-associated increases in histamine concentrations may be involved with the repair process of skeletal muscle following strenuous exercise (231).

Is Histamine a Consequence of Exercise Induced Muscle Damage and Regulator of Inflammation?

Unaccustomed, strenuous, and/or eccentric muscle contractions can result in injury to muscle and connective tissue (174, 219, 289, 290). The injury is characterized by myofibrillar disruption, loss of muscle strength, increased muscle soreness, (delayed onset muscle soreness; DOMS), and the presence myocellular enzymes and proteins (creatine kinase, myoglobin, lactate dehydrogenase) in the blood (37, 210, 290). This trauma activates an acute inflammatory response (37). As evidence, with increased

creatine kinase levels after muscle damaging exercise, there is an associated increase in neutrophils, monocytes, and cytokines in circulation (66, 202, 210). In addition, immediately after exercise, there is an accumulation of neutrophils and macrophage in the exercised muscle (66, 231). Interestingly, in a model of exercise-induced muscle damaged, an isolated muscle undergoing rhythmic electrical stimulation displays evidence of mast cell degranulation and an elevated number of monocytes in areas with widened space between fibers (296). Although, many aspects of the regulation of muscle regeneration remain unclear and involve unknown molecules, the primary purpose of the inflammatory response is to repair damaged tissue and regenerate muscle (174, 231, 289, 307–309).

Therefore, exercise resulting in muscle damage can provide a model to study histamine’s potential association with exercise related inflammation. Uncovering histamine’s role in the recovery process resulting from muscle damage may provide information concerning the regulation of muscle degradation and repair (37). The few studies examining inflammation and muscle repair have demonstrated that blocking inflammatory signals (IL-10, COX blockers) have resulted in reduced leukocyte (neutrophils and macrophages) recruitment into the muscle, as well as, delayed recovery (30).

Strength loss

Loss of muscle strength is an indicator of exercise-induced muscle damage (231). Immediately after exercise, there is a reduction in maximal volitional force. The strength loss is not the greatest immediately after damaging exercise but peaks 24-48 hrs after exercise (78, 173). This indicates that structural damage is

not the only factor limiting force. Besides a reduction of muscular force due to disruption of sarcomeres and reduced actin and myosin overlap, force loss can be caused by a decreased excitation of the muscle sarcomere, decreased calcium release, or a reduced neural input into the contracting muscle. These contractile properties normally recover within the first ~24 h and voluntary force still remains depressed > 24 hrs following exercise (248).

A portion of the prolonged decrease in muscle force may be a neutrally mediated protective mechanism to limit further muscle damage through reduced motor unit recruitment (78). The neural input, or descending drive, from the motor cortex is a major determinant of voluntary strength. Sensations of pain/discomfort, experienced following muscle damaging exercise, are known to inhibit the descending drive from the motor cortex (217). Sensations of pain/discomfort are a result of increased activity of group III/IV afferent neurons (11, 30, 35, 45). The skeletal muscle group III/IV afferent fibers express histamine H_1 and H_2 receptors which, when activated, lower the threshold for stimulation. Therefore, a portion of the reduced muscle strength may be due to direct histaminergic activation of afferent fibers, along with the associated sensations of pain/discomfort (DOMS), inhibiting motor unit recruitment.

Is Histamine Related to Delayed Onset Muscle Soreness and sensations of Pain?

Delayed onset muscle soreness (DOMS) is the sensation of discomfort or pain associated with movement or palpitation of skeletal muscle. This pain normally peaks 24-48 hours after strenuous and/or unaccustomed exercise. The molecular and cellular responses that induce DOMS are largely unknown but

likely involve direct stimulation and/or alterations in group III/IV afferent nerve sensitivity (150, 217). The group III/IV afferent fibers are polymodal, have small diameters, are lightly to non-myelinated, and appear as a triad with the capillary and mast cells within muscle tissue (269, 279). Both *in vivo* and *in situ* examinations of afferent fibers suggest that histamine may directly stimulate (primary hyperalgesia) and indirectly alter the sensitivity of these afferent fibers (secondary hyperalgesia) (279).

The possible role of histamine as a mediator of pain was suggested by Rosenthal and Minard in 1939 (257). In their early experiments, it was noted that exogenous histamine induced sensations of cutaneous pain, which increased with histamine concentrations (257, 330). Since these early experiments, histamine H_1 receptors have been identified on a subgroup type III/IV afferent fibers and a further subgroup exhibits increased expression of the H_1 receptors during inflammation following injury (151, 269). Activation of the H_1 receptor stimulates/lowers the firing threshold, increases pain sensation, and results in pain induced motor impairment in rodents (179). Conversely, administration of H_1 receptors antagonists resulted in anti-nociception with histamine infusion (179).

Following exercise in which histamine H_1 and H_2 receptors were antagonized, there was a reduction in the expression nerve growth factor (NGF) and glial cell line-derived nerurotrphic factor (GDNF) (78, 254). These two factors are believed to play a central role as modulators of pain in secondary hyperalgesia (181, 237, 298, 318, 327), as well as aid in the maintenance and functioning of neural circuits (6, 254). NGF and GDNF are produced in inflamed

tissue and skeletal muscle after ischemia and nerve injury (231, 313) and are present within muscle 2-h to 2 days after exercise (140). These factors are taken up into the nerve terminals of afferent fibers and transported centrally (6, 163). Within these neurons there is an increase in H_1 receptor expression 24-hrs after the initial injury (150), an increased expression of voltage gated sodium channels (NaV1.8) (25, 279, 305, 330, 331), and an increase in the synthesis and release of neurotransmitters (substance P and CGRP) from the afferent nerves in the central nervous system (137, 158, 260, 269). The time course of cellular changes to the afferent fibers are similar to the peak pain experienced from exercise-induced muscle damage. Additionally, experiments blocking NGF revealed a blunting of pain only when the blocking occurred before/during exercise, but not following exercise (202).

These results suggest that histamine may exert a direct and indirect influence on skeletal muscle pain resulting from exercise. The increased afferent feedback can inhibit motor neuron output and may be involved with the reductions in strength, as they appear to follow similar time lines. Although, previous studies seem to suggest that strength loss is due to reduced voluntary activation not due to muscle soreness *per se* (244). However, the association of histamine, pain, and alterations of muscle strength following muscle damage has not been fully explored.

Antihistamines and Antihistamine Use

In 1937, pyrilamine, the first antihistamine, was produced by Dr. Daniel Bovet (281). This drug was viewed as a major achievement in treating allergic diseases and for his work Dr. Bovet received the 1957 Nobel Prize in Medicine (189, 288). Since the production pyrilamine, countless other antihistamines have been developed (288). The newest forms of antihistamines became established in the early 1980s (265, 288) and they have high receptor specificity.

Worldwide, these histamine-blocking agents are the largest selling drug group (75). In 1984, it was reported that 30 million Americans took an antihistamine at some point during the year. Endurance athletes use of anti-allergy medication is five times greater than the general population and they are two times more likely to use antihistamines than sprint/power athletes (1, 2). Antihistamine use in endurance athletes may be due to their higher incidence of allergies compared to other sub populations of athletes and the general public (2, 116, 117). Additionally, oral antihistamines are frequently used by marathon and ultramarathon runners to treat gastrointestinal reflux, ulcers, exercise induced anaphylaxis, GI bleeding, and stress ulcers (18, 196, 199).

The widespread use of histamine blocking agents in the public and especially in endurance athletes suggest the need to further examine the role of histamine in the exercise response.

CHAPTER III

EXPLAINATION OF THE METHODOLOGY

The experimental protocols described in this dissertation were approved by the Institutional Review Board of the University of Oregon (Protocol# 05262016.047 and 02022017.003) and the University of Utah (Protocol#000308100). Written and informed consent was obtained by all volunteer subjects following an explanation of experimental procedures for each individual study. The studies were run in accordance with the guidelines set by the Office of Human Subjects Compliance of the University of Oregon, University of Utah, and of the Declaration of Helsinki.

General Experimental Approach

The studies detailed in Chapters IV and VI were completed at the University of Oregon, in the Evonuk Environmental Physiology Core Laboratory and the Bowerman Sports Science Clinic. The study detailed in Chapter V was completed at the University of Utah, Veterans Administration Medical Center in the Geriatric Research, Education, and Clinical Center.

All studies were conducted as randomized double-blind placebo-controlled cross-over interventions. Volunteer subjects completed the testing once under placebo conditions (Placebo) and again under histamine H_1 and H_2 receptor antagonism (Blockade) conditions. This experimental approach is highly recommended for intervention based studies (193) to control for intrasubject variability and increase the likelihood of identifying physiological differences between the Placebo and Blockade interventions. Additionally, blinding the

investigators as well as the subjects to the drug treatment limited potential preconceived bias on study outcomes. In all studies, investigators were unblinded to the treatments upon completion of data analysis.

In the study detailed in Chapter IV, the importance of the exercise-induced elevation in skeletal muscle histamine concentration to the endurance exercise response was evaluated, i.e. is histaminergic signaling an important pathway in facilitating endurance exercise in humans? Eleven competitive cyclists performed six 10-km time trials on separate days. The first two trials served as familiarization trials. The next two were performed after consuming either Placebo or Blockade and resting for 120 min prior to the trial. The final two trials were performed after consuming either Placebo or Blockade and cycling at a workload eliciting a VO_2 of 50% of peak for 120 min prior to the trial. The main outcome variable was the time to completion of the 10-km time trials. Value added variables include heart rate, blood pressure, blood lactate and glucose, cardiac output, isometric muscle strength of the quadriceps, and rating of perceived exertion. The value added variables were collected during the 120 min of rest and exercise preceding the time trials. In addition, blood lactate and glucose, isometric muscle strength of the quadriceps, and rating of perceived exertion were measured prior to and following the time trials.

Chapter V examined the potential relationship between the exercise-induced increase in intramuscular histamine concentration to the elevation in skeletal muscle blood flow during exercise, i.e. is the exercise-induced elevation in skeletal muscle histamine acting as a vasodilator to contribute to exercise

hyperemia? This study was completed in conjunction with the University of Utah. In this study, sixteen volunteers performed dynamic single-leg knee extension exercise on two separate days. After receiving either the Placebo or Blockade, subjects performed exercise of increasing intensity from 20, 40, 60, and 80% of peak power output. This step-wise increase in exercise intensity was followed by a 60 min bout of single-leg knee-extension exercise at 60% of peak power output. Then subjects performed another step-wise increase in exercise intensity at the same 20, 40, 60, and 80% of peak power output. The main outcome variable was femoral blood flow, which was compared between each exercise intensity, before and after a 60 min sustained bout of exercise, during the 60 min of sustained exercise, and between Placebo and Blockade conditions. Value added variables were VO_2, intramuscular pH, intramuscular oxygen saturation, blood pressure, cardiac output, and rating of perceived exertion. The value added variables were collected during each step-wise increase in exercise intensity and during the 60 min of steady state knee-extension exercise.

Chapter VI of this dissertation examined whether the exercise-induced elevation in skeletal muscle histamine concentration is related to the elevation in circulating leukocytes and cytokines following exercise, i.e. is the exercise-induced elevation in skeletal muscle histamine concentration a signal to initiate a systemic inflammatory response? In this study, 12 volunteers completed two rounds of a 72 hr study observation period, one following Placebo the other after Blockade. Subjects initially completed a bout of muscle damaging exercise and were evaluated 6, 12, 24, 48, and 72 hrs after exercise to capture the time course

of inflammation, strength change, and muscle soreness. The main outcome variables of this study were the concentrations and types of circulating inflammatory cells and inflammatory signaling molecules. Value added variables included measures of muscle force and muscle soreness.

Volunteer subjects

A total of 39 subjects completed the three studies and no subject participated in more than one study. Subjects were deemed healthy following standard screening and were free of any medical condition with known associations or influence on blood pressure or blood flow (e.g. diabetes, anxiety/depression, atherosclerosis, chronic obstructive pulmonary disease)(44, 103, 165, 216). All subjects were asked to abstain from caffeine and alcohol consumption as well as strenuous exercise for 24 hrs before data collection due to their acute influence on blood pressure (132, 218, 322). No subjects were using over-the-counter medications at the time of the study, with the exception of oral contraceptives for females. In the study detained in Chapter V and Chapter VI, female subjects were studied in the early follicular phase of their menstrual cycle or during the placebo phase of their or oral contraceptive. Controlling for menstrual cycle phase in Chapter V and VI was done to reduce potential sex hormone influence on blood flow (Chapter V) and on muscle damage and inflammation (Chapter VI). For Chapter IV, menstrual cycle phase was not controlled for in the female volunteers. All females had negative pregnancy tests prior to any data collection.

Specific Methods

Subject Characterization

In the studies included in this dissertation, a combination of demographic and anthropometric measures including age, height, weight, and body composition in addition to aerobic capacity, anaerobic power, and muscle strength were used to portray the subjects and generalize the study findings to the public. Subject weight was measured using a digital scale (Sartorius model MAPPIU-150FE-L, Elk Grove IL, Chapter IV and VI) or balance scale (Health-o-Meter, Continental Scale Corp, Bridgeview IL, Chapter V) and height was measured using a stadiometer (SECA North America, Chino CA, Chapter IV, V, VI).

Body Composition

Assessment of body composition of the subjects represents a global characterization of health and fitness. Body composition was estimated using a two-component model of measuring fat mass and fat-free mass. Fat mass was estimated using the skin fold technique in which calipers of a set tension are used to obtain the thickness of subcutaneous fat. Thickness measures were made on the right side of the body at the triceps, suprailiac, and thigh sites for females and chest, abdominal, and thigh sites for males according to the American College of Sports Medicine guidelines (8). The sum of the three site thickness measurements in addition to the volunteer's age and race were used in regression equations to calculate body density and the percentage of fat mass. The regression equations were initially developed by Jackson and Pollock (133, 135) to predict body

density in comparison to measures obtained from hydrostatic weighing. This method was only used describe subject characteristics within Chapter IV and VI. Body fat was not calculated with the skin fold technique in Chapter V, as it is not part of the University of Utah usual screening procedures.

More accurate estimates of body composition can be made by expanding beyond the three-site to a seven-site model which includes the triceps, chest, midaxillary, subscapular, suprailiac, abdominal, and thigh site measurements (134). Other techniques to quantify body composition include calculations of body density through underwater weighing or air displacement plethysmography (BODPOD). Bioelectrical impedance is a non-density based measurement in which the resistance of an electrical current through the body is used to estimate fat mass (60, 239). One of the most accurate techniques to measure body composition is dual energy X-ray absorptiometry (DEXA). This technique uses an X-ray tube with a filter to generate low-energy (40kV) and high-energy (70-100kV) photons. The photons are absorbed as they pass through the body and the resulting energy states of the rays are used to quantify tissue densities (239). The DEXA method is sensitive enough for an assessment of bone mineral content as well as fat mass and fate-free mass (three-compartment model). The added accuracy of techniques such as the BODPOD and DEXA would not likely alter the characterization of the subject pool. Therefore, the relatively simple three-site skin-fold estimate of body composition was the preferred method in the present studies as it was not a main outcome measure and only used to depict the subject population.

Anaerobic Power

The amount of power (work per unit time) of the subjects legs were measured using a 10 s Modified Wingate on a cycle ergometer (Excalibur Sport V2; Lode BV, Groningen, The Netherlands). Subjects pedaled at a maximal rate (distance 6m per pedal revolution) against a fixed resistance (force equal to 0.70 Nm; Wingate for Windows software version 1; Lode BV, Groningen, The Netherlands) to calculate work (force · distance). The maximal work over a 1 s time interval was taken as the peak anaerobic power. This measure was performed in the study outlined in Chapter IV. Other tests of anaerobic power include the velocity spectrum test on a computer integrated load cell resistance machine (e.g. Biodex) and sprint running tests. The cycle Wingate test was selected as it is the identical mode of exercise used in the time trials and due to the ease of calculating power.

Aerobic Capacity

Cardiopulmonary fitness was assessed by calculating the highest rate of oxygen transport from the environment to the working muscles during exercise (VO_{2peak}). The normal oxygen consumption of humans at rest is approximately 3.5 $ml \cdot kg^{-1} \cdot min^{-1}$ and can rise to 70-90 $ml \cdot kg^{-1} \cdot min^{-1}$ in elite endurance athletes at maximal work rates. Oxygen consumption of the skeletal muscles during exercise is a function of delivery (heart rate (HR) + stroke volume (SV)) and extraction (oxygen difference between arterial and venous blood (ΔAVO_2)). Measurement of arterial and venous blood gas saturations at the skeletal muscle is an invasive

procedure involving catheters. A non-invasive substitute is to measure the fraction of inspired (F_IO_2) and expired (F_EO_2) oxygen, which approximates the amount transported into and used by muscle.

Oxygen consumption was measured in the studies presented in Chapter IV and V. The exercise used to obtain VO_{2peak} was specific to each study, cycling in Chapter IV and single-leg knee extension in Chapter V. Both studies measured oxygen uptake via a mixing chamber system (Parvomedics, Sandy, UT) either as a standalone unit (Chapter V) or in combination with a mass spectrometer (Marquette MGA 1100, MA Tech Services, St. Louis, MO; Chapter IV).

Measurement of maximal rate of oxygen consumption (VO_2) during large muscle mass exercises (running, swimming) is considered the gold standard of integrated cardiopulmonary-muscle oxidative function (242). Tests of oxygen consumption that utilize small muscle mass exercises (arm crank, single-leg knee extension) do not represent the aerobic capacity of the whole body but are specific to muscle groups (exercises) and are referred to as peak measures (VO_{2peak}). True max tests should be accompanied by high ratings of perceived exertion (19-20), heart rate (>95% of predicted), a respiratory exchange ratio greater than 1.0, plateau of VO_2 measures with increasing workloads, and/or no rise in VO_2 during a verification phase (110% of VO_{2peak} work rate)(242). The oxygen consumption measurements can be made with open circuit techniques, which require knowledge of gas concentrations in the room air (inspired) and expired air, but can also be made with closed circuit measures where the subject breathes a predetermined volume of oxygen from a bag over a set time period. Additional,

yet less precise, tests to estimate oxygen consumption include the use of workload or the length of time to complete a task. The workloads or time variables are used as inputs into previously derived regression analyses to predict maximal oxygen consumption. For example, tests may involve stair stepping (i.e. Harvard Step Test) or walking/running set distances (i.e. Rockport Walking Test, 1.5 mile Run Test). The more precise measure of oxygen consumption using open circuit spirometry was chosen due to the ease of obtaining the measurement and the accuracy provided to determine workloads in the exercise interventions for Chapter IV and VI.

Strength Testing

The primary function of skeletal muscle is to generate force for movement or joint stabilization. Measurement of muscle force generation is also a measure of muscular fitness. Maximal voluntary isometric torque of the knee extensors was determined with a rehabilitation dynamometer (Biodex, System 3, Shirley, NY). Isometric knee extension at 30 degrees was selected for measurement of torque. An isometric muscle contraction was preferred over isokinetic/isotonic contractions in order isolate and gain an accurate measure of force while removing a velocity component present in isokinetic and isotonic contractions. Additionally, these studies were interested in the activation of the quadricep muscles and an isometric contraction allowed for comparison of forces produced voluntarily and with force changes resulting from evoked potentials. Torque testing was performed at 30 degrees of knee flexion because 1) the knee is in mid

extension, 2) it is at an angle where large torque forces are generated and near maximal recruitment (EMG) occurs, and 3) there is a large portion of actin and myosin myofibril overlap (106). The Biodex system provides high temporal resolution and allows for repeat measures between multiple study days. Isometric strength testing using the dynamometer was performed in the study detailed in Chapter VI as this method.

Drug intervention

Histamine H_1- and H_2-Receptor Blockade

To uncover histamine's role in the exercise responsome, the studies in this dissertation used the second/third generation histamine H_1 and H_2 receptor antagonists, fexofenadine hydrochloride (NDC 41167-4122, $C_{32}H_{39}NO_4 \cdot HCL$) and ranitidine hydrochloride (NDC 0173-0393-40, $C_{13}H_{22}N_4O_3S \cdot HCL$) to disrupt histaminergic signaling in humans. Volunteers were given an oral dose of 540 mg of fexofenadine and 300 mg ranitidine 60 min prior to exercise. The quantity of fexofenadine and ranitidine are the manufacturer recommended upper limit of a single dose. Fexofenadine is a selective H_1-receptor antagonist, which reaches peak plasma concentrations within 1 hr after consumption. This dosage of fexofenadine has a 12 hr half-life (263). Ranitidine is a selective H_2-receptor antagonist which reaches peak plasma concentration within 2 hrs after consumption and has a 3 hr half-life (93). This dosage of histamine-receptor antagonists results in more than 90% inhibition of histamine H_1 and H_2 receptors lasting for 6 hours after administration (93). These two antagonists are not

thought to cross the blood brain barrier and affect central histaminergic signaling, unlike first generation antagonists (e.g. diphenhydramine aka Benadryl) which cross the blood brain barrier and cause a reduction in alertness (sedative effects)(263). Importantly, fexofenadine and ranitidine have not been shown to alter blood flow, heart rate, blood pressure, or smooth muscle tone at rest (78, 187, 254).

Histamine H_3 and H_4 receptors were not antagonized as there are limited medications available and these receptors are primarily found within the central nervous system (H_4) or act as self-regulating inhibitors of histamine release (H_3). The studies documented in this dissertation focused on how exercise-induced histamine affected the skeletal muscle and surrounding tissue during exercise. Skeletal muscle and the surrounding tissue are areas where there are a high concentration of H_1 and H_2 receptors (187, 188).

The studies in this dissertation used medications that prevent the ligand, histamine, from interacting with its target receptor by physically covering the receptor and thereby disrupting histaminergic signaling but not altering the baseline cellular activity of the receptor. In contrast, another form of "antihistamine" is an inverse agonist, which is a molecule that binds to the target receptor and decreases cellular activity. Other methods to disrupt histaminergic signaling would be to prevent histamine formation from HDC (e.g. a-fluoromethylhistidine dihydrochloride (a-FMH)), bind histamine to prevent it from interacting with its receptors (histamine reverse antagonists), or prevent histamine from being released from mast cells (e.g. chromolyn). Conversely, to

study histaminergic signaling, the pathway could be stimulated by increasing histamine concentrations without exercise. Increasing histamine concentrations could occur through 1) direct injection, 2) increased release from mast cells (compound 48/80) or, 3) drugs could be given to mimic histamine (histamine receptor agonists). The use of systemic histamine injection or mimetic drugs is not a preferred method to study histamine signaling due to potentially life threatening reductions in blood pressure (similar to anaphylactic responses).

Placebo

A placebo intervention was used to test the normal physiological response in comparison a condition where histaminergic signaling on H_1 and H_2 receptors is blocked. The use of a placebo medication blinds both the subjects and researchers to the trial conditions. The placebo pills used in these studies were manufactured by a compounding pharmacy (Creative Compounds, Wilsonville, OR) or by the Pharmacy at the Veterans Administration Hospital (University of Utah, Salt Lake City, UT). The placebo pills contained the inert fillers of the fexofenadine and ranitidine tablets (silicone dioxide, croscarmellose sodium, hypromellose, iron oxide blends, magnesium stearate, microcrystalline cellulose, polyethylene glycol, povidone, pregelatinized starch, titanium dioxide, triacetin). These fillers are not known to have any cardiovascular effects.

Placebo pills were used instead of no medication to reduce potential subject bias on subjective measures of pain/discomfort and effort as histamine has the potential to influence afferent neurons. Specifically, group III/IV afferent fibers possess both H_1 and H_2 receptors and when stimulated increase sensations

of discomfort/pain. Specific to Chapter VI, previous reports suggest that blocking histaminergic signaling reduces subjective assessment of muscle soreness associated with muscle damage (78). In order to control for subject pre-conceived bias of subjective measurements, psychological studies sometimes employ five groups of subjects. The psychological studies group subjects to receive either 1) no medication, 2) a placebo and told they received a placebo, 3) a placebo and told they received the active medication, 4) an active ingredient and told it was a placebo, or 5) an active ingredient and told it was the active medication (See *Kienle and Kiene 1997* for review (153)). Although, the validity of overly complex randomization schemes has been questioned as current literature reviews and metaanalysis suggest that the "placebo effect" may not be an important confounding factor for drug intervention studies (153). Regardless, a practical limitation of running a study involving multiple subject groups is the large number of subjects needed for biological studies comparing physiologic responses.

The studies in this dissertation assessed the subjective measurements of discomfort/pain and exercise effort as value added variables; therefore, the addition of extra subject groups to test subjective measures may be useful for follow up studies if exercise effort or muscle soreness/pain are markedly different between Placebo and Blockade groups.

Exercise Interventions

Performance Testing

Performance testing is a common and important measure in sport science and physiology research (53). A good performance test has validity, reliability, and sensitivity. Validity, reliability, and sensitivity refer to the testing protocol simulating the real world performance, the variation from repeat testing, and the ability to detect small but important changes in test outcomes, respectively (53). Time trials and time to exhaustion tests are the two most common forms of performance tests. In time trials, subjects complete a set distance as fast as possible, During time to exhaustion tests, subjects exercise at a set intensity for as long as possible. Time trials are preferred over time to exhaustion tests as they have demonstrated low variability between repeat trials (120, 126, 127) and lower coefficients of variation when compared to time to exhaustion tests (53). Additionally, time trials have greater validity than time to exhaustion tests as they simulate and have a high correlation to real world performances (225). Therefore, time trials were the main performance test used in the study detailed in Chapter IV.

A 10-km time-trial distance was selected as the duration of time to complete the task was approximately 15-20 min and was of sufficient duration to primarily challenge the aerobic system where muscle blood flow is an important performance determinant, but not so long as to introduce confounding influences on performance (i.e. dehydration, mental fatigue). Additionally, many cyclists have experience performing hard efforts of this duration providing “real world” or

ecological validity and these tests are commonly used in human performance/exercise physiology performance literature (127). The 10-km time-trials were performed on a computer-integrated cycle ergometer (CompuTrainer, RacerMate Inc., Seattle, WA, USA) using the manufacturer's ergometer computer program that provided a race simulation (Figure 2). All time-trials were performed adhering to the standards outlined by Currell (53). During the time-trials there was: 1) no feedback on performance, 2) no distractions, 3) no encouragement, 4) no physiological measures (except for heart rate), 5) no performance cues, 6) temperature and humidity were controlled, and 7) subjects always used their own bike equipment. Tests using these guidelines are highly reproducible (coefficient of variance = 1.1 ± 0.9 %), and highly correlated with on-the-road competitions (r = 0.98)(225).

Figure 2. An example set-up of the bike and time trial simulator used to test the association of histaminergic signaling in endurance exercise performance (Chapter IV).

Single-leg dynamic knee-extension exercise

This exercise set-up was designed at the Muscle Research Center in Copenhagen, Denmark (7). The single-leg knee-extension exercise effectively

isolates the quadricep muscle group and positions the subject in a semi-reclined position in which Doppler ultrasound can be used to assess femoral artery blood flow. In this set-up, the subject is seated in a chair with a boot connected to the crank arm of a cycle ergometer. Contraction of the quadriceps muscles extend the leg and rotates the ergometer crank-arm. The force of the muscle contraction is monitored by a force traducer connected to the flywheel (Figure 3). The momentum of the flywheel allows for passive extension of the quadriceps muscles following contraction. The single-leg knee-extension set-up was used as an exercise intervention due to the isolation of the quadricep muscle group, the relative ease to monitor blood flow to the exercising muscle, as well as comparison to the existing literature examining femoral artery blood flow (5, 16, 17, 68). This exercise intervention was used in the study detailed in Chapter V.

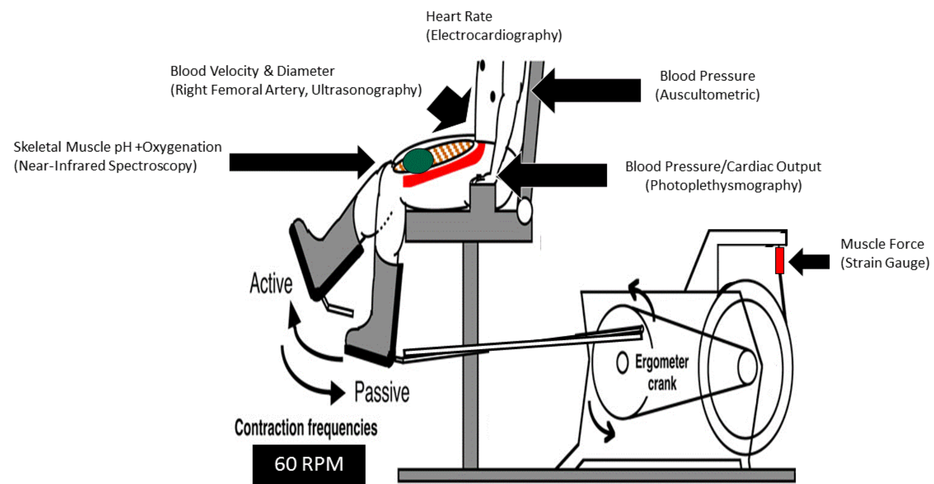

Diagram adapted from Andersen 1985 (7) and Osada 2011 (222)

Figure 3. Diagram of the single-leg knee extension exercise and the variables measured in examining the association histaminergic signaling with let blood flow (Chapter V).

Muscle damaging protocol

An eccentric muscle contraction occurs when the muscle is attempting to contract but the opposing force is greater than the muscle force resulting in a lengthening of the muscle. This type of muscle contraction causes disruptions (damage) in the muscle (actin and myosin myofibril connection and Z-disc) and results in a phenomenon termed delayed-onset muscle soreness (DOMS). This type of exercise was performed in Chapter VI.

Previous studies have shown that intramuscular histamine concentrations increase with endurance type exercise where the muscle rhythmically contracts. This study attempted to combine the rhythmic contractions of conventional endurance exercise with eccentric contractions. The combination of eccentric and endurance exercise will provide a model to simultaneously study histamines association with DOMS and immune function. The subjects in this study performed 300 eccentric contractions of the quadriceps on a resistance dynamometer (Biodex, System 3, Shirley, NY). The 300 knee-extensions were divided into 10 sets of 30 repetitions with a 60 s rest period in between each set. Subjects sat with their back supported, pelvis strapped into the seat, and the resistance pad was placed at 75% of the length of the shank. The knee joint moved through a 75°range from a joint angle of ~20° to 95° of knee flexion. The angular velocity was set to $60°\cdot s^{-1}$ for set 1 and $110°\cdot s^{-1}$ for set 2 through 10. The resistance force of the dynamometer was set at 140% of each subject's maximal isometric force measured at a joint angle of 30° of flexion. This protocol is modeled after Newham et al.(207) in which a moderate level of muscle damage

arose from the 300 eccentric contractions. The whole protocol was completed in approximately 40 min. Preceding and following the eccentric contractions subjects completed 3 maximal voluntary contractions (MVCs) and 3 maximal voluntary contractions + evoked potentials. Between each effort, subjects remained seated at rest for 60 s. The length of time to complete the maximal contractions was approximately 10 min. Therefore, the total amount of exercise time was approximately 60 min, a time duration intended to match the endurance exercise tasks known to elicit a rise in intramuscular histamine concentrations (187, 188, 255).

The eccentric contraction of single-leg knee extension approach using a calibrated dynamometer was preferred over other muscle damaging exercise protocols such as downhill running, calf lowering, and eccentric cycling. The use of single-leg knee extensions isolated a muscle group, allowed for repeated testing on the same individual (left vs right leg; Placebo vs Blockade), permitted a precise measure of total work, and was practical for repeat measures of muscle strength and soreness. Downhill running and eccentric cycling to induce lower body muscle damage are common protocols (38, 78, 230) but these methods are not optimal for cross-over study designs as there are reduced soreness and cellular responses upon future exercise, termed the repeated bout effect, which may last up to 9 weeks (38).

Hemodynamic Measures

The term hemodynamic refers to the movement of blood within the human body. The movement of blood is primarily determined by the heart. The heart's main purpose is to increase pressure within the arterial system (*Blood Pressure*) so that blood will flow from a place of high pressure (*Arterial System*) to a place of low pressure (*Venous System*). The amount of pressure the heart produces is a function of cardiac contraction (*Heart Rate*), the amount of blood pumped with each heart beat (*Stroke Volume*), and the resistance to blood flow (*Total Peripheral Resistance/ Systemic Vascular Conductance*).

Histamine acts as a vasodilator during immune/inflammatory responses, during reactive hyperemia, during tumor formation and growth, during heat stress within the skin, and with post-exercise vasodilation (9, 187, 188, 226, 273, 325). If histamine may also acts as a vasodilator during exercise this could reduce total peripheral resistance and alter hemodynamic measures. Therefore cardiac output, heart rate, arterial pressure, stroke volume, total peripheral resistance and systemic vascular conductance were measured during steady state exercise in Chapter IV. Additionally, Chapter V measured blood flow within the femoral artery, heart rate, arterial pressure, total peripheral resistance, and systemic vascular conductance at multiple exercise intensities and during extended exercise.

Heart rate and Arterial pressure

Heart rate was monitored using a three-lead electrocardiogram in the studies described in Chapter IV and Chapter V. Arterial pressure was measured on the left brachial artery using an automated auscultometric sphygmomanometer (Tango+, SunTech Medical, Raleigh, NC, USA) and in Chapter IV and using finger photoplethysmoraphy (Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands). The finapres provides an indirect assessment of brachial arterial pressure based on volume/pressure changes at the index/middle finger (finapres = finger arterial pressure) (129). This method has been validated against intra-arterial pressure transducers within the brachial artery at rest and during Valsalva maneuvers in normotensive and hypertensive individuals (129). Beat-by-beat arterial pressure waveforms were used to calculate stroke volume and in conjunction with heart rate as inputs in the "modelflow method" then estimate cardiac output and provides a reliable value for the change in cardiac output with exercise (299). This method has a high correlation coefficients (0.91-0.98) and low errors <2.0 L compared to echocardiography (299). Mean arterial pressure was calculated as diastolic pressure plus 1/3 pulse pressure (systolic pressure - diastolic pressure).

Cardiac Output

Cardiac Output is the absolute amount of blood pumped by the heart over time. Assuming minimal right to left heart shunting (absence of a patient foramen ovale and 2 to 3% of cardiac output from bronchial and thebesian circulations at

rest (107)) the total amount of blood pumped systemically also traverses through the pulmonary system. Therefore, indirect estimates of cardiac output can be made with the measurements of inhaled and exhaled gasses. In the study in Chapter IV, cardiac output was estimated by using an open-circuit acetylene (C_2H_2) washin method in which subjects breathed a gas mixture containing 0.6% acetylene, 9.0% helium, 20.9% oxygen, and balanced nitrogen for 8 breaths via a two-way non-rebreathing valve attached to a pneumatic sliding valve. During the washin phase, breath-by-breath acetylene and helium uptake were measured by a respiratory mass spectrometer (Marquette MGA 1100, MA Tech Services, St. Louis, MO) and volume was measured via a pneumotach (model 3700, Hans Rudolph, Kansas City, MO) linearized and calibrated by using test gases before each testing day. Gas concentrations of inspired and expired helium and acetylene were measured with each breath. The difference between inhalation and exhalation concentrations of the gases is assumed to be the volume absorbed into the blood in the pulmonary circulation. This method is limited by the assumption of solubility of acetylene in the blood (solubility may change based on blood temperature (as summarized by 18) and that there is limited/no shunting of blood past pulmonary circulations. This method is based on the Fick principle in that the total uptake and release of any substance in an organ/tissue/system is the product of blood flow to the region and arterial to venous difference of the substance. Gases such as carbon dioxide (CO_2) and nitrous oxide (N_2O) have also been used for the washin breathing method. As cardiac output is a determinant of exercise performance, the accuracy of the acetylene wash-in method was required to detect

potentially subtle differences in cardiac output between the Placebo and Blockade conditions that might affect exercise capacity (Chapter IV).

Cardiac output was also estimated non-invasively via finger photoplethysmoraphy (Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands) and recorded via a data acquisition device (Medwave Vasotrac APM250A; Biopac, Goleta, CA). This method estimates cardiac output based on the difference in systolic and diastolic blood pressures measured in the finger. The finapres system is not as accurate as the acetylene wash-in technique but provides a reasonable measure when assessing changes in cardiac output and can be integrated with ease into the single-leg knee extension exercise set-up (Chapter V).

Alternatives for estimating cardiac output include 1) dilution methods that involve arterial injections of an indicator of known volume and concertation (dye or thermal) with the analysis of downstream concentrations to obtain a time dilution curves, 2) an echocardiogram to obtain the volume of the left ventricle in combination with heart rate, and 3) impedance cardiography which measures transthoracic impedance and changes in electrical conductivity due to blood flow in the chest.

Femoral artery blood flow

Femoral artery blood flow, Chapter V, was determined via duplex ultrasonography using a linear-array ultrasound transducer (Logiq e9, GE Medical Systems, Milwaukee, WI). This method uses a probe, which produces sound

waves that bounce off tissues and the difference between outgoing vs incoming wave velocities to create a 2-dimensional image (Figure 4). The image allows for the measurement of artery diameter and the shift in sound waves (Doppler Shift) is used to calculate the velocity of blood flowing past the probe (Figure 4). The width of the common femoral artery was made 2-3 cm proximal to the bifurcation of the superficial and deep femoral artery branches of the leg when the subjects were seated in an upright position. Diameter measurements were made during diastole using digital calipers that are integrated into the ultrasound system software.

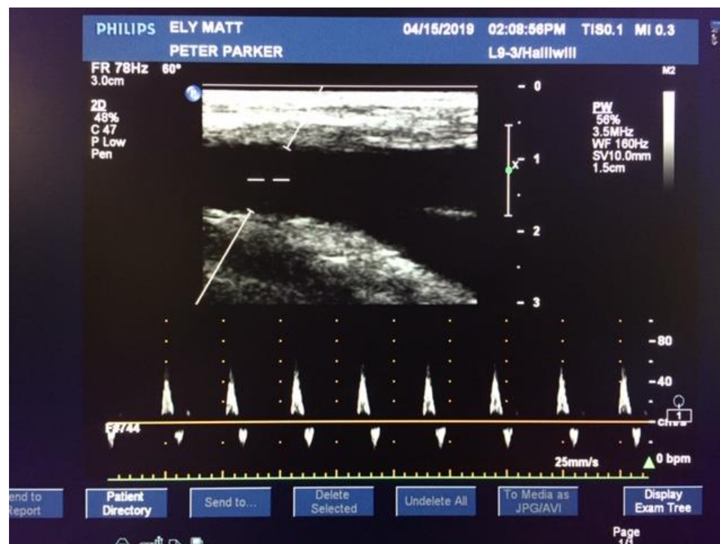

Figure 4. Example of the ultrasound image of the common femoral artery and blood flow velocity produced by the Doppler shift.

Blood flow velocities were calculated from the forward and reverse Doppler frequency shifts using an intensity-weighted algorithm from the Doppler ultrasound that were then corrected for errors associated with the thin beam measurements specific to the ultrasound system (36). Femoral blood flow was

calculated as artery cross sectional area multiplied by the mean femoral blood velocity over a 60 sec duration and reported as milliliters per minute.

Femoral blood flow was calculated as:

$$\textit{Femoral blood flow} = \pi \left(\frac{\text{diameter}}{2}\right)^2 \times \textit{mean blood velocity} \times 60,$$

Where femoral blood flow is in $ml \cdot min^{-1}$, the mean blood velocity is in $cm \cdot s^{-1}$, the femoral diameter is in cm, and 60 was used to convert from $ml \cdot s^{-1}$ to $ml \cdot min^{-1}$. Peripheral resistance was calculated as the mean arterial pressure divided by femoral blood flow (expressed as $mmHg \cdot min \cdot ml^{-1}$) and systemic vascular conductance as the reciprocal of resistance (expressed as $ml \cdot min^{-1} \cdot mmHg^{-1}$).

Doppler ultrasound derived blood velocity measures of the common femoral artery are common in research. The femoral artery is easily accessible, has a large diameter reducing errors in estimating blood flow due to small deviations in diameter, and because the femoral artery is located outside the muscle and is not compressed during muscle contractions (249). The measurement of blood flow using Doppler ultrasound provides high temporal resolution, is non-invasive, is highly correlated with the thermodilution technique ($r = 0.996$), and has a low coefficient of variation between multiple measures (249).

Local or limb blood flow can also be made using plethysmography, Plethysmography is a non-invasive estimate of limb blood flow based on the rate of limb volume/girth expansion with the occlusion of venous blood flow. Disadvantages of plethysmography is that there are no differentiations of limb blood flow to specific tissues (muscle, bone, skin etc.), the venous occlusion may

restrict arterial blood inflow, and measures can only be made at rest and not during muscle contractions (324).

Indicator methods have also been used to estimate blood flow. These methods involve diffusing a set volume and concentration of an indicator, such as a dye (Evans Blue Dye, indocyanine green) or cold saline (~0°C) into the arterial blood leading to the tissue of interest and then analyzing the concentration of the dye or temperature of the blood exiting the tissue. This method assumes the tissue blood flow is proportional to the change in dye concentration or temperature relative to the infusion concentration or temperature. A drawbacks to this method includes the invasive catheterization of arteries and veins (259). A spectroscopy unit with duel-channel laser diodes (NIRS) in combination with indocyanine green can also estimate local blood flow without the use of catheters but the use of this technology is less reliable than direct sampling (33).

Skeletal muscle pH and oxygenation index

Skeletal muscle pH and oxygen saturation are related to the microcirculatory blood flow and nutrient/metabolite transfer between the tissue and circulating blood (292). Near-infrared spectroscopy, the act of measuring the absorption and reflectance of near infrared light (~ 700 nm to 1,000,000 nm) can be used to estimate tissue oxygenation and pH. This technique was developed in the 1930s by Millikan (as reviewed by 22). Tissue oxygenation is estimated based on the absorption properties of hemoglobin. Oxygenated and deoxygenated hemoglobin absorb light at 800 nm and deoxygenated hemoglobin absorbs light at

760 nm, the difference between the two can give an indication of the oxygenation state of the tissue (180). The infrared light penetrates approximately 2.5 cm deep and due to the relatively small thickness of the skin, skin blood flow has a minimal influence on light absorbance, therefore the NIRS absorption indicates the tissue oxygenation of the small blood vessels, capillaries, and intracellular fluid (180). Spectra in the 725-880 nm wavelength range are used for determination of pH (293) and a correlation of spectral shifts to changes in pH during exercise using this technique were validated against invasive intramuscular pH probes (293). Importantly, this method of evaluating skeletal muscle oxygen saturation and pH has been validated during exercise, tissue ischemia, and following pharmacologic alterations in blood flow (180, 292, 293).

Specific to Chapter V, muscle pH and oxygen saturation were made with a near-infrared spectroscopy device (Reflectance Medical Inc. Oximeter 1100, Westborough, MA). This near-infrared spectrometer measured interstitial pH and oxygenation (SmO_2) of the vastus lateralis muscle (293). The sensor was placed 1/3 the distance between the greater trochanter and the knee joint and held in place by a special mounting pad that conformed to the shape of the thigh and prevented the sensor from moving on the skin during exercise. The sensor was further held in place with black self-adherent wrap (Coban, 3M, Maplewood MN) to minimize ambient luminosity.

Reliance on NIRS as a primary outcome measure has been debated. Many investigators have relied on NIRS to provide an estimate of skeletal muscle oxygen saturation, but this method requires the light to penetrate the skin and

assumes a minimal skin blood flow. If skin blood flow is minimal, the NIRS may provide a reasonable estimate of skeletal muscle oxygen saturation but if skin blood flow is elevated, the extra red blood cells and hemoglobin passing under the sensor and will alter the NIRS measures. The elevation in skin blood flow that occurs with elevated body temperature during extended exercise bouts would artificially inflate values that are attributed to skeletal muscle. Additionally, myoglobin has similar infrared light absorption properties to hemoglobin and the saturation of myoglobin may have an impact on the accuracy of NIRS (180). Finally, increased blood temperature during exercise may also affect the absorption of infrared light due to increased kinetic energy of hemoglobin (292). As a result of these confounding variables influence on infrared light absorption, it has been recommended that NIRS be used an indicator of trends in muscle oxygenation and not as a precise measures of oxygen saturation.

Blood Testing

Blood is a fluid that is circulated around the body to transfer nutrients and metabolic byproducts from one tissue to another. Blood is primarily composed of water but also contains soluble gases, mineral ions, proteins, hormones, and cells. Analysis of blood can provide a systemic snapshot of the internal environment. Exercised-induced increases in skeletal muscle histamine may affect glucose delivery and/or lactate removal from skeletal muscle during exercise, which would then influence exercise duration and intensity. Therefore, in the study described in Chapter IV, blood was analyzed for glucose and lactate

concentrations. Histamine may also directly and indirectly influence the type and concentration of white blood cells and inflammatory chemokines within the blood. Therefore, in Chapter VI, the type and concentration of white blood cells and inflammatory chemokines were measured. Lastly, histamine may be linked to the quantity of muscle damage incurred during exercise; therefore, markers of muscle damage in circulation were quantified in Chapter VI.

In the time trial studies detailed in Chapter IV, blood samples were obtained from the left and right earlobes with single use safety lancets (Unistik 3, Owen Mumford, Oxfordshire, UK). The blood was immediately analyzed for glucose (Precision Xtra, Abbot Diabetes Care, Alameda, CA) and lactate (Nova Biomedical, Waltham, MA). Samples of glucose and lactate were made in duplicate and values were averaged. Ear sticks were chosen due to the fast sampling time and convenience for the subject.

During the delayed onset muscle soreness and inflammation study detailed in Chapter IV, a venipuncture method was used to collect blood from the veins in the antecubital space of the left and right arm. Blood was collected before, immediately after and 6, 12, 24, 48, and 72 hours after exercise using a Saftey-Lok blood collection set (Becton, Dickinson and Company, Franklin Lakes NJ, USA). Blood was collected into three 3 mL vacutainers coated with K2EDTA (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes NJ, USA). Blood was then aliquoted for separation and quantification (See Appendix A for step by step procedures) of white blood cell populations via flow cytometry (Gallios, Beckman Coulter Life Sciences, Indianapolis IN, USA) using the

manufactures data acquisition software (Kaluza, Beckman Coulter Life Sciences, Indianapolis IN, USA)(Figure 5). A separate aliquot was immediately centrifuged (Marathon 3200R, Fisher Scientific, Pittsburg PA) for 10 min at 1000 RCF and the plasma was extracted and frozen at -80°F until analysis.

Plasma was analyzed for inflammatory cytokines via a bead based flow cytometry kit (Biolegend, LEGENDplex Human Inflammation Panel 1, multi-analyte flow assay kit). This method of cytokine identification uses beads of varying size coated with antigens specific to cytokines of interest (IL-1B, INFα2, IFN-γ, TNFα, MCP-1, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33). The size and fluorescence of the beads allows for separation and quantification of the cytokine by flow cytometry (Gallios, Beckman Coulter Life Sciences, Indianapolis IN, USA). Plasma was also analyzed for creatine kinase, a marker of muscle damage, using an activity assay (Sigma-Aldrich, St. Louis MO, USA.

Muscle Pain, Discomfort, and Soreness

Pain and discomfort are an inherent part of exercise and sport but they have been under appreciated in exercise science education and research (217). The neurobiological mechanisms that contribute to the pain/discomfort sensations may be different during exercise and following muscle-damaging exercise (DOMS) but histamine may be involved in both sensations. Histamine has been shown to stimulate peripheral nociceptors (Group III/IV afferent fibers) and initiate the sensation of cutaneous pain in both murine and human studies (151, 217). These afferent fibers are present in high amounts within skeletal muscle and

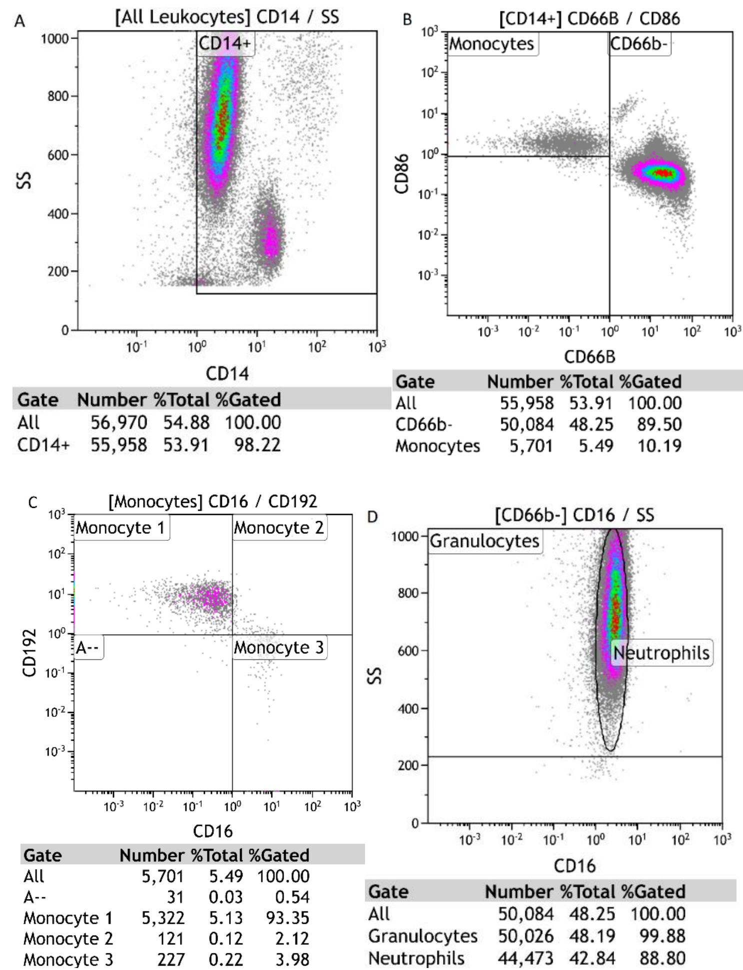

Figure 5. A representative gating procedure to identify A) leukocytes (CD14+), B) Monocytes (CD 86+, CD66-), C) Monocyte 1, 2, & 3 sub-populations, D) Granulocytes and Neutrophils.

the receptors associated with the nerves terminate along the walls of arterioles and surrounding connective tissue. These fibers receive algesic signals from active and damaged skeletal muscle (217). As histamine concentrations increase within the skeletal muscle during exercise, it would be of importance to study histamine's association with exercise-induced pain and discomfort. The studies in this dissertation assessed exercise effort, pain, and discomfort during and after exercise using a rating of perceived exertion, a visual analog scale, and algometry.

These methods allowed for the quantification of the subjective measures of pain/discomfort and exercise effort.

Rating of Perceived Exertion:

The concept of perceived exertion refers to the strain on the musculoskeletal, cardiovascular, and pulmonary systems at rest and during exercise (32). This concept is related to exercise intensity and the method to assess the strain involves asking volunteers to rate their level of exertion “right now” on a Likert Scale (31, 32). A 0-10 sale for exertion was employed for the study detailed in Chapter V while a 6-20 scale was used for the study in Chapter IV. An advantage of the 6-20 scale is that the numbers approximate heart rate. Both scales were developed by Gunner Borg (32). Other exertion scales have been made over the years but all rely on the premise of taking a subjective measure and applying a quantitative value so that statistical analysis could be made.

Visual Analog Scale

Muscle pain and discomfort were evaluated by means of a visual analog scale (VAS) questionnaire (217). The scale allows the subjects to provide a subjective rating of their general muscle soreness by marking a single vertical line along a 100 mm continuous scale with descriptive anchors at 0 mm indicating “no pain/discomfort”, 50 mm “moderate” pain/discomfort,” and 100 mm “severe pain/discomfort” (Figure 6). The distance from the left end (0 mm) of the scale to the vertical mark allowed for quantification of the pain/discomfort level.

Assessments of pain/discomfort were focused on the quadriceps muscle group that underwent the muscle damaging protocol in Chapter VI. The use of a VAS was sensitive enough to detect changes in muscle soreness following muscle damaging exercise (78) as well as between drug interventions in exercising protocols (78, 182, 200, 218).

Figure 6. A visual analog scale (VAS) used for evaluating muscle pain and discomfort. Adapted from O'Connor et al.(217)

Algometry, Pain pressure threshold

The pain pressure threshold is the minimum pressure which induces pain or discomfort (86). The pain pressure threshold was evaluated using algometry at the vastus lateralis and rectus femoris of the subject's exercised leg in Chapter VI. The rectus femoris was evaluated along the midline of the front of the leg and between 8 to 15 cm above the patella and the vastus lateralis was evaluated 10 to 20 cm above the lateral epicondyle of the femur (Figure 7). The sites were marked with a pen for visit-to-visit repeatability. Pain pressure threshold was evaluated with a Wagner digital algometer (Model FPX25, Wagner Instruments, Greenwich CT, USA), according to the methods of Fischer (86) in which the 1 cm^2 flat rubber tip is pressed firmly and perpendicularly into the muscle with an increasing

force of approximately 5 N s^{-1}. Pressure was stopped when the volunteer indicated the onset of pain/discomfort. Each site was evaluated 2 times and values within 5 N were accepted, if not additional trials were performed and all values were averaged. Of note, the use of algometry does not in itself change perception of pain, as multiple measures made to the same site over a three-day period did not affect pain scores (215).

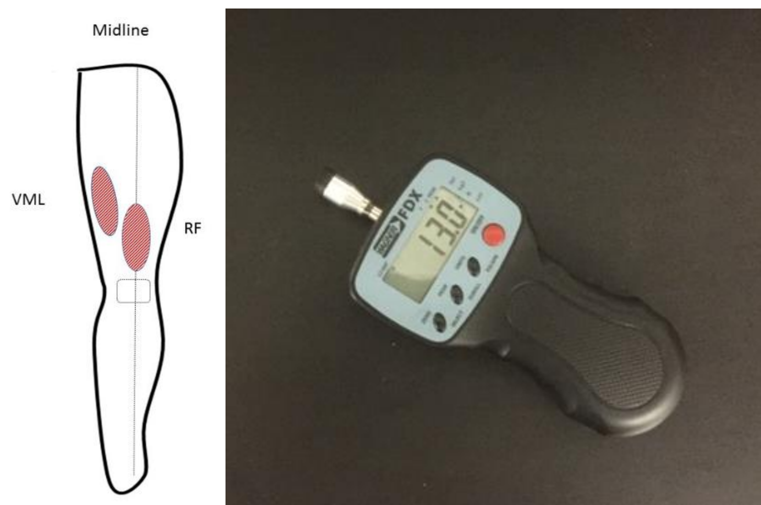

Figure 7. Sites of pain pressure threshold testing and the algometer.

There is no "Gold Standard" for measuring pain, but current guidelines suggest that the use and integration of several pain measures as a way to understand its complexity and interindividual variance (217). Methods to initiate pain include electrical, pressure, thermal, and chemical stimuli and pain parameters such as threshold (onset of pain), tolerance (length of time a stimulus can be tolerated), and intensity (category scales) can all be used to quantify the overall state of being.

Muscle function & damage

The primary function of skeletal muscle is to generate force for movement or joint stabilization. When muscle damage occurs, the ability to generate force is decreased. The decrease in muscular force may be due to either a decrease in actin and myosin overlap resulting from disruption of sarcomeres, a decreased excitation of the muscle sarcomere, a decreased calcium release, or to a reduced neural input into the contracting muscle. The neural input, or descending drive, from the motor cortex is a major determinant of the timing and strength of voluntary contractions. Inhibition of neuronal firing from the motor cortex is known to increase with increased sensation of pain (217). Blocking histamine interaction with group III/IV afferent fibers may decreased sensations of pain and reduce the motor neuron inhibition (151, 285) allowing greater voluntary force production, as speculated in previous DOMS research (78). Additionally, motor neuron drive parallels the sense of effort and inhibitory feedback from group III/IV afferent neurons may alter the motor neuron drive and influence muscle contractile force. Therefore, voluntary strength tests in conjunction with peripheral stimulation of motor neurons, in an attempt to bypass central motor neuron recruitment, were performed to differentiate the strength loss from muscle ultrastructure damage from the decreased motor neuron activity in the study detailed in Chapter VI.

Serial Strength Testing

Strength testing is used as an indirect measure of muscle damage (50, 78, 173, 174) and was previously used to assess histamines effect on muscle function

(78, 194). In the study detailed in Chapter VI, serial maximal strength tests of the quadricep muscles were made pre, 2, 4, 20, 24, 48 and 72 hours after an eccentric bout of exercise. Subjects performed 3 maximal contractions on a dynamometer (Biodex, System 3, Shirley, NY) with a 60 s rest period between each trial.

Evoked potentials and supramaximal stimulation

To gauge the amount of motor neuron activation to the quadricep muscles, the femoral nerve was stimulated with a single pulse to activate the musculature when the subject was at rest and while performing a maximal voluntary contraction. Volitional activation of a muscle occurs when force is produced by the recruitment of motoneurons through increased drive from the motor cortex. A stimulation of the femoral nerve, near the peak in voluntary force, may provide an acute increase in force production (Figure 8). The increase in force production represents the amount of musculature that has not been voluntarily activated by the central nervous system (23, 245). The combination of evoked twitch force at rest and the increase in force upon a maximal stimulation (supramaximal stimulation) can be used to evaluate strength changes during fatigue and muscle damage (23, 26, 244, 248). The increase in force may reflect inhibition of motorneurons by afferent feedback or reduced recruitment starting in the motor cortex (217, 244). A central activation ratio (CAR) was calculated by comparing the superimposed evoked force against the force output of a voluntary contraction (MVC+Stimulated force))(192). A CAR of 1.0 indicates a complete voluntary activation of the muscle. Other researchers in this field prefer the term central activation failure (1-CAR) as they feel the measure is more accurate of what the

central nervous system is failing to accomplish (23). Regardless, the intrasubject variability using this technique in healthy subjects is 7.0 ± 1.2% and is sensitive enough to parse out central vs peripheral mediated reductions in voluntary strength (-20 to -40%) that occurs with DOMS (192). Additionally, the superimposed evoked force was compared against the resting evoked twitch to calculate an interpolated twitch (IT ratio = superimposed twitch / resting evoked twitch force X 100)(23, 245).

The use of single stimulations superimposed on a MVC may not provide a precise estimate of muscle activation (23). Other techniques involve giving stimuli doublets, producing tetany, and using stimulations superimposed upon multiple sub-maximal intensity isometric contractions obtain insight into the mechanisms of fatigue and damage. The use of single superimposed stimuli were preferred due to the ease of obtaining the measure and as it is a gross measure of muscle activation during isometric contractions (23).

Specifically, in Chapter VI, the initial stimulation intensity was determined using a specially devised stimulator (Digitimer Constant Current Stimulator Model DS7AH, Welwyn Garden City, Hertfordshire, UK) over the femoral nerve on the cutaneous surface. The stimulation area was medial to the anterior-superior iliac spine, proximal to the inguinal crease, and was marked for reference for follow-up testing. The stimulator provided a single monophasic square-wave stimulus of a 200 watt voltage for a 200 ms duration. The amperage was progressively increased to provide the largest M-Wave and twitch torque. The M-Wave was detected on an oscilloscope (Tetronix TDS 210, Beaverton

OR). A stimulation amperage of 130% of that to produce a maximal twitch force was then used for testing (245). The stimulation amperage was adjusted for each subject and between each testing session.

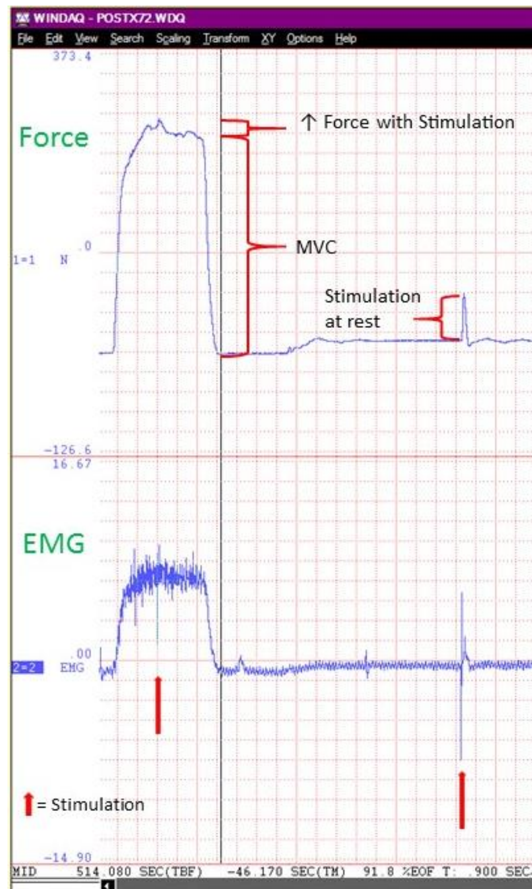

Figure 8. Representative tracing of the maximal voluntary contraction (MVC) force of the quadricep muscles with a force increase from a superimposed stimulation of the femoral nerve (Top). The electromyographical activity of the vastus lateralis muscle during the MVC and super imposed stimulation (Bottom).

Limitations to this technique are that stimulation does not just activate motor neurons but may also cause antidromic as well as orthodromic potentials which may inhibit the voluntary stimulation in addition to possibly stimulating

synergistic or antagonist muscles (95, 303). Additionally, the stimulus intensity and location to elicit a maximal M-wave over the femoral nerve was made at rest while the superimposed stimulation was made during muscle contraction. During a maximal contraction, there is movement of the muscle tissue and possibly the associated motor nerves under the skin. The movement of the nerve will influence the amount of stimulus reaching the nerve, and therefore the stimulus intensity may not be consistent with repeat trials.

Electromyography

Electromyography (EMG) is the process of recording the electrical activity of the muscle. Myoelectric signals are generated by active muscle fibers that are associated with action potentials from motor neurons. The size and duration of the EMG signal give an indication of the activity of the muscle. In the study in Chapter VI, surface electromyography (EMG) was used evaluate the amount of muscle activated during maximal voluntary contractions and from stimulated muscle contractions.

A single muscle, the vastus lateralis, was evaluated with an EMG (Motion Lab Systems Inc. Baton Rouge, LA) which included a built-in amplifier and ground. The electrode was placed in parallel to the approximate axis of the muscle fibers at a distance approximately 25% of the distance between the anterior superior iliac spine and the Gerdy prominence (29) and was held in place with adhesive tape. The location of the electrode was marked with ink for placement of the electrodes on future evaluations. The maximal amplitude of the EMG signal was compared between maximal contractions. Unfortunately, the area under the

EMG curve could not be analyzed as the duration the subjects were able to hold a contraction diminished as muscle soreness peaked.

Limitations of using EMG are the relatively unknown area and depth of tissue that are sensed by the electrodes and whether there is background electrical signals (crosstalk) from co-activated muscles or inactive muscles in the vicinity of the electrode (29).

Hydration

Dehydration and hypohydration are causes of fatigue. During extended exercise bouts subjects will lose body water as sweat and water vapor in the process of humidifying air as it enters the lungs. Body weight is a simple and crude way of assessing hydration changes. Therefore, body weights were monitored to exclude/account for dehydration as a possible causative factor of fatigue during the 10-km time-trials in Chapter IV. Nude body weight was recorded before and after all testing visits. Weights were recorded to the nearest 0.1 g (Sartorius model CIS IS64FEG-S, Elk Grove IL). Fluid intake/loss was documented with the combination of measured body weights, including those taken before and after urination/defecation, and changes in water bottle weights.

Statistical Analysis

Statistical inferences were drawn from a combination of paired t-tests, 2-way and 3-way repeated measures analysis of variance (ANOVAs) using SigmaPlot (v12, Systat,San Jose, CA, USA) or Statistical Analysis Software (SAS version 9.2; SAS Institute. Cary, NC, USA). F-values were corrected for sphericity where appropriate and Tukey’s post hoc tests were used when main or interaction effects were observed. Regression models were run using SAS Proc GLMSELECT (Proc MIXED, SAS version

9.2; SAS Institute. Cary, NC, USA). For all tests, significance was set at $P < 0.05$. Variables describing the distribution of the subject population, including anthropometric and demographic measures are presented as means ± standard deviation (SD). Data describing physiological variables between conditions and changing over time are presented as means ± standard error of the mean (SEM) to display the precision of the interventions.

CHAPTER IV

HISTAMINE RECEPTOR-ANTAGONISTS SLOW 10-KM CYCLING PERFORMANCE IN COMPETITIVE CYCLISTS

This study could not have been completed without the contributions of Dylan C. Sieck, Joshua E. Mangum, Emily A. Larson, Leandro C. Brito, Christopher T. Minson, and John Halliwill. Dylan, Josh, Emily, and Leandro provided much needed assistance on all the data collection sessions and contributed to the intellectual development of the manuscript. ChristopherT. Minson and John R. Halliwill assisted in the intellectual development and the writing of the manuscript. I was involved with all aspects of this project including the development of the protocol, completion of all data collection, compilation of the data, analysis of the data, and development of the manuscript. The manuscript for this study is currently published online and will be available in print form in the July 2019 issue of the Journal Medicine and Science in Sport and Exercise.

Introduction

Skeletal muscle interstitial histamine concentrations are low at rest, but increase in active musculature during moderate-intensity endurance exercise as histamine is released by mast cell degranulation and through *de novo* synthesis (255), secondary to increased activity of the enzyme histidine decarboxylase (HDC) (13, 255). Mast cell degranulation has been shown to occur with stimuli associated with exercise such as hypoxia, increased temperature, vibration, and hyperosmolality (190, 279, 294). While HDC is present in high concentrations within mast cells, there is also evidence it may be expressed within skeletal muscle myocytes (13, 270) and endothelial cells (270, 312). HDC continually synthesizes minute quantities of histamine in resting muscle (270, 320),

but further activity can be induced by stimuli associated with exercise such as hypoxia (138), increased temperature (267, 271), and decreased pH (267). Along these lines, experiments in exercising mice or using repeated electrically stimulated contractions in isolated muscle have demonstrated that the magnitude of increased HDC activity is proportional to the duration of exercise (14, 82). Since histamine is rapidly degraded by cytosolic and membrane bound enzymes or reabsorbed by mast cells, it has a short half-life (~100 s) (22, 130, 204). Therefore, histamine may have an important autocrine and paracrine action within skeletal muscle during sustained activity (212, 328).

In mice, blocking histamine's actions negatively affected moderate-intensity long-duration exercise tasks, but did not affect outcomes of short-duration high-intensity activities in humans. Specifically, histamine H_1 receptor antagonism in mice decreased multi-hour duration gnawing activity (328), and the speed and duration components of multi-hour walking tests (84, 212). Similarly, blocking histamine H_2 receptors tended to reduce walking endurance in mice, but the effect was smaller than blockade of H_1 receptors (212). In contrast to the exercise studies in mouse models, blocking H_1 receptors in humans had no effect on isokinetic muscle strength or endurance (194), maximal aerobic exercise, steady state submaximal exercise, or high intensity intermittent exercise (195, 196, 236). However, because the maximum length of human testing was brief (at most, 30 min, using high-intensity intervals alternated with rest periods), these studies do not reveal the effect of histamine on sustained endurance task performance. It is unknown if the different outcomes between human and mouse studies are due to differences in histamine's actions between species or are related to the intensity and/or duration of the exercise tests.

In humans, 60 min of moderate-intensity exercise increased histamine concentrations within skeletal muscle (255). This intramuscular histamine activated H_1 and H_2 receptors on the vascular endothelium (164) and smooth muscle, respectively, to induce a dilation of resistance arterioles, and increase local blood flow for several hours following exercise (187, 188). Histamine plays a similar vasoactive role in immune and inflammatory responses, in which increased local histamine concentrations increase blood flow and capillary permeability (56, 57, 177). It is unknown if histamine has a similar vasodilatory influence on blood flow within the muscle during endurance exercise. Given the proximity of histamine containing and producing cells to the skeletal muscle vasculature and the physiological actions of histamine, it is a reasonable premise that exercise induced increases in intramuscular histamine contribute to dilation and increased blood flow during exercise.

If histamine aids in increasing blood flow during exercise, blocking this action could decrease nutrient and oxygen delivery as well as carbon dioxide and metabolite removal. This perfusion to metabolism mismatch may have a larger impact on longer-duration exercise compared to short-duration exercise, and could be an explanation for the decreased endurance task performances observed in mice (82, 84, 212, 328) but absent in short-duration exercise studies in humans (194–196, 236) in response to histamine blockade.

Therefore, the purpose of this study was to gain insight into histamine's role in endurance exercise in humans, and potentially reconcile the contrasting results between human and animal studies. Specifically, on multiple days, human subjects performed short-duration high-intensity exercise performance tests following a time-matched period

of rest or endurance exercise. Subjects performed the tests in a condition where histamine's effects on H_1 and H_2 receptors were blocked and in a placebo condition. Prior to the study, it was hypothesized that blocking both H_1 and H_2 receptors would increase the time-to-completion of a fixed-distance time-trial compared to placebo, and the effect would be greater following an endurance-exercise bout.

Methods

Subjects

This study was approved by the Institutional Review Board of the University of Oregon. Eleven (3 female, 8 male) healthy, non-smoking individuals volunteered for the present study. Each volunteer gave written and informed consent prior to participation and the study conformed to the principles of the Declaration of Helsinki. All volunteers were competitive cyclists with a USA cycling race Category 1, 2, or 3 classification and a history of racing within the past year. No subjects were using over-the-counter or prescription medications at the time of the study, with the exception of oral contraceptives. The multi-day study was organized around training, work, and family schedules, therefore for female volunteers, menstrual cycle phase was not controlled for within or between subjects.

Experimental design

The study consisted of seven visits: one screening, two familiarization, and four double-blind placebo-controlled testing visits. All volunteers were required to abstain from caffeine, alcohol, and strenuous exercise for 24 h prior to each visit. A 24-h food

diary was provided to the subjects prior to the first testing visit, and food intake was matched as close as possible by the subjects before testing visit 2, 3, and 4.

Screening visit

An initial screening visit was completed to expose the subjects to all testing procedures and obtain demographic and anthropometric information (age, height, weight) including measurement of 3-site skin-fold body-fat estimate (triceps, supra-iliac, and mid-thigh for females; chest, abdominal, mid-thigh for males) according to the American College of Sports Medicine guidelines (161). Subjects then completed a 10-s Wingate test for anaerobic power, a cycling VO_{2Peak} test for aerobic capacity, and an isometric knee-extension strength test.

Familiarization Visits

Subjects performed two familiarization 10-km cycling time-trials that were separated by 3 to 7 days. Prior to each time-trial, an isometric knee-extension strength test was performed. Following a 10-min warm-up at a self-selected pace, blood glucose and lactate as well as rating of perceived exertion were measured, and then subjects performed a 10-km time-trial. If the difference in time-to-completion of the two visits was large (~30 s), subjects were asked to perform a third familiarization (this was done for one subject, as noted in the results). Immediately following the time-trials (within 30 s) blood glucose and lactate as well as rating of perceived exertion were measured. Isometric knee-extension strength was measured 3 min following the time trial completion.

Testing Visits

All testing visits were scheduled so that the 10-km time-trials occurred at the same time of day as the familiarization time-trials to reduce the potential influence of circadian rhythms on muscle blood flow (34) and exercise performance (247). Subjects were randomized after the familiarization visits to complete testing visits 1, 2, 3 and 4, in one of four possible orders (A, B, C, D), as shown in Table 5. Subjects completed a 10-km time-trial after 120-min seated rest (Rest) in the first two testing visits (1 and 2) and after 120-min cycling exercise (Exercise) in the last two visits (3 and 4), as shown in Figure 9. Subjects either received placebo pills (Placebo) or combined H_1/H_2 histamine receptor antagonists (Blockade) for the first two visits (Rest) and again for the second two visits (Exercise).

Table 5. Order of testing visits

Testing visit	1	2	3	4	
Activity prior to time-trial (Preceding Condition)	120-min Rest		120-min Exercise		*n*
Order A	Placebo	Blockade	Placebo	Blockade	3
Order B	Blockade	Placebo	Blockade	Placebo	3
Order C	Placebo	Blockade	Blockade	Placebo	3
Order D	Blockade	Placebo	Placebo	Blockade	2

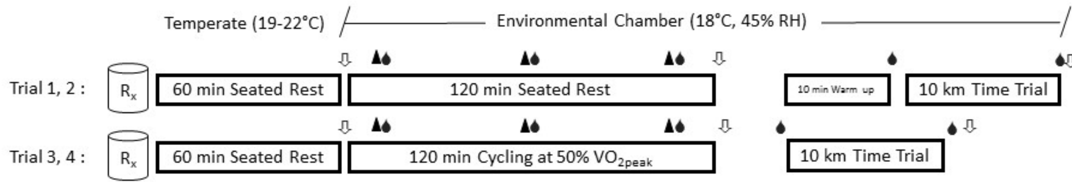

Figure 9. Study time line. After ingestion of Placebo or Blockade (R_x), volunteers performed either 120-min seated Rest (Visit 1 and 2) or 120-min cycling Exercise at 50% of VO_{2peak} (Visit 3 and 4). Each arrow (⇩) represents a time where isometric knee-extension strength and body weight were measured. The blood drop (🌢) indicates a time point where blood was sampled. The black triangle (▲) represents a 5-min period when VO_2, cardiac output, blood pressure, and perceived exertion were recorded. Testing visits 1 and 2 included a 10-min warm-up period in preparation for the 10-km time-trial; this was not included following the 120-min of cycling exercise.

Upon arriving to the lab, subjects ingested either Placebo or Blockade pills with ~90 ml of water and were seated in a temperate room (19-22°C) for 60-min before the start of Rest/Exercise. A measure of nude body weight was obtained, and then subjects were moved to an environmental chamber controlled at 18°C and 45% relative humidity (Tescor, Warminster, PA) for the remainder of the visit. Baseline measures of isometric knee-extension strength, heart rate, blood pressure, cardiac output, blood glucose and lactate, and rating of perceived exertion were made before 120-min seated Rest/Exercise. For Rest, subjects sat in a padded phlebotomy chair. For Exercise, subjects were seated on their own bikes on a CompuTrainer (RacerMate Inc., Seattle, WA, USA) computer-integrated cycle ergometer and the workload was set at a wattage corresponding to 50% of peak oxygen uptake. The CompuTrainer was set to vary resistance to maintain a constant work output, allowing the subjects to self-select and vary pedal cadences throughout the 120-min. Measures of cardiac output, heart rate, blood pressure, blood glucose and lactate, and perceived exertion were made at 15, 60, and 105 min during Rest/Exercise. Subjects were provided with ~ 90 ml of Gatorade every 20 min, for a total

of 533 ml (18 oz) during the 120-min. In addition to Gatorade, subjects were given water *ad libitum* during Exercise. At the completion of Rest/Exercise, a measure of isometric knee-extension strength was recorded. After Rest, subjects completed a 10-min warm-up at a self-selected power output prior to the time-trial (Visit 1 and 2), whereas after Exercise, subjects transitioned directly to the time-trial without additional warm-up (Visit 3 and 4). Immediately after the time-trial, perceived exertion, blood glucose and lactate, isometric knee-extension strength, and nude body weights were obtained (Figure 1). For the majority of subjects (7 of 11), each testing visit was performed 7 days apart, and for all subjects, they were separated by at least 72 h.

The objective of the time-trial was to finish as quickly as possible, with time being the criterion measure of performance. The time-trials were performed adhering to the standards outlined by Currell (53). During the time-trials there was: 1) no feedback on performance, 2) no distractions, 3) no encouragement, 4) no physiological measures (except for heart rate), 5) no performance cues, 6) temperature and humidity were controlled, and 7) subjects always used their own bike equipment. Time-trials using these guidelines often have a low day-to-day coefficient of variation and are likely to show meaningful differences for an intervention (53). Time-trials in competitive cyclists also have a high logical validity as they are “real world” type tests, are highly reproducible (coefficient of variance = 1.1 ± 0.9 %), and highly correlated with on-the-road competitions ($r = 0.98$)(225).

Measurements

10-s Wingate

Subjects were seated on a cycle ergometer (Excalibur Sport V2; Lode BV, Groningen, The Netherlands) and performed a 5-min warm-up at power output of 100 Watts at a self-selected cadence. Immediately following the warm up, subjects completed a modified (10 s) Wingate test of anaerobic power. The torque factor was set to 0.70 Nm (Wingate for Windows software version 1; Lode BV, Groningen, The Netherlands). The final two subjects were unable to complete the Wingate test due to computer-ergometer interface malfunctions (reported in Table 1).

VO_{2Peak}

After the Wingate test, subjects performed an additional 5- to 10-min warm-up, cycling at 1.5 Watts per kilogram of body weight at a self-selected pedal cadence. Subjects then performed an incremental cycle ergometer exercise test (Lode Excaliber, Groningen, The Netherlands) comprised of 1-min workload increments of 25-30 watts to determine peak oxygen uptake (VO_{2peak}). The workload increment was based on the subject's self-reported training load and intensity. Whole-body oxygen uptake was measured throughout the test via a mixing chamber system (Parvomedics, Sandy, UT) integrated with a mass spectrometer (Marquette MGA 1100, MA Tech Services, St. Louis, MO). The test was terminated when subjects were unable to maintain a pedal cadence of 40 revolutions per min. Subjects had obtained a respiratory exchange ratio of 1.13 ± 0.06 (mean ± SD), a heart rate of 95 ± 4% (mean ± SD) of age-predicted maximum, and reached subjective exhaustion [rating of perceived exertion on the Borg

scale of 19–20, (31)]. The VO_{2peak} test was performed for estimation of a 50% workload for testing visits 3 and 4.

Knee-Extension Strength Testing

Maximal voluntary isometric force of the right knee extensors was determined using a custom-built knee-extension apparatus and interfaced with a commercially available strain gauge (DP25-S, Omegadyne Inc. Sunbury, OH, USA). The knee angle was set to 45˚ of knee extension and subjects performed two to three maximal knee extension contractions with ~30 s rest between attempts. The greatest contractile force was accepted as a maximal value using the other trials as verification. Additional trials were performed as needed if trials did not agree within 10%.

10-km Time-Trial

The 10-km time-trials were performed on a computer-integrated cycle ergometer (CompuTrainer) using the subjects own racing bike. The time-trial was implemented using a manufacturer cycle ergometer computer program that provided a race simulation. Subjects were instructed to complete the time-trial as rapidly as possible and were blinded to feedback (i.e. time, pedal cadence, heart rate, power) but were given verbal cues indicating remaining distance at each 1 km, and at 500 m remaining. At the completion of each 1 km, split time and heart rate (H1 heart rate sensor, Polar, Lake Success, NY) were recorded but not shared with the subject.

Histamine H_1- and H_2-Receptor Blockade

Oral administration of 540 mg of fexofenadine, a selective H_1-receptor antagonist, reaches peak plasma concentrations within 1 h and has a 12 h half-life (263). Oral administration of 300 mg ranitidine, a selective H_2-receptor antagonist, reaches peak plasma concentration within 2 h and has a 3 h half-life (93). This dosage of histamine-receptor antagonists results in more than 90% inhibition of histamine H_1 and H_2 receptors lasting for 6 h after administration (93, 287). Fexofenadine and ranitidine are not thought to cross the blood-brain barrier or to have sedative effects (93, 263, 287). Importantly, histamine H_1- and H_2-receptor antagonism does not alter blood flow, heart rate, blood pressure, or smooth muscle tone at rest (78, 79, 187, 188, 254).

Placebo

The placebos were manufactured by a compounding pharmacy (Creative Compounds, Wilsonville, OR) and contained the inactive ingredients of the fexofenadine and ranitidine tablets.

Body Weight

Nude body weight was recorded before and after all testing visits. Weights were recorded to the nearest 0.1 g (Sartorius model CIS IS64FEG-S, Elk Grove IL). Body weights were monitored to exclude/account for dehydration as possible causative factor of fatigue during the 10-km time-trials. Fluid intake/loss was documented with the combination of measured body weights, including those taken before and after urination/defecation, and changes in water bottle weights.

Rating of Perceived Exertion

A subjective rating of exertion was documented on a 6-20 scale before and at 15, 60, and 105 min Rest/Exercise, and before and immediately following each time-trial (31).

Blood Glucose/Lactate

Blood was obtained from the left and right earlobes with single use safety lancets (Unistik 3, Owen Mumford, Oxfordshire, UK). Blood was analyzed for glucose (Precision Xtra, Abbot Diabetes Care, Alameda, CA) and lactate concentrations (Nova Biomedical, Waltham, MA) in duplicate and values were averaged.

Heart Rate and Arterial Pressure

Heart rate was monitored using a three-lead electrocardiogram and arterial pressure was measured on the right brachial artery using an automated auscultometric sphygmomanometer (Tango+, SunTech Medical, Raleigh, NC, USA). Mean arterial pressure was calculated as diastolic pressure plus 2/3 pulse pressure (systolic pressure - diastolic pressure) and reported in mmHg.

Cardiac Output, Stroke Volume, Total Peripheral Resistance, and Systemic Vascular Conductance

Cardiac output was estimated by using an open-circuit acetylene washin method. This method allows for noninvasive estimation of cardiac output. Subjects breathed a gas mixture containing 0.6% acetylene, 9.0% helium, 20.9% oxygen, and balanced nitrogen

for 8 breaths via a two-way non-rebreathing valve attached to a pneumatic sliding valve. During the washin phase, breath-by-breath acetylene and helium uptake were measured by a respiratory mass spectrometer (Marquette MGA 1100, MA Tech Services, St. Louis, MO) and total volume was measured via a pneumotach (model 3700, Hans Rudolph, Kansas City, MO) linearized and calibrated by using test gases before each testing day. Stroke volume was determined by dividing cardiac output by heart rate. Total peripheral resistance was calculated as the mean arterial pressure divided by cardiac output (expressed as $mmHg \cdot min \cdot ml^{-1}$) and systemic vascular conductance as the reciprocal of resistance (expressed as $ml \cdot mmHg^{-1} \cdot min^{-1}$).

Statistical Analysis

Results were not separated for a sex comparison as only 3 females volunteered for the study. Menstrual cycle phase was not controlled for in the females as there is a strong consensus that there is no change in aerobic (77, 89, 282) nor anaerobic performance (94) over the menstrual cycle, and females train and compete throughout all phases of their menstrual cycle.

Statistical inferences were drawn from a combination of paired t-tests and 2- or 3-way repeated measures ANOVAs with *a priori* contrasts, depending on the sampling of outcome measures, and models were run using SAS (Proc MIXED, SAS version 9.2; SAS Institute Inc. Cary, NC, USA). For all tests, significance was set at $P < 0.05$. All data are presented as mean ± SEM, unless stated otherwise (i.e., Table 1).

In addition, the practical importance of the time-to-completion was examined by the following procedures. First, a change score and 95% confidence interval (CI) between

Blockade and Placebo time-to-completion was calculated. Second, the change score and 95% CI were compared to a zone of indifference, determined as the coefficient of variation from the familiarization time-trials (i.e., a metric of day-to-day variability), as a test for equivalence (238). This procedure provides insight into the likely range of true population differences beyond statistical inference, using a comparator that is more meaningful than zero (i.e., null change) (19). Third, the taxonomic classification of the change score and confidence intervals for the strength of the effect was identified, based on the work of Batterham and Hopkins (19) and Piaggio (238). In this construct, performances outside of the day-to-day variation, regardless of statistical inference tests, represent a meaningful change in performance, as noted previously in the performance literature (48, 152, 276). Lastly, a traditional effect size (Cohen's d_z) was calculated for each preceding condition as well as a common language effect size (CLES, (159)), an expression of the probability of an outcome, for ease of understanding for the non-science public.

Results

Subject characteristics

Subject's demographic and anthropometric characteristics obtained from the screening visit including age, height, weight, body fat percentage, peak power, VO_{2peak}, isometric knee-extension strength, and 50% VO_{2peak} workload are presented in Table 6.

Table 6. Subject Characteristics

n	11 (3F, 8M)
Age (years)	27 ± 5, (19-33)
Height (cm)	176.6 ± 9.2, (135.5-196)
Weight (kg)	71.29 ± 12.44, (50.84-95.96)
Body Fat (%)	16.1 ± 9.6, (4.7-38.5)
Peak Anaerobic Power (W)*	897 ± 262, (526-1303)
$VO_{2\ peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	58.7 ± 6.3, (49.2-70.1)
Isometric knee-extension Force (N)	603.7 ± 224.4, (202.4-1030.8)
50% $VO_{2\ peak}$ Workload (W)	160 ± 30, (100-220)

Values are means ± SD, (Range). * n = 9 for Peak Anaerobic Power, n = 11 for all others.

Responses during Familiarization Visits and Time-Trial Reproducibility

One subject performed three familiarization time-trials, as their time-to-completion in the second familiarization was 27.7 s slower than the first. For this subject, only the two fastest times were used for analysis. There were no differences in time-to-completion between the first and second familiarization time-trial (1008.6 ± 25.9 s vs. 1001.7 ± 26.7 s; $P = 0.128$). The subjects demonstrated consistency in their efforts as the coefficient of variation between trials was 0.97%. The last kilometer of the time-trial was the fastest and the first kilometer was the slowest ($P < 0.05$), but pacing for each kilometer of the time-trial did not differ between trial 1 and 2 ($P = 0.504$)(Figure 10). Likewise, while heart rate increased within the time-trials (main effect for time $P < 0.05$), it did not differ between trial 1 and 2 ($P = 0.381$)(Figure 10). There were no differences in blood glucose and lactate concentrations or strength change between trials (Table 6).

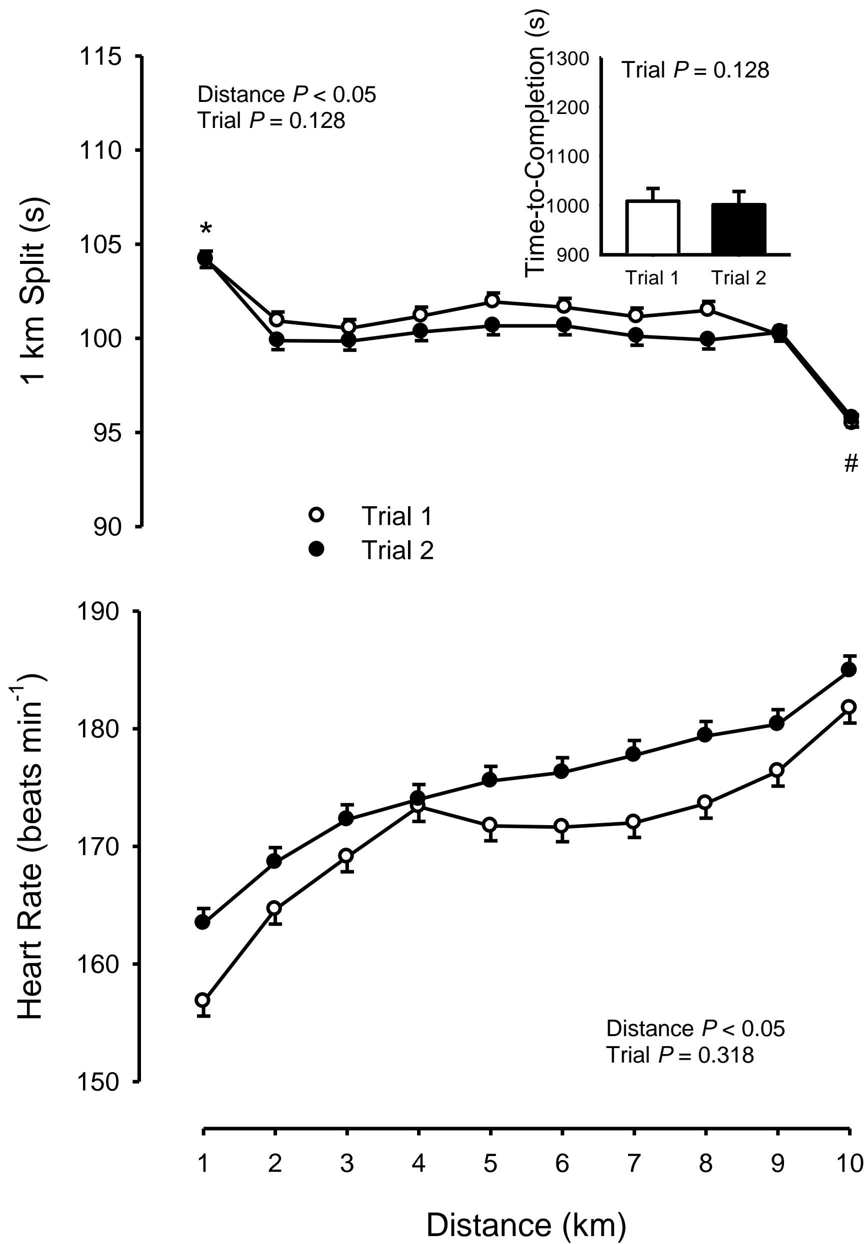

Figure 10. Familiarization time-trials. **Upper panel:** Comparison of the two visits for split time at 1-km increments, with 10-km time-to-completion shown as inset. * denotes slower than at 2-10 km ($P < 0.05$); # denotes faster than 1-9 km ($P < 0.05$) within the trials. **Lower panel:** Comparison of the two visits for heart rate at 1-km increments. Values are means ± SEM.

Table 7: Familiarization Trials

		Trial 1	Trial 2	*P* value Trial	Time	Trial*Time
Glucose (mg/dl)	Pre	77 ± 3	81 ± 5	0.895	<0.05	0.198
	Post	98 ± 8	95 ± 6			
Lactate (mmol/l)	Pre	1.2 ± 0.1	1.2 ± 0.1	0.476	<0.05	0.912
	Post	11.8 ± 0.8	11.9 ± 0.9			
Muscle Strength (N)	Pre	607 ± 13	593 ± 13	0.814	<0.05	0.403
	Post	546 ± 13	555 ± 13			
RPE	Pre	6 ± 1	6 ± 1	<0.05	<0.05	<0.05
	Post	18 ± 1	19 ± 1			

Values are means ± SEM. RPE, Rating of Perceived Exertion

Responses during 120-min Rest and Exercise

During 120-min Rest, there were no changes in any hemodynamic or metabolic measure in either Placebo or Blockade (Table 7). During 120-min Exercise, subjects cycled at a power output of 160 ± 30 W (mean ± SD) and, as expected, all hemodynamic and metabolic measures were elevated above pre-exercise and were higher than Rest ($P < 0.05$), with the exception of blood lactate concentrations ($P = 0.819$) and stroke volumes ($P = 0.448$) which did not differ between Exercise and Rest. There were no differences in hemodynamic, metabolic, or perceptual measures within or between the Placebo and Blockade conditions during 120-min of Exercise.

Table 8. 120-minutes of Rest and Steady State Cycling

	Phase	Drug	Pre	10	60	110	Drug	Phase	Drug*Phase	Time	Drug*Time	Phase*Time	Drug*Phase*Time
Heart Rate (beats·min^{-1})	Rest	Placebo	57 ± 1	56 ± 2	58 ± 2	54 ± 2	0.281	<0.05	0.518	<0.05	0.904	<0.05	0.879
		Blockade	55 ± 3	55 ± 3	56 ± 3	55 ± 2							
	Exercise	Placebo	63 ± 3	134 ± 5	138 ± 5	141 ± 6							
		Blockade	63 ± 3	127 ± 7	134 ± 5	138 ± 6							
VO_2 (l·min^{-1})	Rest	Placebo	0.301 ± 0.018	0.289 ± 0.017	0.297 ± 0.018	0.300 ± 0.016	0.405	<0.05	0.345	<0.05	0.889	<0.05	0.944
		Blockade	0.276 ± 0.018	0.294 ± 0.013	0.303 ± 0.013	0.291 ± 0.016							
	Exercise	Placebo	0.385 ± 0.028	2.225 ± 0.120	2.259 ± 0.148	2.301 ± 0.143							
		Blockade	0.362 ± 0.025	2.342 ± 0.140	2.385 ± 0.176	2.453 ± 0.188							
Respiratory Exchange Ratio	Rest	Placebo	0.84 ± 0.02	0.79 ± 0.02	0.83 ± 0.03	0.82 ± 0.02	0.820	<0.05	0.208	0.563	0.961	0.119	0.709
		Blockade	0.83 ± 0.02	0.83 ± 0.02	0.83 ± 0.02	0.84 ± 0.01							
	Exercise	Placebo	0.86 ± 0.02	0.89 ± 0.02	0.87 ± 0.02	0.84 ± 0.02							
		Blockade	0.86 ± 0.02	0.87 ± 0.01	0.85 ± 0.03	0.83 ± 0.02							
Respiratory Rate (breaths·min^{-1})	Rest	Placebo	15 ± 1	14 ± 1	14 ± 1	14 ± 1	0.126	<0.05	0.792	<0.05	0.409	<0.05	0.683
		Blockade	16 ± 1	16 ± 2	14 ± 1	14 ± 1							
	Exercise	Placebo	15 ± 1	22 ± 1	22 ± 1	23 ± 1							
		Blockade	14 ± 1	23 ± 1	24 ± 1	24 ± 1							
Systolic Pressure (mmHg)	Rest	Placebo	127 ± 5	128 ± 5	126 ± 5	132 ± 5	0.483	<0.05	0.628	<0.05	0.933	<0.05	0.827
		Blockade	129 ± 5	126 ± 4	126 ± 4	130 ± 4							
	Exercise	Placebo	137 ± 5	190 ± 8	193 ± 10	185 ± 9							
		Blockade	134 ± 4	190 ± 7	181 ± 9	185 ± 8							
Diastolic Pressure (mmHg)	Rest	Placebo	74 ± 2	74 ± 2	77 ± 2	78 ± 2	0.991	<0.05	0.824	<0.05	0.444	<0.05	0.995
		Blockade	73 ± 2	76 ± 2	75 ± 3	81 ± 3							
	Exercise	Placebo	77 ± 2	61 ± 3	64 ± 5	59 ± 4							
		Blockade	77 ± 2	61 ± 3	60 ± 3	63 ± 4							
Mean Arterial Pressure (mmHg)	Rest	Placebo	91 ± 3	92 ± 2	94 ± 2	96 ± 2	0.624	<0.05	0.635	0.064	0.569	0.264	0.893
		Blockade	92 ± 3	92 ± 3	92 ± 3	97 ± 2							
	Exercise	Placebo	97 ± 2	104 ±4	107 ± 4	101 ± 4							
		Blockade	96 ± 2	104 ± 3	100 ± 3	103 ± 5							
Cardiac Output (l·min^{-1})	Rest	Placebo	6.43 ± 0.46	6.04 ± 0.51	5.68 ± 0.46	6.30 ± 0.63	0.706	<0.05	0.821	<0.05	0.948	<0.05	0.783
		Blockade	6.39 ± 0.45	5.75± 0.30	5.81 ± 0.40	5.72 ± 0.35							
	Exercise	Placebo	6.55 ± 0.34	14.29 ± 0.59	14.70 ± 0.64	14.51 ± 0.70							
		Blockade	5.79 ± 0.63	14.18 ± 0.90	14.80 ± 1.07	15.09 ± 1.29							
Stroke Volume (ml)	Rest	Placebo	113 ± 8	109 ± 9	99 ± 7	118 ± 12	0.829	0.448	0.999	0.900	0.880	0.282	0.450
		Blockade	121 ± 12	108 ± 9	106 ± 10	108 ± 10							
	Exercise	Placebo	108 ± 9	107 ± 5	107 ± 5	104 ± 6							
		Blockade	94 ± 7	114 ± 9	111 ± 9	110 ± 101							
Toal Peripheral Resistance (mmHg·min·l^{-1})	Rest	Placebo	14.6 ± 0.6	15.9 ± 0.8	17.0 ± 0.8	16.1 ± 0.9	0.287	<0.05	0.985	<0.05	0.495	<0.05	0.627
		Blockade	15.0 ± 1.0	16.4 ± 0.8	16.4 ± 1.0	17.6 ± 1.1							
	Exercise	Placebo	15.2 ± 0.7	7.4 ± 0.4	7.5 ± 0.5	7.2 ± 0.5							
		Blockade	16.9 ± 0.7	7.7 ± 0.6	7.1 ± 0.6	7.2 ± 0.6							
Systemic Vascular Conductance (ml·mmHg^{-1}·min^{-1})	Rest	Placebo	70 ± 4	65 ± 5	61 ± 4	65 ± 6	0.983	<0.05	0.642	<0.05	0.679	<0.05	0.796
		Blockade	70 ± 4	62 ± 3	64 ± 4	59 ± 3							
	Exercise	Placebo	67 ± 3	140 ± 9	138 ± 9	143 ± 9							
		Blockade	60 ± 2	138 ± 10	149 ± 11	148 ± 12							

Table 8 Continued. 120-minutes of Rest and Steady State Cycling

Glucose (mg/dl)	Rest	Placebo	81 ± 5	89 ± 3	98 ± 4	93 ± 4	0.674	<0.05	0.809	0.115	0.829	<0.05	0.754
		Blockade	85 ± 4	90 ± 5	93 ± 4	93 ± 4							
	Exercise	Placebo	82 ± 5	67 ± 3	72 ± 2	72 ± 2							
		Blockade	80 ± 3	66 ± 3	71 ± 3	72 ± 2							
Lactate (mmol/l)	Rest	Placebo	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	0.424	0.819	0.789	0.195	0.934	<0.05	0.982
		Blockade	1.1 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	1.5 ± 0.1							
	Exercise	Placebo	1.2 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.1 ± 0.1							
		Blockade	1.2 ± 0.1	1.4 ± 0.2	1.2 ± 0.1	1.2 ± 0.1							
Rating of Perceived Exertion	Rest	Placebo	6 ± 0	6 ± 0	6 ± 0	6 ± 0	0.560	<0.05	0.560	<0.05	0.982	<0.05	0.982
		Blockade	6 ± 0	6 ± 0	6 ± 0	6 ± 0							
	Exercise	Placebo	6 ± 0	9 ± 1	11 ± 1	12 ± 1							
		Blockade	6 ± 0	10 ± 1	11 ± 0	12 ± 1							

Values are means ± SEM.

Responses during Time-Trials

Time-to-completion of the time-trial was slower for Blockade compared to Placebo (+10.5 ± 3.7 s, Range -15.9 to +53.1 s, $P < 0.05$), as shown in Figure 11. There was a trend for time-to-completion to be slower after 120-min Exercise versus Rest ($P = 0.057$), but this was not further compounded by Blockade in comparison to Placebo ($P = 0.716$). The pattern of pacing (Figure 11) was similar to the familiarization time-trials, the first km was the slowest, the last km was the fastest ($P < 0.05$), and was not different in Blockade versus Placebo ($P = 0.307$). On average, pacing of each km was slower following Exercise versus Rest ($P < 0.05$) but did not differ between Blockade and Placebo ($P = 0.926$). Heart rate during the time-trial was higher after 120-min Exercise versus Rest ($P < 0.05$) and lower for Blockade compared to Placebo ($P < 0.05$), as shown Figure 11. Heart rate increased throughout the time-trial ($P < 0.05$), but the pattern was not affected by Blockade versus Placebo ($P = 0.566$) or by prior Exercise versus Rest ($P = 0.998$).

Changes in Body Weight

There were no differences in nude weight at the beginning of the testing visits ($P = 0.547$). There was a trend for lower nude weights upon completion of 120-min Exercise compared to Rest ($P = 0.081$), but this was not affected by Blockade versus Placebo ($P = 0.846$). Subjects completed the Exercise trials slightly hypohydrated (Placebo -1.25 ± 0.18%, Blockade -1.46 ± 0.18% of pre-trial body weight). In five of the time-trials that were preceded by 120-min exercise, subjects exceeded a 2% weight loss by the end of the time-trial. The distribution of those five trials included two subjects on both their Placebo and Blockade visits, and one subject during only their Blockade visit.

The one subject that exceeded 2% weight loss in only the Blockade condition completed the time trial 15.9 s faster than the Placebo condition, opposite of the general trend of the other subjects. Therefore, in consideration of the overall pattern across subjects, as described above, dehydration exceeding a 2% body weight loss does not appear to be an important confound for interpreting the effect of Blockade versus Placebo.

Analysis of Change Scores

As noted above, the coefficient of variation between repeat performances from the familiarization time-trials was 0.97%. This was compared to a change score, calculated from time-to-completion as [(Placebo – Blockade) / Placebo)] and expressed as percent (%) change ± 95% CI, along with individual change scores (Figure 12). Following 120-min Rest, the mean change score was -0.87% (95% CI: -2.02 to 0.29%) and following 120-min Exercise, the mean change score was -1.20% (95% CI: -2.45 to 0.05%). Based on the taxonomic classifications of Batterham and Hopkins (19) and Piaggio (238), these change scores represent a “possibly trivial” effect of Blockade compared to Placebo following Rest but a “likely harmful” effect following Exercise. The size of the effects were 0.505 and 0.646 for Rest and Exercise, respectively. Using the common language effect size (159), one can state that there is a 69% chance of Blockade slowing 10-km time-trial performance when preceded by Rest and a 75% chance of Blockade slowing 10-km time-trial performance when preceded by 120-min Exercise.

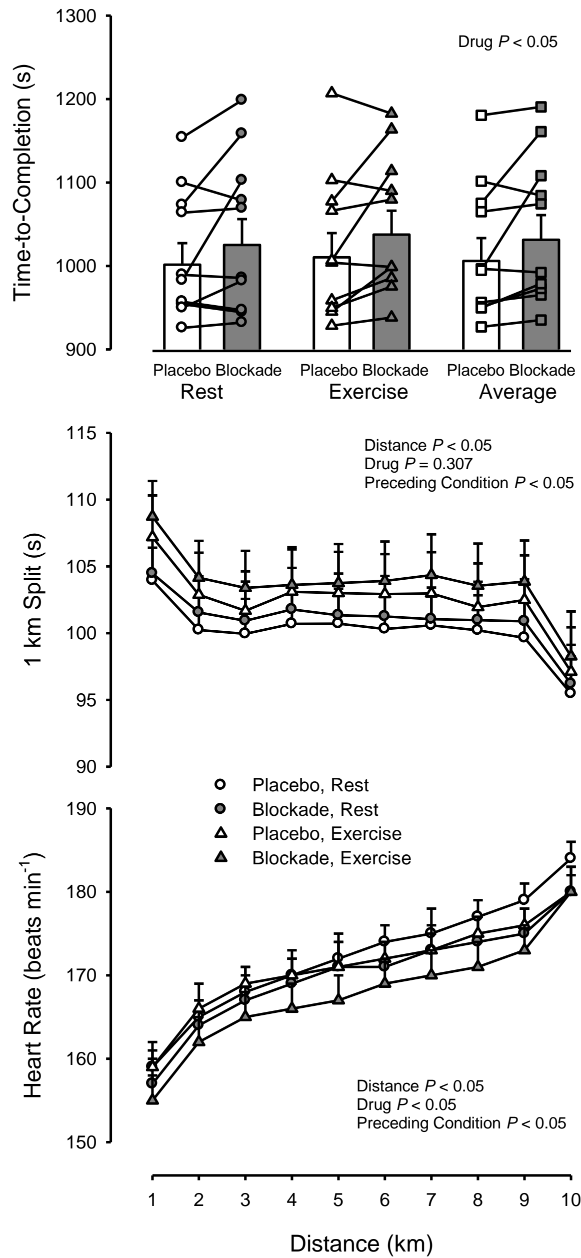

Figure 11. Testing visit time-trials. **Upper panel:** Effect of prior Exercise versus Rest and Blockade versus Placebo on 10-km time-to-completion. Values are means ± SEM, as well individual values. **Middle panel:** Effect of prior Exercise versus Rest and Blockade versus Placebo on split time at 1-km increments. The first km was the slowest and the last km was the fastest ($P < 0.05$). Values are means ± SEM. **Lower panel:** Effect of prior Exercise versus Rest and Blockade versus Placebo on heart rate at 1-km increments. Values are means ± SEM.

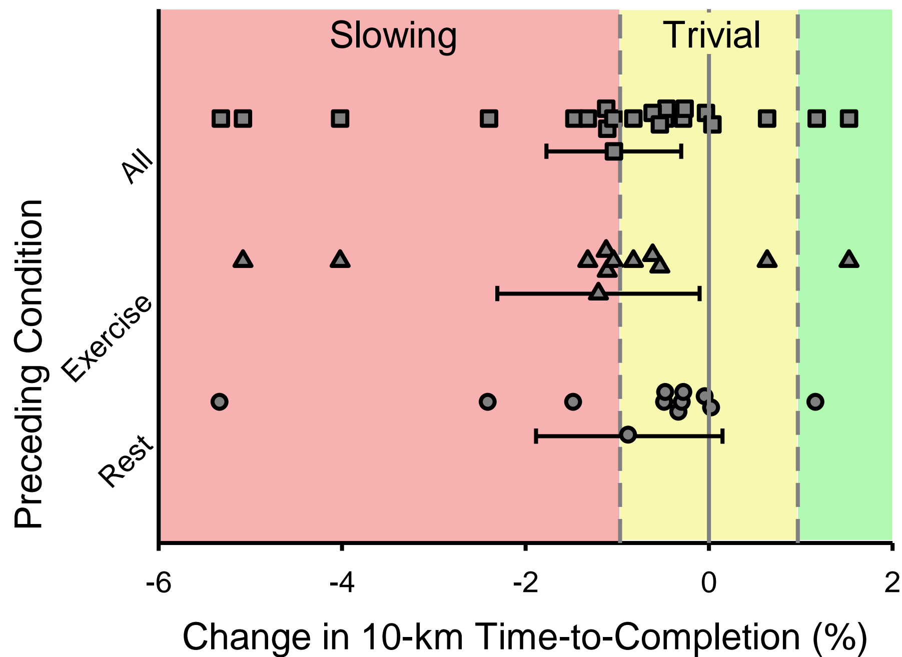

Figure 12. Effect of Blockade versus Placebo as a percent change in 10-km time-to-completion, with and without prior exercise (Exercise or Rest). Values are means ± 95% confidence intervals, as well as individual values. Yellow shaded area represents a zone of indifference (± 0.97%) based on performance variability measured during the familiarization trials.

Additional Outcome Measures

There were no differences in blood glucose, blood lactate, rating of perceived exertion, or isometric knee-extension muscle strength preceding the time-trial in any condition (Table 9). Blood glucose increased from before to after Rest ($P < 0.05$), decreased from before to after Exercise ($P < 0.05$), but was not affected by Blockade versus Placebo ($P = 0.809$). Blood lactate increased from before to after the time-trial ($P < 0.05$). The rise was greater following Rest versus Exercise ($P < 0.05$), but there was no effect of Blockade versus Placebo ($P = 0.496$). Rating of perceived exertion increased from before to after the time-trial ($P <0.05$), but there was no effect of Exercise versus

Rest (*P* = 0.663) or of Blockade versus Placebo (*P* = 0.827). Isometric knee-extension strength was highly variable within and between subjects and therefore showed no clear indication of any change based on prior exercise (*P* = 0.586) or drug condition (*P* = 0.722).

Subjective observations

Upon completion of both time-trials within the Rest and Exercise conditions, cyclists were asked to subjectively assess if they believed one medication resulted in an easier/faster, harder/slower, or no change in time-trial performance. Of the 22 comparisons, there were 5 reports of “no change” between the medications, 6 reports of “harder/slower” for Placebo, and 11 reports of “harder/slower” for Blockade.

Discussion

The main finding of the present investigation was that taking combined histamine H_1 and H_2 antihistamines slowed time-to-completion of a 10-km time-trial in competitive cyclists. These results corroborate the evidence suggesting blocking histamine’s actions reduces endurance in mice, and expands the observations in humans beyond the prior reports that were restricted to short-duration high-intensity tasks. A strength of the research design was the use of highly fit competitive cyclists and repeated familiarization trials. The cyclists demonstrated low variability in the 10-km familiarization trials (CV =

Table 9. Time Trials

		Rest		Exercise		*P* value						
		Placebo	Blockade	Placebo	Blockade	Drug	Phase	Drug* Phase	Time	Drug* Time	Phase* Time	Drug*Phase* Time
Glucose (mg/dl)	Pre	76.6 ± 1.5	72.5 ± 3.9	85.2 ± 2.9	80.7 ± 4.2	0.185	0.490	0.500	0.350	0.971	< 0.05	0.463
	Post	92.3 ± 4.8	83.8 ± 5.1	75.2 ± 6.1	75.5 ± 4.9							
Lactate (mmol/l)	Pre	1.5 ± 0.1	1.5 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	0.496	<0.05	0.512	<0.05	0.390	<0.05	0.711
	Post	11.2 ± 0.8	10.0 ± 0.7	7.1 ± 1.1	6.8 ± 1.0							
Muscle Strength (N)	Pre	525 ± 52	511 ± 52	504 ± 60	504 ± 61	0.722	0.586	0.862	0.902	0.994	0.946	0.997
	Post	530 ± 57	516 ± 54	496 ± 55	487 ± 58							
RPE	Pre	7 ± 1	6 ± 0	7 ± 1	7 ± 1	0.827	0.663	0.513	<0.05	0.528	1.00	0.827
	Post	18 ± 0	18 ± 0	18 ± 0	19 ± 0							

Values are means ± SEM. RPE, Rating of Perceived Exertion.

0.97%), and this metric allowed for an in-depth analysis of performance, beyond traditional statistics, into the importance of the outcomes. This innovative approach supports our statement that blocking histamine's actions would result in a 69% chance of slowing, and the slowing would have a "possibly trivial" effect on time-to-completion in a cycling 10-km time-trial, when not preceded by significant exercise activity. In contrast, blocking histamine's actions in a 10-km time-trial preceded by a period of sustained exercise would result in a 75% chance of slowing of performance, and the slowing would have a "likely harmful" effect on time-to-completion. These results suggest that exercise-induced skeletal muscle histamine production plays an integral role in endurance exercise capacity. Additionally, in the setting of competitive athletics, the combination of common over-the-counter H_1 and H_2 receptor antihistamines have negative influences on performance that are as large as the performance enhancing effects of altitude training or caffeine ingestion.

Sources of histamine during exercise

Mast cells synthesize, amass, and release histamine (211). Due to their location, in close contact with nerve endings and of the microcirculation of skeletal muscle, mast cells have been described as part of the vascular control network responsible for monitoring microcirculatory homeostasis (213). In response to exercise in humans, histamine is both released by mast cells and produced *de novo* by HDC within skeletal muscle (255). Mast cell degranulation commonly occurs in response to antigen-immunogloblin interactions during allergic reactions, but has also been shown to occur

with non-immunologic stimuli such as direct disruption of the cell membranes (213) and exposure to substance P, bradykinin, adenosine, nerve growth factor (141), chemokines, hypoxia (279), hyperosmolaility, superoxides, increased temperature, and vibration (97, 190, 294). In resting skeletal muscle, HDC enzyme activity generates minute quantities of histamine (271, 320). HDC mRNA expression, protein abundance, and activity are increased with exercise (14, 82, 255). HDC activity appears to be enhanced by increased temperature (267, 271), decreased pH (267), hypoxia (138), and several signaling molecules such as interleukin-1 (13, 81, 82, 320), all of which may play a role during repeated muscle contractions. Along these lines, in response to exercise, HDC activity is positively correlated with the duration of muscle activity (212).

Role of histamine in exercise responses

Histamine has a short half-life (~ 100 s) (130), as it is degraded rapidly by diamine oxidase in the vascular endothelium and blood (22). Therefore, histamine likely acts as a local autocrine and/or paracrine signal within exercising skeletal muscle, as there is evidence that interstitial levels do not change within inactive skeletal muscle (e.g., arms during leg exercise)(255). While its specific actions during exercise are unknown, some insight can be gained from studying histamine in other contexts, such as stimulation of afferent fibers, recovery from exercise, and pathophysiological reactions.

Contributing to afferent feedback

Histamine has the potential to modulate feedback from exercising skeletal muscle carried by muscle afferent Group III/IV nerve fibers. The Group III/IV afferent fibers,

also termed chemo- and metabo-receptors are associated with the exercise pressor response (151), and are one third of a “triad” in that they are in close proximity to mast cells and capillaries (27). These afferent fibers express both H_1 and H_2 histamine receptor subtypes (151) that, when bound to histamine, increase firing rate of the nerves and, through a reflexive central autonomic loop, increase heart rate. Thus, it is possible that histamine receptor antagonism reduced the activity of afferent nerves, diminishing their stimulation of the cardiovascular control centers. This would be consistent with the reduced heart rate seen during time-trials with histamine-receptor antagonism (Figure11). With reduced afferent feedback, it is possible that muscle perfusion was not well matched to metabolic demands of the exercising muscle.

Contributing to vasodilation

During inflammatory and immune responses, histamine binds to H_1 and H_2 receptors, causes vascular dilation by relaxation of smooth muscle, and increases the size of inter-cellular gaps between endothelial and pericyte cells within post-capillary venules (31, 36, 45, 58, 59). The vasodilation and increased size of the gaps results in plasma effusion into tissues. Similarly, following a moderate-intensity bout of endurance exercise, histamine interacts primarily with H_1 receptors on the vascular endothelium and H_2 receptors on the vascular smooth muscle, leading to a sustained vasodilation of resistance arterioles (sustained post-exercise vasodilation), and contributes to post-exercise hypotension (110, 187, 188). Germane to the present study, histamine blockade in endurance-exercised mice resulted in a reduced presence of nitric oxide metabolites in

the quadriceps muscles (212), suggesting that endothelium-mediated vasodilation may be stimulated by histamine during exercise.

If histamine has a vasodilatory effect within active skeletal muscle vasculature during exercise, this could facilitate oxygen delivery, nutrient delivery, and metabolite clearance during exercise. Thus, blocking these local effects may influence systemic blood flow, oxygen uptake kinetics, and skeletal muscle metabolism (e.g. glucose uptake, lactate production/removal) and, in doing so, have a negative effect on exercise performance. In this investigation, we did not attempt to measure skeletal muscle blood flow, but given the large vascular bed of exercising skeletal muscle, histamine-mediated vasodilation could affect arterial pressure. However, we did not detect any change in systemic hemodynamics (total peripheral resistance or systemic vascular conductance) during 120 min of moderate-intensity steady-state exercise (Table 8). Since arterial blood pressure is highly regulated at rest and during exercise, it is possible that the impact of any potential reduction in skeletal blood flow was masked by an offsetting increase in blood flow in another vascular bed, mediated by arterial baroreflex pathways.

Similarly, there were no changes in oxygen uptake (Table 8) or any other hemodynamic or metabolic measure during exercise (Table 8). These results are similar to those presented by Peterlin (236) who showed no change in total peripheral resistance or respiration after H_1 receptor antagonism in individuals performing 20 min of cycling at 50% $VO_{2\ peak}$. Thus, it remains to be seen whether histamine contributes importantly to exercise hyperemia, and whether histamine-receptor antagonists reduce skeletal muscle blood flow during exercise. However, such an effect would be consistent with the reduced performance that was observed in the present study.

Contributing to glucose delivery

Histamine is presumed to be a major controller of the microcirculation, and may play a role in facilitating movement of glucose (and other nutrients) from the microcirculation to active myocytes. During recovery from exercise, histamine receptor antagonists were found to decrease intramuscular glucose concentrations (as assessed via microdialysis) (234). Further, blocking histaminergic vasodilation following endurance exercise reduced skeletal muscle glucose delivery and blunted whole body insulin sensitivity by 25% (233, 234). Interestingly, reduced glucose delivery to skeletal muscles may have a larger impact on muscle glucose uptake in individuals with the highest VO_2 (79). In mice, histamine H_1 receptor antagonists have been shown to reduce intramuscular glycogen content following a 3 h prolonged walking task (212). Thus, several studies in mice and humans suggest the possibility that histamine receptor blockade could affect muscle glucose uptake during exercise. However, in the present study, we found no differences in circulating blood glucose concentrations either during the steady state conditions or following the time-trial in response to histamine blockade. Of note, because glucose was sampled from the ear in this study, it served as an indicator of systemic concentrations and did not reflect uptake within the skeletal muscle. Thus, it may be that blood glucose is well maintained, but less is functionally available to the exercising muscle. If so, one would predict a greater reliance (and use) of muscle glycogen, as seen in mice (212).

Overall, we note that performance in an endurance task is diminished, and we have identified several potential mechanisms that could explain why, but we were unable

to pinpoint a mechanism for the slower performance based on currently available information.

Methodological considerations

The open-circuit acetylene wash-in method produced reasonable and physiological estimates of cardiac outputs, as well as calculated values for stroke volume, total peripheral resistance, and systemic vascular conductance during the 120-min of Exercise. However, resting values are somewhat elevated and more variable than what is generally reported, and are suggestive of an anticipatory response to upcoming exercise. Although we took great care in performing this procedure, the variability in the measure indicates that these non-exercise values should be interpreted with caution.

The 10-km distance for the time-trial for a number of reasons. First, as the duration of time to complete the task was approximately 15-20 min, it was of sufficient length to primarily challenge the aerobic system where muscle blood flow is an important performance determinant, but not so long as to introduce confounding influences on performance (i.e. dehydration, mental fatigue). Second, many cyclists have experience performing hard efforts of this duration, providing “real world” or ecological validity. Third, for comparison purpose, it is a common test in the human performance/exercise physiology literatures (127). Lastly, the 10-km time-trial using laboratory cycle ergometry has been shown to be highly reproducible with a low day-to-day coefficient of variation (1-5%) in highly fit cyclists (120, 126, 127). Indeed, important to the interpretation of the findings was that the subjects displayed low day-to-day variability in their 10-km efforts from the familiarization trials. The volunteers in this study had a day-

to-day variability in performance of 0.97%. This is similar to that reported in other studies examining highly fit competitive athletes (19, 120, 121, 126, 139, 228, 297). This is especially impressive given that the subjects were not given feedback on heart rate, power output, 1 km split time, or time-to-completion. The 0.97% CV was used as a zone of indifference for comparing changes between the placebo and blockade conditions, i.e., comparison of the slowing of time-to-completion from placebo to blockade compared to the day-to-day variation. The slowing of performance when in the Rest preceding condition was -0.87% (95% CI -2.02 to 0.29%) (Figure 12). In contrast, following a 120 min period of cycling, the change in performance from placebo to blockade conditions was -1.2% with a 95% CI -2.45 to 0.05%. Based on the classification system set up by Batterham and Hopkins (19) and equivalence testing by Piaggio (238), the slowing of performance from the blockade is "possibly trivial" (ES = 0.505) following a period of rest and "likely harmful" following the exercise bout (ES = 0.646) (Figure 12).

Importance

This study demonstrates the importance of histamine in endurance exercise. This discovery of histamine, commonly associated with immune and inflammatory responses, as being intimately involved with endurance exercise, may open up new avenues of research as the field of physiology attempts to account for the beneficial effects of exercise beyond the improvement of traditional risk factors (205). Additionally, these findings have implications for competitive athletes, as the difference between a gold and silver medal can be a mere 0.52%, while a fourth place finish may trail as little as 2% behind the winning time (225). In an effort to capture the valuable seconds that determine

athletic victories, competitive athletes are continuously optimizing training methods and exploring legal ergogenic aids. For example, endurance athletes partake in altitude training, carbohydrate supplementation, and caffeine ingestion which have been demonstrated to improve performance ~2%, 2-3%, and up to 5% respectively (91, 140). However, competitive athletics has largely ignored the potential for antihistamines (potential ergolytic drug), commonly used to treat allergies and acid reflux, to hinder performance. The volunteers in the current study received a one-time dose of antihistamines prior to exercise and were asymptomatic for allergies and gastrointestinal reflux; therefore, the current findings may not apply to symptomatic athletes or those taking daily antihistamine medications. But, this question is of particular relevance to endurance athletes, as they are more likely to be diagnosed with allergies and/or allergic rhinitis than the general population (2), are five-times more likely to use allergy medication than non-athletes (1), and are two-times more likely to use oral antihistamines than sprint athletes or the general population (2). Additionally, oral antihistamines are frequently used by marathon and ultramarathon runners to treat gastrointestinal reflux and ulcers (196). The present study suggests that histamine plays an important role during endurance exercise. Furthermore, when this role is disturbed, it may have detrimental effects for competitive athletes chasing narrow margins of victory.

Conclusion

In conclusion, blocking histamine's actions had a "possibly trivial" effect" on 10-km time-trial performance following a period of rest and a "likely harmful" effect following 120 min of cycling. These findings corroborate prior observations in mice

regarding the effect of antihistamines on endurance exercise, and expand the work in humans beyond the scope of earlier studies that focused on short-duration high-intensity tasks.

The results from the experiment performed in Chapter IV entitled "Histamine-Receptor Antagonists slow 10-km cycling performance in competitive cyclists" suggests that exercise-induced skeletal muscle elevations in histamine concentrations serve an important purpose in the endurance exercise response. This study measured multiple systemic hemodynamic and metabolic variables and only detected a difference in heart rate between Placebo and Blockade conditions. Given that histamine is produced within the active skeletal muscle, it likely exerts local effects that are buffered by other pathways so systemic processes are not disturbed. The study outlined in Chapter V will further investigate the potential role exercise-induced increases in skeletal muscle histamine and histaminergic signaling at the muscle to influence the leg blood flow and the exercise response.

CHAPTER V

EFFECT OF HISTAMINE RECEPTOR-ANTAGONISTS ON LEG BLOOD FLOW DURING EXERCISE

This study was completed in conjunction with Dr. Stephen Ratchford, Dr. Heather Clifton, Taylor La Salle, Dr. Joel Trinity, and Dr. Walter Wray of the Department of Exercise and Sport Science of the University of Utah. Dr. John Halliwill, Dr. Joel Trinity, and Dr. Walter Wray assisted in the intellectual development of the protocol. All individuals contributed to the data collection process. Dr. Halliwill assisted in the data analysis.

INTRODUCTION

Histamine, an important component of immune and inflammatory responses, is released and produced within the affected tissue to increase blood flow by its vasodilatory actions on arterioles and capillaries (22, 176–178, 271, 273, 290). Beyond immune and inflammatory responses, endogenously produced histamine is associated with increased skin blood flow during heat stress (325), with elevated limb blood flow in reactive hyperemia (9, 10, 243), with elevated limb blood flow during sustained post-exercise vasodilation (187, 188), and with increased tissue blood flow during tumor growth (226). In all these situations, histamine receptor antagonists have attenuated the elevations in blood flow (58, 69, 70, 80, 226, 243, 266, 321, 325). Recent evidence shows that histamine concentrations increase within the skeletal muscle interstitial fluid during exercise (197, 255), however, no known study has explored the role of histamine in contributing to the rise of exercising skeletal muscle blood flow.

Histaminergic contribution to exercise hyperemia may be dependent on the duration or intensity of muscle contractions. The duration of repeated muscle contractions may be an important aspect of histamines influence on blood flow as the enzymatic activity of histidine decarboxylase, the enzyme which produces histamine, appears to be positively correlated with exercise duration (14, 212). Along these lines, blocking of histaminergic signaling did not affect the performance outcomes of exercises that lasted seconds (single muscle contractions) to few minutes in duration (194, 195) and instead appear to decrease the ability to perform tasks that are 15 minutes to hours long in duration (Chapter IV (212, 328)). The tests that were of short duration were of maximal exercise intensity and relied primarily on anaerobic metabolic pathways while those that were of longer duration were performed at sub-maximal intensities. Sub-maximal intensity exercises require adequate skeletal muscle blood flow for nutrient delivery and metabolite removal, which is integral in staving off many factors implicated in fatigue (e.g. hydrogen ion build up, reduced oxygen delivery, intramuscular glycogen depletion) and maintaining exercise output. It is possible that skeletal muscle histaminergic pathways are a link in the normal rise in skeletal muscle blood flow to maintain nutrient delivery and metabolite removal during repeated contractions typical of endurance exercises.

Therefore, the purpose of this experiment was to examine histamine's influence in the regulation of skeletal muscle blood flow during exercise. As histamine's contribution to hyperemia may be exercise intensity and/or duration dependent, limb blood flow was quantified at multiple intensities before and after an extended bout of exercise. Specifically, femoral blood flow was measured in subjects who performed knee-

extension exercise at 20, 40, 60, and 80% of peak power output before and after 60 min of knee-extension exercise at 60% of peak power in a condition where histamine's effect on the H_1 and H_2 receptors were blocked and in a placebo-controlled condition. Prior to the study, it was hypothesized that blocking histamine's actions would decrease femoral artery muscle blood flow at high exercise intensities and the effect would be greater following a bout of endurance exercise.

METHODS

Subjects

This study was approved by the Institutional Review Board of the University of Utah and the Salt Lake City Veterans Affairs Medical Center. Each volunteer gave written and informed consent prior to participation and the study conformed to the principles of the Declaration of Helsinki. No subjects were using over-the-counter or prescription medications at the time of the study, with the exception of oral contraceptives. Female participants were studied during the early follicular phase of their menstrual cycle or during the placebo phase of their oral contraceptive to minimize any potential cardiovascular effects of sex-specific hormones. All testing was performed in a temperate environment (21-23°C, 13-46% rh) at the Veterans Affairs Salt Lake City Geriatric, Research, Education, and Clinical Center in the Utah Vascular Research Laboratory (altitude ~1400 m).

Screening visit

An initial screening visit was completed to expose the subjects to all testing procedures and to obtain demographic and anthropometric information (age, height,

weight). Subjects also completed a unilateral knee-extension test to determine peak power output.

Experimental design

This study consisted of two double-blind placebo-controlled testing visits. Subjects performed experiments after a 4 h fast and abstaining from caffeine, alcohol, and strenuous exercise for 24 hours.

Testing Visits

Subjects completed two testing visits that were performed at least 7 days apart and at the same time of day to reduce the potential for circadian rhythms to influence skeletal muscle blood flow (34). Initially, 60 min before the start of unilateral knee-extension exercise, subjects ingested either placebo pills (Placebo) or combined H_1 and H_2 histamine receptor antagonists pills (Blockade) with approximately 90 ml of water. Subjects were then seated on the ergometer for the remainder of the visit. After 60 minutes of seated rest baseline measures of femoral blood flow, blood pressure, heart rate, cardiac output, oxygen consumption (VO_2), muscle pH, muscle oxygenation index, and a rating of perceived exertion were measured.

Absolute femoral blood flow values as well as the rate of rise in blood flow was measured at 20, 40, 60, and 80% of single-leg knee-extension peak power. Femoral blood flow was then measured every 10 min during 60 min of steady state exercise at 60% their individual peak power. Following the steady state exercise, femoral blood flow was again measured at 20, 40, 60, and 80% of single-leg knee-extension peak power. Exercise was held at each exercise intensity for 5 min and blood flow was recorded in the final 1-2 minutes. Each increase in workload (20, 40, 60, 80%) was separated by 2 min of

seated resting recovery (Figure 13). A 20 min seated rest period was provided before and following the steady state exercise. Additionally, blood pressure, cardiac output, VO_2, muscle pH, and muscle oxygen saturation were measured with each increase in exercise intensity and VO_2, muscle pH, and muscle oxygenation index were measured for 3 min at 10, 30, and 50 min during the steady state exercise (Figure 13).

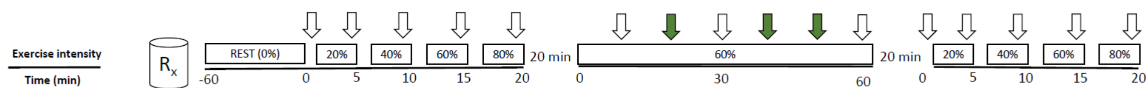

Figure 13. Study time line. After ingestion of Placebo or Blockade (R_x), volunteers performed three phases of exercise. Phase 1 and 3 included 3 min of knee-extension at a rate of 60 contractions per min at 20, 40, 60 and 80% of peak power. Phase 2 consisted of 60 min of knee-extension at a rate of 60 contractions per min at 60 % of peak power. Each phase was separated by 20 minutes of seated rest. Each arrow (⇩) represents a time point where blood flow, blood pressure, heart rate, cardiac output, VO_2, muscle oxygenation index, tissue pH, and perceived exertion were measured.

Measurements

Knee-extension peak test

Subjects were seated on an adjustable chair with a cycle ergometer (Monark 828E, Vansbro, Sweden) positioned behind the chair. Resistance was created with a friction driven flywheel turned upon knee extension via a metal bar connected between a crank arm of the bike and a boot worn by the subject. Resistance was increased 5 W every minute until the subject reached volitional fatigue. This peak test was performed for estimation of workload increments in testing visits 1 and 2.

Knee-extension exercise

Subjects were seated in a semi-recumbent position on the same chair-monarch bike equipment interface as the peak test. Subjects exercised for 3 minutes at 20, 40, 60, and 80% of peak power and rested for 2 minutes between each workload. For steady state subjects exercised at 60% of peak power for 60 min. In all phases, subjects were required to maintain a leg extension rate of 60 per minute.

Histamine H_1- and H_2-Receptor Blockade

Oral administration of 540 mg of fexofenadine, a selective H_1-receptor antagonist, reaches peak plasma concentrations within 1 h and has a 12 h half-life (262). Oral administration of 300 mg ranitidine, a selective H_2-receptor antagonist, reaches peak plasma concentration within 2 h and has a 3 h half-life (93). This dosage of histamine-receptor antagonists results in more than 90% inhibition of histamine H_1 and H_2 receptors lasting for 6 h after administration (93, 287). Fexofenadine and ranitidine are not thought to cross the blood-brain barrier or to have sedative effects (93, 264, 287). Importantly, it has been shown that histamine H_1- and H_2-receptor antagonism does not alter blood flow, heart rate, blood pressure, or smooth muscle tone at rest (78, 79, 187, 188, 254).

Placebo

The placebos were manufactured by the University of Utah pharmacy and contained the inactive ingredients of the fexofenadine and ranitidine tablets.

Doppler Ultrasound

Measurements of blood flow velocity and vessel diameter were measured from the common femoral artery in the exercising leg, 2-3 cm proximal of the bifurcation of

the superficial and deep branches of the femoral artery. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area, where the best spacial resolution was achieved. Velocity measurements were made at 5 MHz with a linear array transducer (Logiq e9, GE Medical Systems, Milwaukee, WI). Care was taken to avoid aliasing the blood velocity spectra by using scale adjustments, especially during exercise. All blood velocity measures were obtained with the probe appropriately positioned to maintain an insonation angle of 60˚ or less. The sample volume was maximized according to the vessel size and was centered within the vessel based on real-time ultrasound visualization. Femoral blood velocity was estimated using the commercially available software (Logic e9). Femoral blood flow (mL/min) was calculated by multiplying the mean blood velocity over 60 seconds by the cross sectional area of the common femoral artery during resting baseline measures. The common femoral artery diameter during rest was used in the calculation of exercise blood flow as earlier studies have indicated that there is a low coefficient of variation (CV ~1.5%) in repeated diameter measurements of the common femoral artery during systole and diastole, from rest to exercise, at increasing exercise intensities, and between multiple trial days (227, 249, 275).

$$Femoral\ blood\ flow = \pi \left(\frac{\text{diameter}}{2}\right)^2 \times mean\ blood\ velocity \times 60,$$

Heart Rate, Arterial Pressure, and Vascular Conductance

Heart rate was monitored using a three-lead electrocardiogram and arterial pressure was measured on the left brachial artery using an automated auscultometric sphygmomanometer (Tango+, SunTech Medical, Raleigh, NC, USA). Mean arterial pressure was calculated as diastolic pressure plus 1/3 pulse pressure (systolic pressure -

diastolic pressure) and reported in mmHg. Leg vascular conductance was calculated as blood flow / mean arterial pressure and is expressed in ml·min^{-1}·mmHg^{-1}).

Cardiac Output

Cardiac output was estimated non-invasively via finger photoplethysmoraphy (Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands) recorded via a data acquisition device (Medwave Vasotrac APM250A; Biopac, Goleta, CA). The finapres provides accurate indirect assessment of intraarterial pressure (129). Beat-by-beat arterial pressure waveforms are used to calculate stroke volume and in conjunction with heart rate as inputs in the "modelflow method" can then estimate cardiac output and provides reliable values for the changes in cardiac output with exercise (299). This method has a high correlation coefficients (0.91-0.98) and low errors of ~2.0 L compared to echocardiography (299).

Skeletal Muscle pH and Muscle Tissue Oxygenation Index

Intramuscular microvascular blood pH and tissue oxygenation of the vastus lateralis muscle was estimated using near-infrared spectroscopy (Reflectance Medical Inc. Oximeter 1100, Westborough, MA). Near-infrared spectroscopy is commonly used to estimate capillary oxygen saturation in the muscle (180). Near-infrared spectra in the 725-880 nm wavelength range are correlated with changes in hydrogen ion concentrations. This method of correlated spectra with changed in muscle pH has been validated against invasive intramuscular probes during exercise (293). The sensor was placed 1/3 the distance between the knee joint and the greater trochanter and the sensor was held in place by a mounting pad that conformed to the shape of the thigh and prevented sensor movement on the skin during exercise. The sensor was further held in

place with black self-adherent wrap (Coban, 3M, Maplewood MN) that also limited ambient luminosity.

Oxygen Uptake

Whole-body oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were measured during single-leg knee extension exercise using a mixing chamber system (TrueOne 2400, Parvo-Medics, Sandy UT). Respiratory exchange ratio was calculated as VCO_2/VO_2.

Rating of Perceived Exertion

A subjective rating of exertion was documented on a 1-10 scale at during the last minute of each exercise intensity, as well as at 10, 30, and 50 minutes into steady state exercise (31).

Statistical Analysis

Statistical inferences were drawn from a combination of paired t-tests and 2 or 3-way repeated measures ANOVAs with *a priori* contrasts. Models were run using SAS Proc GLMSELECT (Proc MIXED, SAS version 9.2; SAS Institute. Cary, NC, USA). For all tests, significance was set at $P < 0.05$. All data are presented as means ± SEM, except for data characterizing the subjects, which are presented as means ± SD. (Table 1).

RESULTS

Subject characteristics

Sixteen (7 female, 9 male) healthy, non-smoking individuals volunteered for the present study. Subject's demographic and anthropometric characteristics obtained from

the screening visit including age, height, weight, body mass index, knee-extension peak power, and 60% workload are presented in Table 10.

Table 10. Subject Characteristics

N	16 (7F, 9M)
Age (years)	25 ± 5
Height (cm)	174.9 ± 8.1
Weight (kg)	73. 9 ± 12.7
Body Mass Index	24.1 ± 3.3
Knee Extension Peak Power (Watts)	50 ± 10
60% Workload (Watts)	30 ± 5

Values are means ± SD.

Two subjects were excluded from blood flow and femoral vascular conductance analysis due to large differences (0.07 and 0.08 cm) in resting femoral artery diameter in the Placebo and Blockade trials. For comparison, there was a range from 0.00 to 0.02 cm in femoral artery diameter for subjects that were included in the analysis. Therefore, 14 subjects were used in the analysis as we could be confident in that blood velocities were being recorded at similar positions of the common femoral artery.

Ramped Exercise Response Pre- and Post- Steady State

Subject's workload increased from 10 ± 2, 20 ± 4, 29 ± 6, to 39 ± 9 Watts (Mean ± SD) for the 20, 40, 60, and 80% workloads. As expected heart rate, mean arterial pressure, femoral blood flow, and femoral vascular conductance increased with increasing workloads while muscle tissue oxygenation and intramuscular pH decreased with increasing workloads ($P < 0.05$)(Figure 14). Cardiac output increased from rest (5.73 ± 0.22 L/min) through each increase in workload (20% 7.57 ± 0.28, 40% 8.00 ± 0.34, 60% 8.87 ± 0.32, 80% 9.26 ± 0.32 L/min; $P < 0.05$).

Femoral vascular conductance, muscle tissue oxygenation, and intramuscular pH were higher in POST compared to PRE ($P < 0.05$). Blockade resulted in an elevated femoral blood flow at all exercise intensities in both PRE and POST compared to Placebo ($P<0.05$). Blood flow was elevated 6.4 ± 5.2, 10.6 ± 5.8, 15.3 ± 5.2, and $9.1 \pm 4.8\%$ at 20, 40, 60, and 80% of peak force, respectively, with Blockade compared to Placebo. In addition, Blockade resulted in a lower intramuscular pH in both PRE and POST compared to Placebo ($P < 0.05$). Blockade had no effect on heart rate, mean arterial pressure, cardiac output, femoral vascular conductance, or tissue oxygenation compared to placebo (Figure 14).

There was an increase in VO_2, ventilation, respiratory exchange ratio, and rating of perceived exertion with increased workloads ($P < 0.05$). Respiratory exchange ratio and perceived exertion were elevated during POST compared to PRE ($P < 0.05$) but there was no effect of Blockade compared to Placebo (Figure 15).

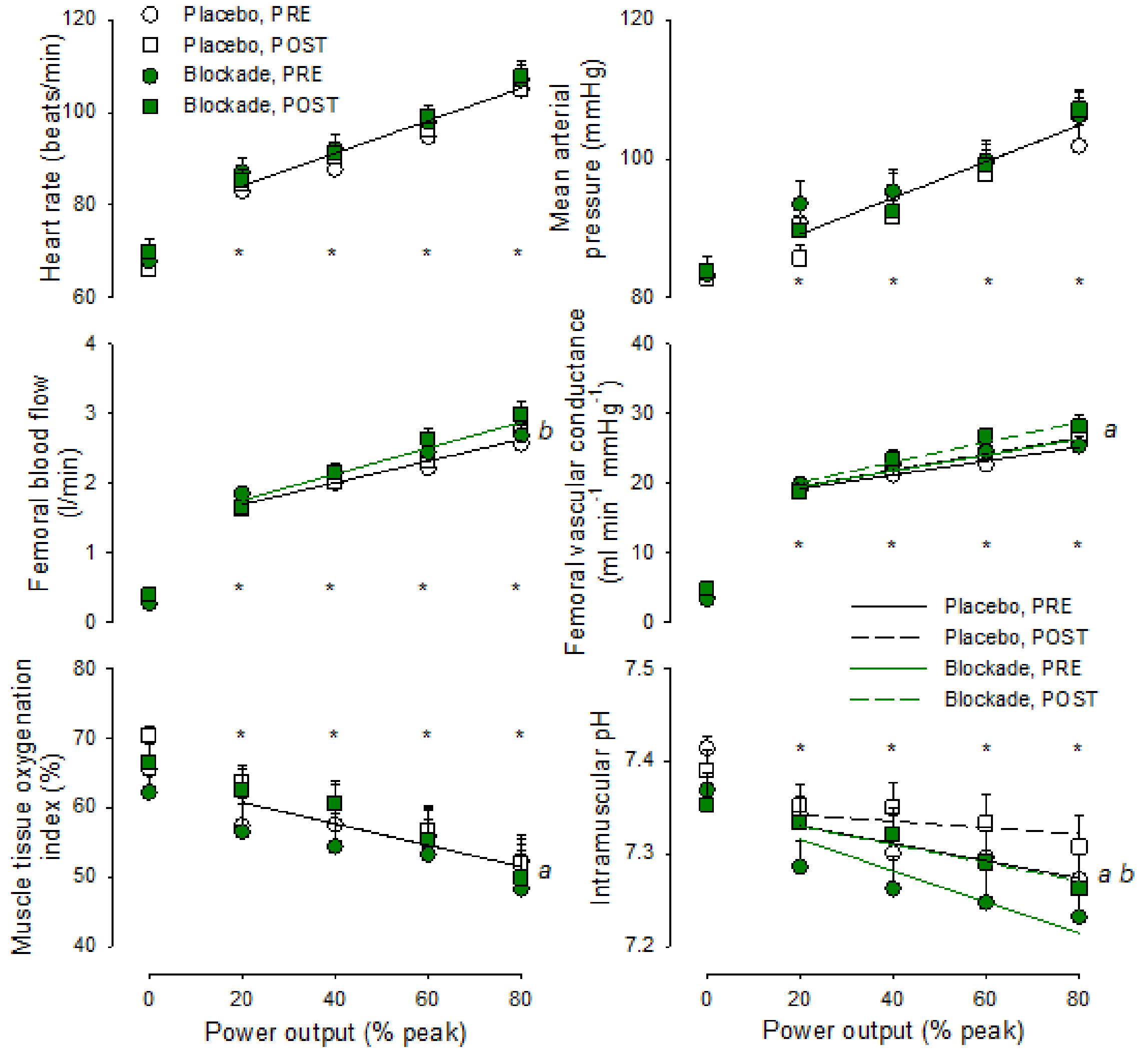

Figure 14. Hemodynamic measures, muscle tissue oxygenation, and muscle pH during pre-steady state (PRE) and post-steady state (POST) conditions. * = $P < 0.05$ Exercise vs Resting at specified power outputs; a = $P < 0.05$ Main effect for POST vs PRE; b = $P < 0.05$ Main effect for Blockade vs Placebo.

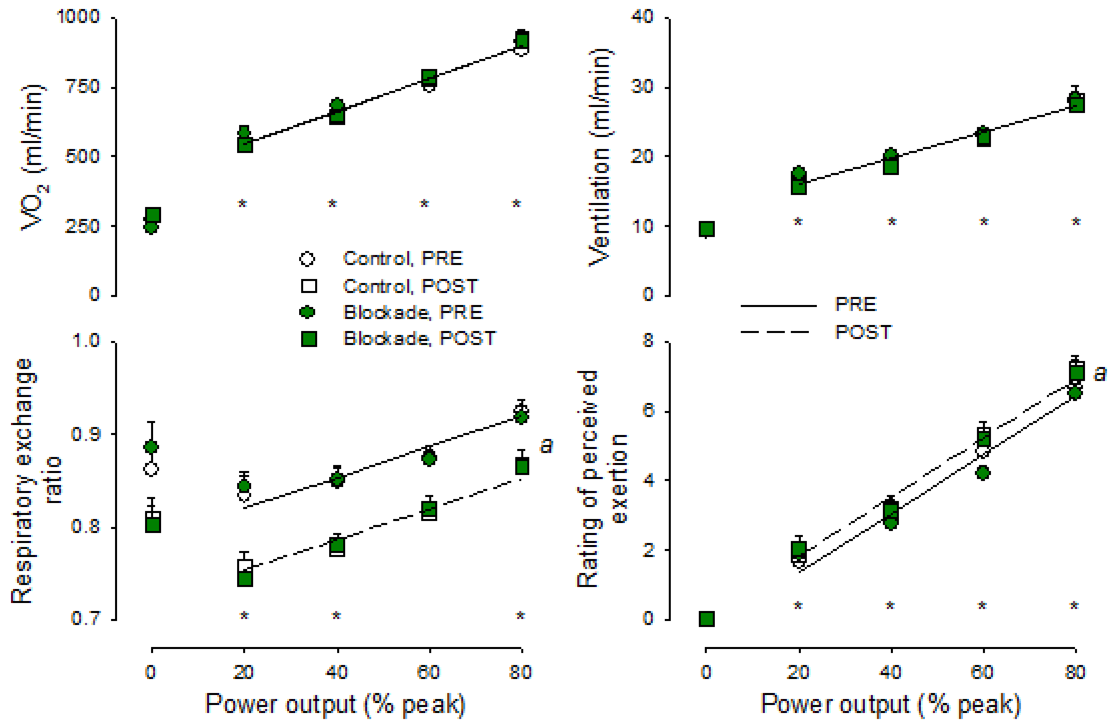

Figure 15. Metabolic measures during pre-steady state (PRE) and post-steady state (POST) conditions. * = $P < 0.05$ Exercise vs Resting at specified power outputs; a = $P < 0.05$ Main effect for POST vs PRE.

Steady State

Subjects maintained 29 ± 2 watts (mean±SD) for the full 60 minutes of steady state knee extension exercise. Heart rate, mean arterial pressure, muscle tissue oxygenation, and intramuscular pH were stable throughout the 60 min and were not different between Blockade and Placebo conditions (Figure 16). Femoral blood flow and femoral vascular conductance did not change over the 60 minutes of steady state exercise but femoral blood flow (12.7± 2.8%) and vascular conductance (16.2 ± 3.5%) were greater in Blockade compared to placebo ($P < 0.05$, Figure 16).

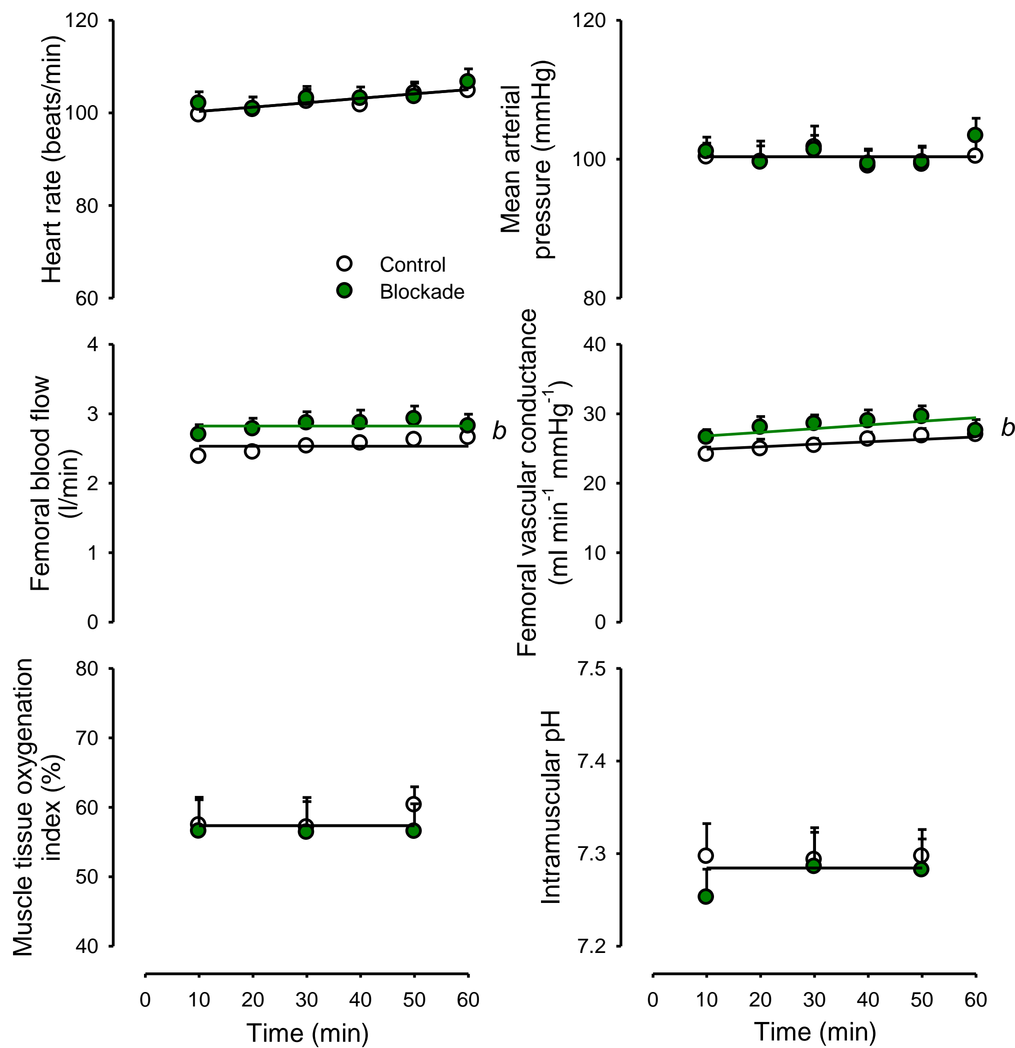

Figure 16. Hemodynamic measures, muscle tissue oxygenation, and muscle pH during 60 min of steady state exercise. b = P < 0.05 Main effect for Blockade vs Placebo.

Oxygen consumption and ventilation were stable throughout the 60 minutes and were not different between Placebo and Blockade. Respiratory exchange ratio decreased throughout the 60 minutes of exercise (P<0.05) and this change was similar between Placebo and Blockade (P > 0.05). Rating of perceived exertion increased through the 60

minutes of exercise ($P < 0.05$) and the increase was similar for Placebo and Blockade ($P > 0.05$; Figure 17).

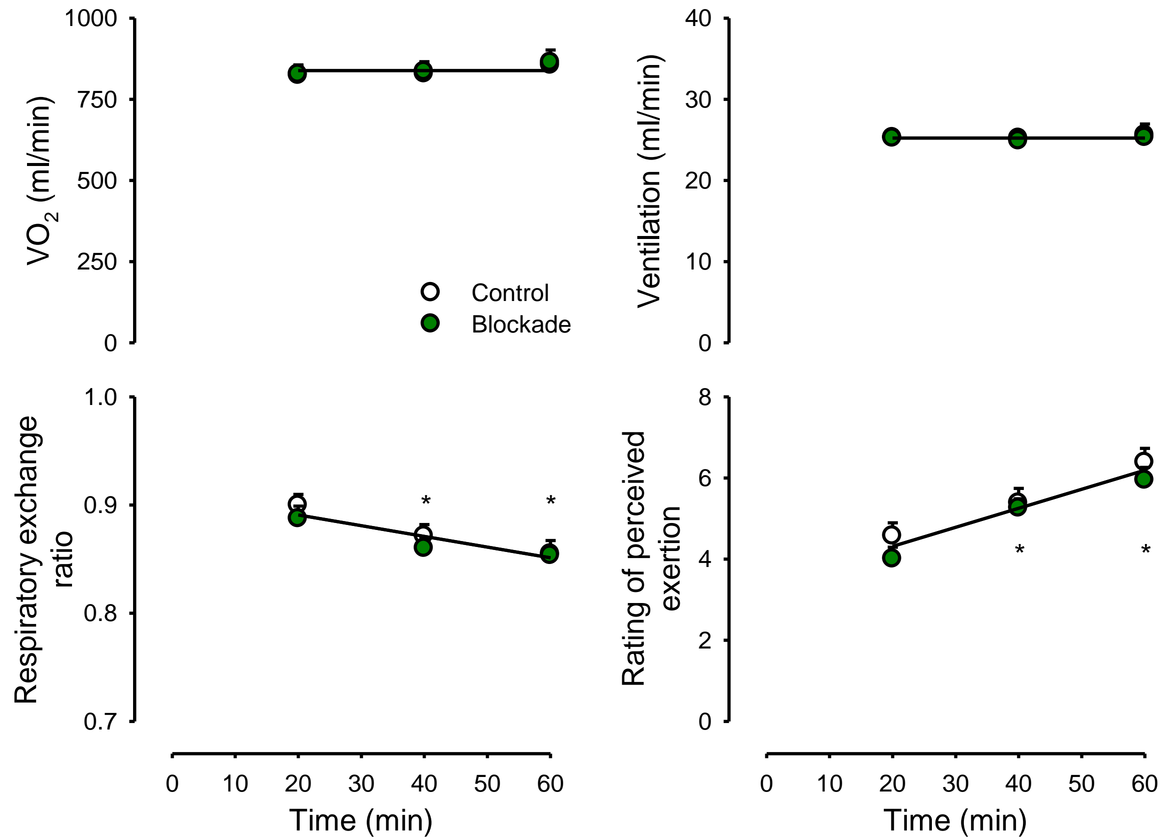

Figure 17. Metabolic measures during 60 min of steady state exercise. * = $P < 0.05$ Exercise vs Resting at specified power outputs.

DISCUSSION

The main finding of the present study was that blocking histamine's actions during exercise resulted in an elevation of skeletal muscle blood flow during exercise. The elevated skeletal muscle blood flow occurred acutely with increasing exercise intensities, during an extended steady state exercise bout, and again with increasing

exercise intensities after the extended exercise bout. These findings are opposite of the proposed hypotheses, that exercise induced elevations in skeletal muscle histamine concentrations aid in the exercise hyperemic response, and are counter to the current understanding of histamine's actions in non-exercise situations.

Legs blood flow during increasing exercise intensities

It is well documented that femoral blood flow increases linearly with intensity during single leg knee-extension exercise (17, 33, 96, 249, 324). Similar to previous investigations, the present results show within the Placebo condition, that exercise blood flow increased linearly with increasing exercise intensities from 20 to 80% of single-leg knee extension peak power (Figure 14). The elevation in blood flow occurred concomitantly with linear increases in heart rate, mean arterial pressure, femoral vascular conductance, oxygen consumption, ventilation, respiratory exchange ratio, and perceived exertion while muscle oxygenation index and pH decreased ($P < 0.05$; Figure 14 and Figure 15).

Leg blood flow during steady state exercise

To our knowledge, this is the first study that has examined femoral blood flow during steady state exercise longer than 25 min in duration. Most examinations of femoral blood flow are at fixed workloads for 3 to 10 min in duration and involve short rest periods before elevating the work load or administering an intervention (4, 5, 17, 33, 63, 96, 250) and therefore do not assess the constancy of blood flow over extended exercise bouts. In only two known instances has femoral blood flow been examined over

a prolonged exercise bout and these were time-controls that were incorporated into larger intervention studies. The time-controls were 25 min in duration at 19 Watts in five individuals (275) and 60 min in duration at 20W in two individuals (249). These two time-controls documented low coefficients of variation (CV ~9 to 14%) in blood flow over multiple time points (249, 275).

In the present study, absolute blood flow values during the 60 min of steady state exercise at 60% of peak were similar to those obtained during 60% of peak in the acute exercise (Figure 14) and were maintained throughout steady state (Figure 16). Associated with the maintained blood flow were constant heart rates, mean arterial pressure, tissue oxygen consumption, intramuscular pH, VO_2, and ventilator rates (Figure 16, 17). As exercise progressed, the respiratory exchange ratio decreased and perceived exertion increased (Figure 17).

Leg blood flow during increasing exercise intensities after prolonged steady state exercise

Elevations in femoral blood flow from increases in exercise intensity are nearly identical before compared to after 120 min of sedentary rest (17, 324). In comparison, femoral blood flow after a short exercise shows a faster rise at exercise onset (128) and time to reach steady state (88). This suggests that local vasodilators may still be present within the exercising skeletal muscle from preceding exercise that may aid in the subsequent exercise hyperemic response, but no known study has examined femoral blood flow using increases in exercise intensity following a prolonged exercise bout. The closest example identified was by Paterson *et al*. in 2005 (227) where similar elevations in femoral blood flow were noted during single-leg knee extension exercises that were

alternating 6 min of exercise at 80% of peak power and 6 min of exercise with no resistance for a total of 24 min.

The current study results suggest that after the steady state exercise (POST) blood flow increased linearly from 20 to 80% of peak power and the absolute blood flows and the rate of rise was not different from PRE (Figure 14). Blood flow and systemic arterial pressure were not different PRE to POST steady state but there was a significant rise in leg vascular conductance PRE to POST steady state (Figure 14), this indicates that there is either a reduced vasorestrictor or increased vasodilator influence on the leg vasculature resulting from the extended exercise. A maintained arterial pressure indicates that there must have been a decreased conductance in another vascular bed to offset the increased conductance in the leg at each exercise intensity. There was also an attenuated drop in intramuscular pH with increasing exercise intensities PRE to POST steady state ($P<0.05$) which may be associated with the increased blood flow (increased nutrient delivery and metabolite removal).

Skeletal muscle histamine and its hypothesized influence on exercise hyperemia

In 1945 Anrep (10) suggested that histamine may mediate exercise hyperemia as histamine concentrations were noted to rise in exercising muscles in humans. Histamine concentrations increase within the muscle from mast cell degranulation and increased production from HDC (255). The stimuli to cause the degranulation and increase production are unknown but *in situ* and *in vitro* studies have shown stimuli associated with exercise such as hypoxia (138), increased temperature (267, 271), vibration, hyperosmolality (190), and decreased pH (267) all act individually to increase histamine

release or production. It would be assumed that as exercise intensity and duration increased, that the positive allosteric influences of these factors would lead to increased skeletal muscle histamine concentrations. In fact microdialysis examination of intramuscular histamine concentrations show a 3-4 fold increase with 60 min of activity (255). T hese studies indicate that multiple exercise related pathways may contribute to the rise of histamine and suggest that it is an important part of the skeletal muscle response to endurance or prolonged exercise. Although, the physiological actions of histamine during exercise are unknown.

Based on the vasodilatory actions of histamine in inflammatory and immune responses, following tissue damage, in reactive hyperemia, and during sustained post-exercise vasodilation (58, 69, 70, 80, 177, 178, 187, 188, 226, 266, 271, 273, 290, 321) it was hypothesized that exercise associated increases in intramuscular histamine would aid in exercise hyperemia. It was also hypothesized that histaminergic vasodilation would increase with exercise intensity and duration based on 1) exercise hyperemia is governed primarily by mechanical factors at light workloads while vasodilator substances are more important for increasing blood flow at higher intensities (250, 284), 2) vasodilatory substances, such as prostaglandins and histamine, increase with exercise intensity (11, 33, 324) and 3) a positive correlation between exercise duration and histidine decarboxylase mRNA transcription and enzymatic activity (14, 82, 212, 254).

Effect of blocking histaminergic signaling on leg blood flow

Contrary to the hypotheses, blocking histamine did not result in a reduced femoral blood flow at any exercise intensity or duration. Blocking histamine's actions resulted in

an increased blood flow response that was similar in magnitude from 20 to 80% of peak power output (P < 0.05; Figure 14). The elevated blood flow remained during the steady state exercise (Figure 16), and was not further exacerbated at multiple exercise intensities following the steady state exercise (Figure 14) (P<0.05). The elevation of blood flow occurred without any noticeable change in heart rate, mean arterial pressure,, oxygen consumption, ventilation, respiratory exchange ratio, or perceived exertion between Placebo and Blockade conditions (P>0.05;Figure 14, 15). Similarly, other studies found no discernable effect of histamine receptor antagonists on heart rate, mean arterial pressure, or ventilation during submaximal prolonged exercise compared to Placebo. (194, 195, 236)

Microcirculation

Associated with the increased blood flow was a statistically significant reduction in intramuscular pH (P<0.05, Figure 14). Histamine is an intrinsic regulator of the microvasculature. Histamine initiates the contraction of endothelial and pericyte cells on the venule side of the capillary, widening intercellular gaps, resulting in increased capillary permeability (41, 42, 101, 155, 270). It is postulated that the rise in skeletal muscle histamine concentration is determined by the tissues need for blood to maintain circulatory homeostasis (270). In the present study, Blockade resulted in a progressively lower pH as exercise intensity increased (Figure 14). It is possible that Blockade altered the permeability of the capillaries and reduced hydrogen ion transport/diffusion out of the tissue resulting in the lowered tissue pH (Figure 14). Alternatively, blocking histamine may have altered skeletal muscle metabolism and shifted the balance of ATP production toward glycolysis resulting in elevated hydrogen ion production, but we are unaware of

any studies suggesting histamine's influence of shifting cellular metabolism. Future studies combining femoral blood flow (Doppler) and capillary permeability (NIRS+ICG, labeled albumin, and/or magnetic resonance spectroscopy) may be necessary to link the histaminergic pathway with skeletal muscle blood flow and permeability of the capillary during exercise.

Importance

During exercise, skeletal muscle arterioles dilate and blood flow rises in proportion to the metabolic demands of the tissue (145). The vasodilation occurs primarily through a modulation of neural vasoconstrictor and locally derived vasodilators released by or near the active musculature (145). The primary mediator leading to vasodilation is unknown and the current thinking is that there is a redundancy in their formation and synergy in their actions. As evidence, blocking the actions of one (324), two (33, 275), or even three (198) of these known vasodilators have produced negligible, if any reductions in skeletal muscle blood flow during acute exercise (<10 min duration) in healthy individuals. The majority of studies have investigated the influence of vasodilators on exercise hyperemia at the start of exercise (33, 63, 96, 250) but the factors regulating steady state exercise blood flow may be different from those initiating exercise hyperemia (283). It is in this context that the examination of histamine was important as very few studies have examined the role of vasodilators during prolonged steady state leg exercise in humans (275).

The current study results are important because if histamine plays minor role in blood flow during exercise this pathway would have profound effects on individuals with

blood flow impairment such as heart failure or peripheral vascular disease. The administration of “antihistamines” in these individuals may alter blood flow or perfusion responses and impair intramuscular homeostasis and exercise capacity.

Conclusions

Blocking the actions of histamine, a molecule often associated with inflammatory and immune responses, resulted in an increased skeletal muscle blood flow during exercise. The elevated blood flow occurred over exercise intensities ranging from 20-80% of peak force and in exercise of 60 minutes in duration. These results suggest that exercise-induced elevations in histamine concentrations are involved in the exercise response.

CHAPTER VII

HISTAMINE RECEPTOR-ANTAGONISTS DO NOT ALTER THE SYSTEMIC INFLAMMATORY RESPONSE OR RECOVERY FROM MUSCLE-DAMAGING EXERCISE

This study could not have been completed without the contributions of Karen W. Needham, Jonathan K. Ivankovic, Brendan W. Kaiser, Dylan C. Sieck, Joshua E. Mangum, Emily A. Larson, and John Halliwill. Karen provided assistance with the blood processing, leukocyte isolation and quantification, and the multi-analyte flow assays. Jon assisted with subject visits and data recording. Brendan, Dylan, Josh, and Emily provided much needed assistance on all the data collection sessions. John R. Halliwill assisted in the intellectual development project. I was involved with all aspects of this project including the development of the protocol, completed all data collection, compiled the data, analysis of the data, and developed the manuscript.

INTRODUCTION

Following high-intensity exercise, prolonged endurance exercise, or muscle damaging exercise there is an increase in the number of circulating white blood cells (leukocytosis) (39, 51, 111, 210, 230, 280). Exercise also leads to a transient increase in cytokines that are believed to originate from within the contracting skeletal muscle (185, 232). The combination of increased leukocytes and chemokines create a pro-inflammatory state with a primary purpose to repair damaged tissue and regenerate muscle (174, 231, 289, 307–309). The place of origin of the acute rise in circulating leukocytes is unknown (280) but the cells enter the previously active skeletal muscle in the hours to days after exercise cessation (39, 66, 173, 231). Although the skeletal muscle

leukocytes aid in muscle repair and remodeling, many aspects in the regulation of muscle repair are unclear and involve unknown molecules (231).

Histamine is a molecule that is released and produced within the contracting skeletal muscle from mast cell degranulation and *de novo* production from the enzyme histidine decarboxylase (255). Following exercise, histamine mediates a dilation of resistance arterioles within the previously active muscle (187, 188, 255). The dilation results in an elevation of local blood flow that can last upward of 120 min (187, 188). The purpose of the histamine-mediated increase in post-exercise blood flow could be part of a post-exercise inflammatory response to deliver the circulating leukocytes to the muscle tissue.

Outside of excise, histamine plays a major role in the inflammatory response. In the area surrounding tissue damage, there is a high concentration of histamine (177). The histamine initiates the margination of capillary endothelial and pericyte cells to increase permeability (22) and permits neutrophil and macrophage infiltration into damaged tissues. Histamine is also associated with the expression of adhesion molecules on endothelial cells, acts directly and indirectly in the production of cytokines/chemokines, and aids in chemotaxis (22, 146, 224). Interestingly, mice bred without histidine decarboxylase or treated with H_1 receptor antagonists display reduced macrophage infiltration and have extended skin wound healing times (166, 214, 221).

Exercise resulting in muscle damage can provide a model to study histamine's association with exercise related inflammation and recovery. Following muscle damage induced by eccentric contractions, there are disruption of sarcomeres and reduced actin and myosin overlap. These disruptions affect the ability to generate force (231). The

change in muscle force is an indicator of the extent of tissue damage and the recovery of force signals repair to the tissue (231). The decrease in muscle force may not be limited to peripheral factors such as actin and myosin overlap but may also be caused by central factors including motor unit recruitment. Sensations of muscle pain/discomfort by activation of Groups III/IV afferent nerves following muscle damage decrease motor unit recruitment (151, 285). These afferent neurons can be directly stimulated by histamine, and histamine may alter two neurotrophic factors that are involved with longer term sensitization and activity of these fibers (78, 181, 237, 279, 298, 318, 327). Monitoring muscle pain/discomfort will also be an indication of histamine's role in the recovery from exercise-induced muscle damage.

Therefore, the purpose of this study was to gain insight into histamine's role in the exercise inflammatory response and recovery of muscle function from muscle-damaging exercise. Specifically, human subjects performed unilateral knee-extension eccentric contractions to induce muscle damage. Circulating leukocytes and cytokines as well as muscle strength were measured periodically for 72 hours after exercise. Subjects performed the tests in a condition where histamine's effects on H_1 and H_2 receptors were blocked and in a placebo condition. It was hypothesized that H_1/H_2 receptor antagonism would result in an extended duration of elevated circulating leukocytes, a reduced expression of inflammatory cytokines, and delated recovery of muscle strength.

METHODS

Subjects

This study was approved by the Institutional Review Board of the University of the University of Oregon. Each volunteer gave written and informed consent prior to

participation and the study conformed to the principles of the Declaration of Helsinki. Twelve healthy volunteers (3 female, 9 male) completed the study and were considered sedentary or recreationally active based on exercise habits over the previous 12 mo (157). No subjects were using over-the-counter or prescription medications at the time of the study, with the exception of oral contraceptives. Female participants were studied during the early follicular phase of their menstrual cycle or during the placebo phase of their oral contraceptive to minimize any potential effects of sex-specific hormones on muscle damage and immune responses. Demographic and anthropometric information (age, height, weight) including measurement of 3-site skin-fold body-fat estimate (triceps, supra-iliac, and mid-thigh for females; chest, abdominal, mid-thigh for males) were made prior to the initial study day. All volunteers were required to abstain from caffeine, alcohol, and exercise for 24-hr before and for the duration of each observational period.

Experimental Design

The study consisted of two double-blind placebo-controlled testing and observation segments that were of 72-h in duration (Figure 18). To avoid possible cross limb (e.g. left to right leg) adaptations that protect muscle from damage/soreness resulting from eccentric contractions, cross-over trials were separated by at least 30 days. A food diary was provided to the subjects prior to the first testing visit. The food diary encompassed the 24-h prior to the initial exercise as well as the 72-hr observation period after exercise. Participants reported to the laboratory at 0700 after an overnight fast. After a confirmed negative pregnancy test for women, subjects were block randomized into one of four different testing orders (Table 11) which alternated both the leg to be exercised (left or right) as well as drug (placebo or histamine H_1 and H_2 receptor antagonists

(Blockade)) conditions (Table 11). Prior to the second segment of the study, subjects were provided with their food diary from the first segment and asked to mimic food intake (timing, type, and quantity) as close as possible in order to control potential influences of carbohydrate intake on the inflammatory state or protein intake on skeletal muscle remodeling (65).

Table 11. Order of testing visits

Testing visit	Segment 1 Leg	Segment 1 Drug	Segment 2 Leg	Segment 2 Drug	*n*
Order A	Right	Placebo	Left	Blockade	3
Order B	Left	Blockade	Right	Placebo	3
Order C	Right	Blockade	Left	Placebo	3
Order D	Left	Placebo	Right	Blockade	3

n = number of subjects per order

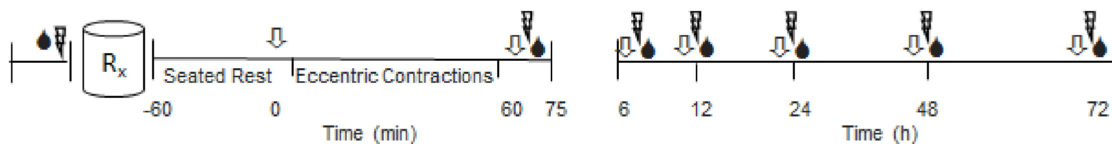

Figure 18. Study time line. The 72-hr study and observational period was conducted twice after the subject consumed either Placebo or Blockade (Rx). Each blood drop (♦) indicates a time point at which venous blood was sampled. The lightning bolt represents a time in which a visual analog scale and algometry were used to asses muscle pain/discomfort. The arrow (⇩) represents a time in which muscle strength was assessed.

Following an initial blood draw and assessment of muscle pain/discomfort, subjects

consumed the Placebo or Blockade pills orally with ~6 oz of water and sat in a padded phlebotomy chair for 60-min. Next measurements of muscle strength (MVC) and

voluntary activation (interpolated twitch) were taken. Then subjects performed 300 eccentric contractions of the leg extensors followed another assessment of muscle strength, muscle pain/discomfort, and a post-exercise blood draw. Subjects then went about their regular day but came back to the lab at 6, 12, 24, 48, and 72-hrs after the completion of exercise for assessment of muscle strength, pain/discomfort, and to provide a venous blood sample (Figure 18).

Histamine H_1- and H_2-Receptor Blockade

Oral administration of 540 mg of fexofenadine, a selective H_1-receptor antagonist, reaches peak plasma concentrations within 1-h and has a 12-h half-life (262). Oral administration of 300 mg ranitidine, a selective H_2-receptor antagonist, reaches peak plasma concentration within 2-h and has a 3-h half-life (93). This dosage of histamine-receptor antagonists results in more than 90% inhibition of histamine H_1 and H_2 receptors lasting for 6-h after administration (93, 287). Fexofenadine and ranitidine are not thought to cross the blood-brain barrier or to have sedative effects (93, 264, 287). Importantly, it has been shown that histamine H_1- and H_2-receptor antagonism does not alter blood flow, heart rate, blood pressure, or smooth muscle tone at rest (78, 79, 187, 188, 254).

Placebo

The placebos were manufactured by a compounding pharmacy (Creative Compounds, Wilsonville, OR) and contained the inactive ingredients of the fexofenadine and ranitidine tablets.

Eccentric Contractions

Subjects performed 300 eccentric contractions of the quadriceps on a resistance dynamometer (Biodex, System 3, Shirley, NY). The 300 knee-extensions were divided into 10 sets of 30 repetitions with a 60-s rest period in between each set. Subjects sat with their back supported, pelvis strapped into the seat, and the resistance pad was placed at 75% of the length of the shank. The knee joint moved through a 75° range from a joint angle of 20° to 85° of knee flexion. The angular velocity was set to $60°\cdot s^{-1}$ for set 1 and $110°\cdot s^{-1}$ for set 2 through 10. The resistance force of the dynamometer was set at 140% of each subject's maximal isometric force measured at a joint angle of 30° of flexion. Subjects were instructed to resist knee flexion as best as they could. Their leg was then moved passively by the dynamometer back into the extended position. This protocol is modeled after Newham et al. and MacIntyre et al. (173, 207) in which 300 eccentric contractions of the quadricep muscles induced a moderate level of muscle damage and caused a local inflammatory response. The whole protocol was approximately 40-min in duration.

Measurements

Blood Sampling

Venous blood (9 ml) was collected from a superficial vein in the antecubital space into three 3-ml Vacutainers (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes NJ, USA) for collection plasma (K2 EDTA anticoagulant). Blood was then aliquoted for separation and quantification of white blood cell populations via flow cytometry (Gallios, Beckman Coulter Life Sciences, Indianapolis IN, USA) using the manufactures data acquisition software (Kaluza, Beckman Coulter Life Sciences, Indianapolis IN, USA). Separate aliquots were centrifuged at 1,275 RCF (Marathon 3200R, Fisher Scientific, Pittsburg PA) and stored in Cryovials at -80˚C until analysis.

Plasma was analyzed for creatine kinase using an activity assay (Sigma-Aldrich, St. Louis MO, USA). Plasma was also analyzed for inflammatory cytokines via a bead based flow cytometry kit (Biolegend, LEGENDplex Human Inflammation Panel 1, multi-analyte flow assay kit). This method of cytokine identification uses beads of varying size coated with antigens specific to cytokines of interest (IL-1B, INFα2, IFN-γ, TNFα, MCP-1, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, and IL-33). The size and fluorescence of the beads allows for separation and quantification of the cytokine by flow cytometry (Gallios, Beckman Coulter Life Sciences, Indianapolis IN, USA).

Knee extension strength testing

Maximal voluntary isometric force of the knee extensors was determined using a resistance dynamometer (Biodex, System 3, Shirley, NY). The knee angle was set to 30˚ of knee extension and subjects performed three maximal knee extension contractions with 60-s rest between attempts. A single muscle, the vastus lateralis, was evaluated with an EMG (Motion Lab Systems Inc. Baton Rouge, LA) during the strength test. The EMG unit included a built-in amplifier and ground. The electrode was placed in parallel to the approximate axis of the muscle fibers at a distance approximately 25% of the distance between the anterior superior iliac spine and the Gerdy prominence (29) and was held in place with adhesive tape. The location of the electrode was marked with ink for placement of the electrodes on future evaluations. Following the three maximal contractions, three further maximal contractions with a superimposed stimulation of the femoral nerve (supramaximal stimulation) in combination with an evoked twitch at rest were performed (23, 26, 244, 248). The evoked twitch was performed approximately 7-s

after the maximal contraction. The evoked twitch and supramaximal stimulation were performed to parse out central versus peripheral factors related to the decrease in muscle strength experienced following muscle damaging exercise. Activation of the quadriceps during the MVCs were assessed using a superimposed single twitch on the MVC and was compared with the force produced by the evoked twitch (3).

Muscle pain and discomfort

Subjects' perceived muscle pain and discomfort were evaluated by means of a visual analog scale questionnaire. Subjects provided a subjective rating of their general muscle soreness by marking a single vertical line along a 100 mm continuous scale with descriptive anchors at 0 mm indicating "no pain/discomfort," 50 mm "moderate pain/discomfort," and 100 mm "worst pain/discomfort". The distance from the left end (0 mm) of the scale to the vertical mark allowed for quantification of the pain/discomfort level.

Pain pressure threshold

The minimum pressure that induces pain or discomfort, pain pressure threshold (12), was evaluated using algometry at two sites on the subject's thigh, the vastus lateralis and rectus femoris. The vastus lateralis was evaluated 10 to 20 cm above the lateral epicondyle of the femur and the rectus femoris was evaluated along the midline of the front of the leg between 8 to 15 cm above the patella. The sites were marked with a pen for visit-to-visit repeatability. Pain pressure threshold was evaluated with a Wagner digital algometer (Model FPX25, Wagner Instruments, Greenwich CT, USA), according to the methods of Fischer (86) in which the 1 cm^2 flat rubber tip is pressed firmly and

perpendicularly into the muscle with an increasing force of approximately 5 N s^{-1}. Pressure was stopped when the volunteer indicated the onset of pain/discomfort. Each site was evaluated 3 times and values were averaged.

Statistical Analysis

Statistical inferences were drawn from a combination of paired t-tests and 2 -way repeated measures ANOVAs with *a priori* contrasts (v12, Systat,San Jose, CA, USA). For all variables, a separate 2-way repeated measure ANOVA was performed to test for differences in testing order to account for any decreased response from a repeated bout effect. Significance was set at $P < 0.05$. All data are presented as means ± SEM, except for data characterizing the subjects, which are presented as means ± SD. (Table 12).

RESULTS

Subject characteristics

Twelve (3 female, 9 male) healthy, non-smoking individuals volunteered for the present study. Subject’s demographic and anthropometric characteristics obtained from the screening visit including age, height, weight, body mass index, body fat percentage, and knee-extension peak power are presented in Table 12. Trial days were separated by 58 ± 19 days (mean ±SD; range 30-94 days).

Table 12. Subject Characteristics

N	12 (3F, 9M)
Age (years)	24 ± 3
Height (cm)	181.9 ± 12.4
Weight (kg)	78.4 ± 16.4
Body Mass Index ($kg \cdot m^{-2}$)	23.5 ± 3.0
Body Fat (%)	20.5 ± 11.3
Isometric Knee Strength (N·m)	199 ± 47

Values are means ± SD.

300 Eccentric contractions

The amount of work performed in each of the 10 sets of 30 repetitions is shown in Figure 19. By design, the amount of work increased from set 1 to 2. The amount of work subjects performed was greater in set 2-5 than set 1 (set effect $P<0.05$). There were no differences between Placebo (64296 ± 5692 N•m) and Blockade (62146 ± 5047 N•m) in the total amount of total work performed over the 10 sets (drug effect; $P = 0.318$) nor was there an interaction in the amount of work completed in each set between Placebo and Blockade conditions (drug X set effect; $P = 0.884$).

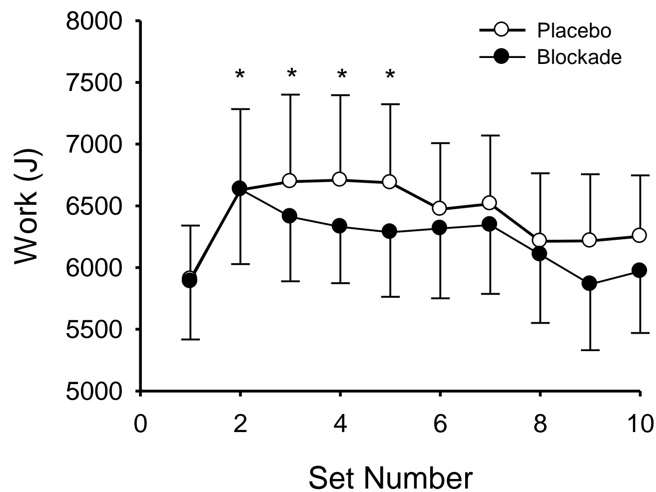

Figure 19. Work completed during each set of 30 eccentric contractions of the quadricep muscles. ○ = Placebo; ● = H_1/H_2 Blockade. Values are means ± SEM. * $P < 0.05$ vs set #1 across both groups (main effect of time). There was no drug effect nor was there a drug x time interaction.

Creatine Kinase

Plasma creatine kinase concentrations were not different pre-exercise between Placebo (62.7±9.9 U/l) and Blockade (63.1 ± 10.5 U/l), and increased above preexercise concentrations at 6, 12, 24, 48 h after exercise (time effect $P<0.05$) (Figure 20). Creatine kinase concentrations increased similarly between Placebo and Blockade conditions (drug effect $P = 0.370$) and the pattern of change was not different between groups (drug x time interaction $P = 0.647$). By convention, an area under-the-curve analysis was performed to represent the extent of damage. The area under-the-curve analysis indicated that there was no difference between Placebo and Blockade in the elevation of creatine kinase over 72-hr ($P = 0.292$)

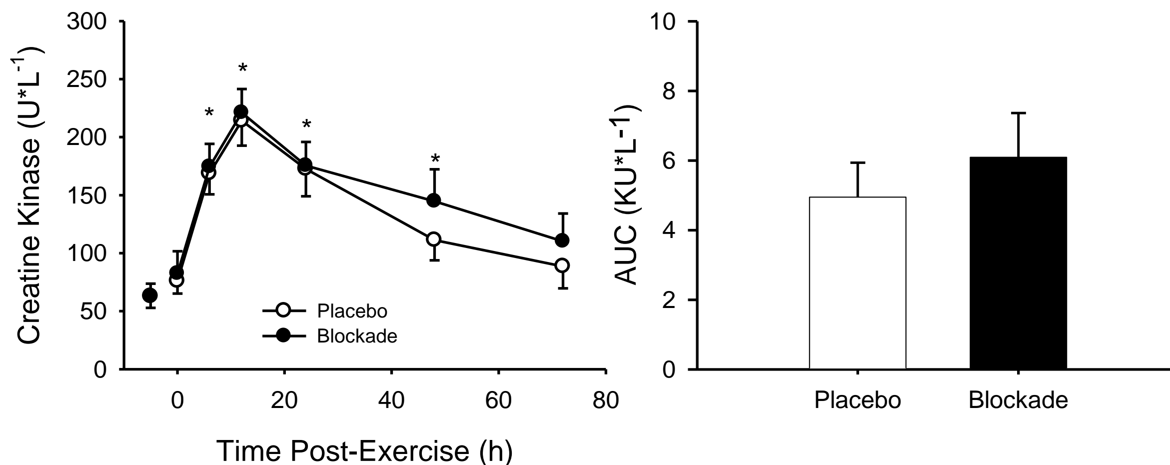

Figure 20. Plasma concentrations of creatine kinase during 72 h of recovery from eccentric exercise. ○ = Placebo; ● = H_1/H_2 Blockade. Values are means ± SEM. * P<0.05 vs preexercise.

Strength

The isometric torque produced by the quadricep muscles did not differ between Placebo and Blockade preexercise (P=0.895, Figure 21). Torque was reduced immediately following exercise and returned to preexercise levels 72-h after exercise (Figure 21). The torque loss of -163±8 N•m peaked 24-h after exercise and represents a 19.5±3.4% reduction in isometric strength. There were no differences in strength loss between Placebo and Blockade (drug effect, P = 0.817) nor in the pattern of strength loss between conditions (drug x time interaction P = 0.906).

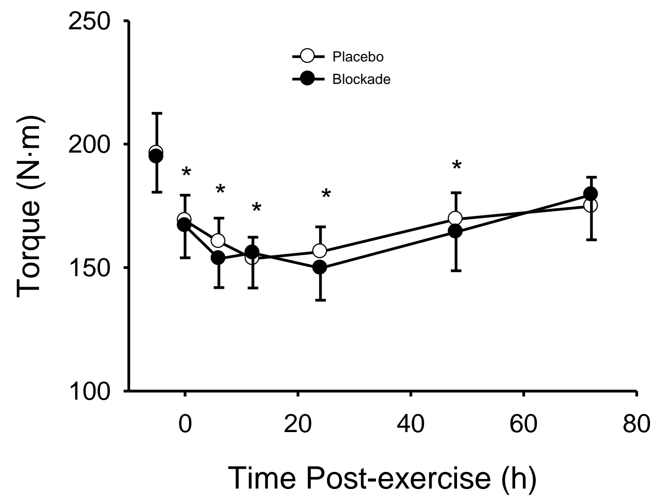

Figure 21. Quadriceps isometric torque during 72 h of recovery from eccentric exercise. ○ = Placebo; ● = H_1/H_2 Blockade. Values are means ± SEM. * P<0.05 vs preexercise.

Evoked Twitch

The amount of torque produced by the evoked twitch was similar preexercise between Placebo (58.5±3.7N•m) and Blockade days (59.1±3.7N•m). Torque decreased after exercise and remained below preexercise for 24-hs after exercise (time effect P<0.05). The reduction in evoked torque was not different between Placebo and Blockade conditions (drug effect P= 0.873) nor was the pattern of change between Placebo and Blockade different over the 72 hours (drug X time interaction P = 0.959) (Figure 22).

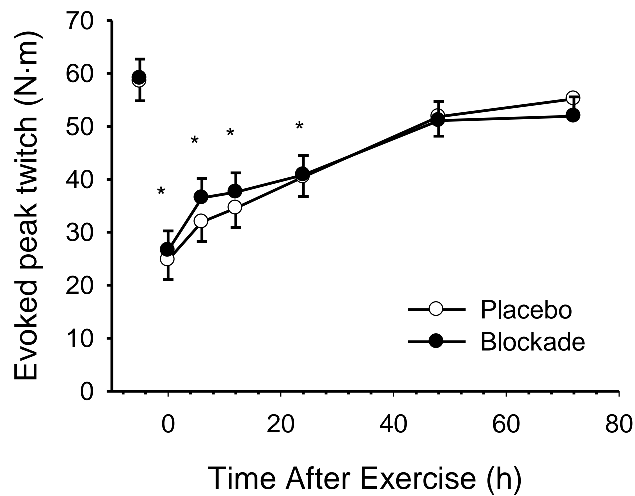

Figure 22. Evoked twitch of the quadricep muscles during 72 h of recovery from eccentric exercise. ○ = Placebo; ● = H_1/H_2 Blockade. Values are means ± SEM. * P<0.05 vs preexercise across both groups (main effect of time).

Central Activation Ratio

Due to difficultly obtaining a plateau in voluntary force, as most subjects produced a peak force early in the contraction which was not maintained, a reliable increase in force from a superimposed twitch could be only be obtained in 7 individuals in both the Placebo and Blockade conditions. Due to high variability in the measures there were no difference in the central activation ratio over time (time effect P = 0.476), between drug conditions (drug effect P = 0.110), nor an interaction effect (drug X time interaction P = 0.578) (Figure 23).

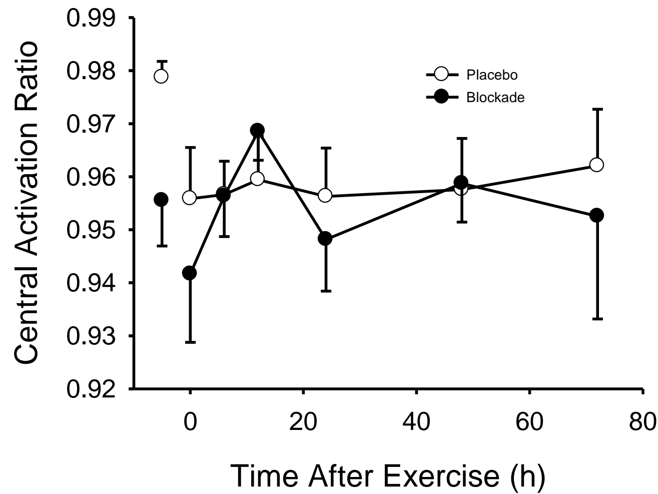

Figure 23. Central activation ratio during 72 h of recovery from eccentric exercise. ○ = Placebo; ● = H_1/H_2 Blockade. Values are means ± SEM, n = 7.

Muscle Pain/Discomfort

Visual analog scale ratings of pain/discomfort were not different between Placebo and Blockade preexercise (P=0.805). The pain/discomfort rating was increased following exercise (time effect P<0.05). Muscle pain peaked 24 to 48 h after exercise but this response was not different between Placebo and Blockade conditions (drug effect P = 0.239) nor was the pattern of change over the 72 hrs different (drug x time interaction P = 0.923)(Figure 24).

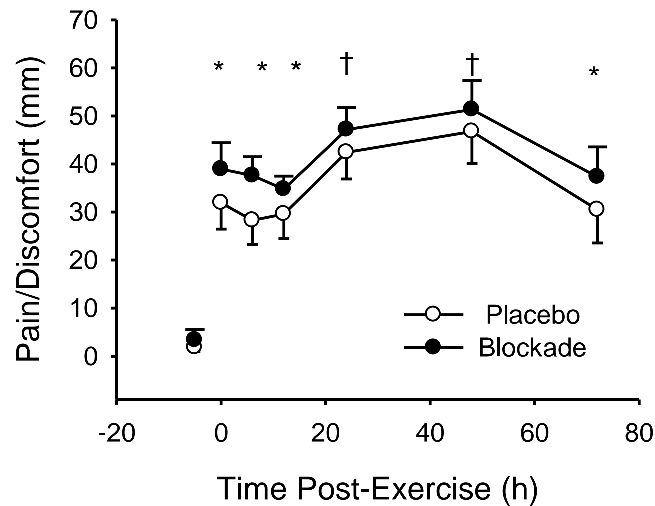

Figure 24. Pain and discomfort rating from a visual analog scale during 72-h after eccentric exercise. ○ = Placebo; ● = H_1/H_2 Blockade. Values are means ± SEM. * P<0.05 vs Preexercise, † P<0.05 vs 0, 6, 12, and 72-hr (main effect of time). There was no drug effect or drug x time interaction.

Algometry

Pain pressure threshold preexercise was not different between Placebo and Blockade for the vastus lateralis (P=0.478) or the rectus femoris muscle (P=0.640)(Figure 25). Pain pressure thresholds were decreased following exercise (more pain) for both muscles at 6, 24, 48, and 72-h after exercise (time effect P<0.05) (Figure 25). For reference, dashed reference lines in figure 25, defines a decrease in pain pressure threshold below 84.1% of baseline as a clinically relevant change in pain sensation (215) . These results indicate that the majority of the soreness the subjects experienced was in the vastus lateralis and represents a clinically relevant change in pain sensitivity.

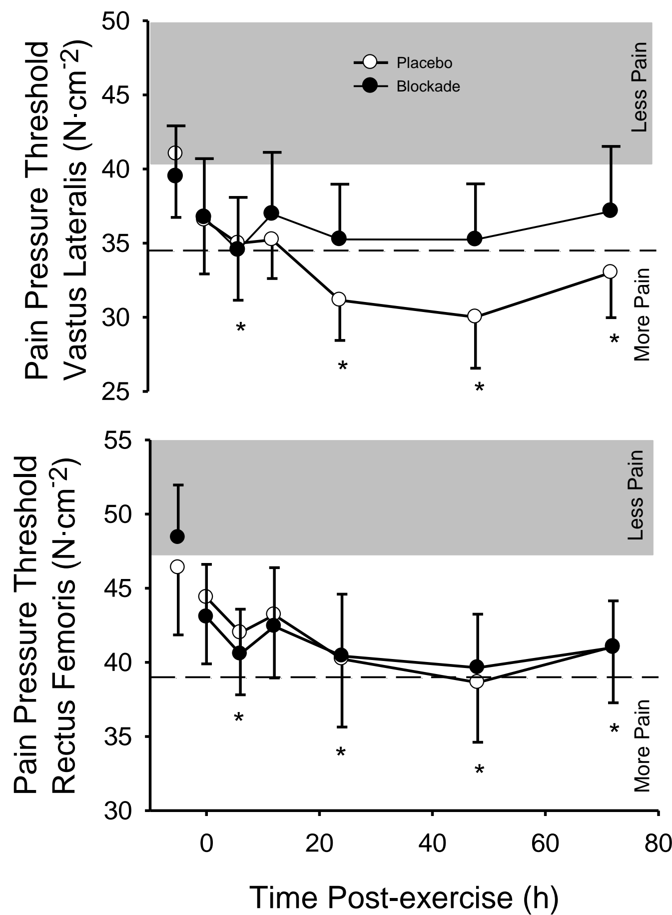

Figure 25. Pain pressure threshold of the vastus lateralis and rectus femoris muscles during 72 h of recovery from eccentric exercise. ○ = Placebo; ● = H_1/H_2 Blockade. Values are means ± SEM. The shaded band represents an increase in pain pressure threshold in that it required more point pressure to induce a perception of pain. Dashed lines represent a drop in pain pressure threshold of 15.9%, a clinically meaningful change in pain perception. There was no drug effect or drug X time interaction. * $P<0.05$ vs preexercise across both groups (main effect of time).

Leukocytes

There was no difference in leukocyte count preexercise between placebo and blockade. Exercise caused an increase in circulating leukocytes that peaked 6-h after exercise (time effect $P < 0.05$). The rise in leukocytes represents a 74±18% increase over

baseline at 6 hours post-exercise. There were no differences between Placebo and Blockade (drug effect P = 0.991) nor was there a change in the pattern of change over time between Placebo and Blockade (drug X time interaction P = 0.828) (Figure 26A). To examine whether testing order may have affected the outcomes between drug conditions a 2-way repeated measures ANOVA was run (Figure 25B). There was a larger increase in Leukocytes Day 1 compared to Day 2 regardless of drug condition (P<0.05). The leukocyte count peaked 6-h after exercise (P<0.05) and then returned to preexercise levels. To remove the order effect Placebo versus Blockade was further examined only during Day 1 (Figure 25C). This analysis of a sub-set of the data did not indicate a difference between Placebo and Blockade (drug effect P = 0.499), over time (time effect p=0.161), nor drug X time interaction (P=0.583) (Figure 26C).

Cell Sorting

During cell separation, there were 105,618 ± 7815 singlets counted per time point. Monocyte cells were identified and separated by the CD14, CD66 and CD86 surface markers and sub populations of monocytes were based off CD 16 and CD 192 surface markers. Neutrophils were identified based off CD66B and CD16 surface markers.

Neutrophils

The percent of neutrophils in the blood preexercise was not different between Placebo (40.6±4.5%) and Blockade at (40.6±1.7%). There were no differences in the percent change in neutrophils between Placebo and Blockade conditions (drug Effect P=0.330), over the 72 hours (time effect P = 0.063), nor in the pattern of change over time between Placebo and Blockade (drug X time interaction P = 0.242)(Figure 27).

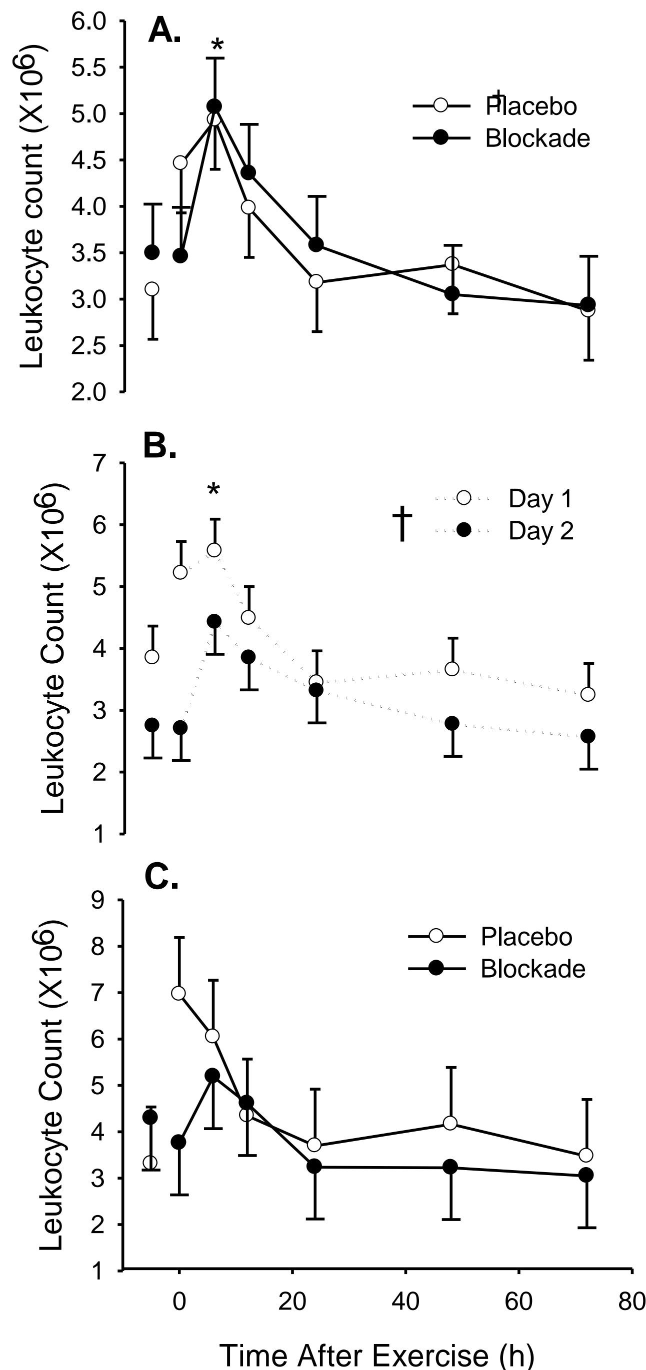

Figure 26. Total Leukocyte count per ml of blood during 72-hr of recovery from eccentric exercise. **A.** Blockade versus Placebo. **B**. Day 1 versus Day 2. **C.** Placebo versus Blockade within Day 1 only. * P<0.05 vs preexercise across both groups (main effect of time). † = Effect of Day (P<0.05). There were no Drug X time or day X time interactions. ○ = Placebo; ● = H_1/H_2 Blockade. Values are means ± SEM.

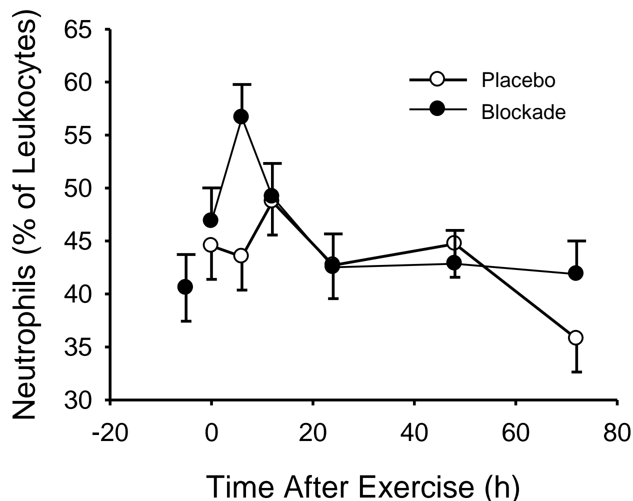

Figure 27. Neutrophils as a percent of total leukocyte count in the blood over 72-hr after exercise. ○ = Placebo; ● = H_1/H_2 Blockade. Values are means ± SEM.

Monocytes

Total monocyte and monocyte sub-populations were not different between Placebo and Blockade conditions before exercise. There was no difference in between Placebo and Blockade conditions for monocytes or monocyte sub-populations over time nor in the pattern of change over time (Table 13).

Inflammatory Cytokines

The Interleukin-12 (IL-12p70) never rose to levels in the blood that exceeded the lowest detectable limits of the bead assay. There were no differences between Placebo and Blockade for any cytokine (Table 12). Monocyte chemoattractant protein-1 (MCP-1) was elevated at 6 and 12 hours after exercise compared to the other time points ($P<0.05$) but the rise was similar between Placebo and Blockade conditions (Table 14). No other cytokine changed over time.

Table 13. Monocyte and monocyte sub-populations during recovery from eccentric exercise

		Time after exercise (h)							P-Value		
		Pre	0	6	12	24	48	72	Drug	Time	Drug*Time
Monocytes % of Leukocytes	Placebo	5.7±1.0	4.7±1.0	5.7±1.1	4.5±0.8	4.9±0.8	4.6±0.9	3.6±0.6	0.505	0.191	0.616
	Blockade	6.1±1.0	4.8±0.7	4.8±0.8	4.2±0.6	5.2±0.6	6.4±1.4	4.6±0.8			
Mon I % of Monocytes	Placebo	59.7±9.5	71.2±7.0	65.2±8.9	76.0±8.0	72.5±8.1	65.1±8.6	59.8±7.1	0.743	0.116	0.600
	Blockade	71.8±9.4	71.3±9.3	75.5±9.5	75.3±9.8	68.2±10.9	66.2±9.7	66.0±8.8			
Mon II % of Monocytes	Placebo	34.3±9.1	25.5±7.2	31.7±9.3	19.8±8.4	23.8±8.2	31.7±8.9	36.6±7.5	0.801	0.067	0.726
	Blockade	25.6±9.6	25.9±9.4	21.4±9.8	20.3±10.3	28.8±11.1	30.1±10.0	31.5±8.9			
Mon III % of Monocytes	Placebo	2.4±0.9	2.1±0.6	2.1±0.7	2.6±0.6	2.3±0.5	2.3±0.7	2.4±0.7	0.897	0.605	0.690
	Blockade	1.8±0.3	2.0±0.5	2.1±0.7	2.6±0.7	2.4±0.5	3.1±0.7	1.5±0.5			

Values are means ± SEM

Table 14. Plasma cytokine changes during recovery from exercise

		Time after exercise (h)							P-Value		
		Pre	0	6	12	24	48	72	Drug	Time	Drug*Time
IL-1B (pg/ml)	Placebo	7.7±1.9	7.5±2.0	8.1±1.8	7.2±2.0	7.3±1.6	7.1±1.7	7.3±1.8	0.918	0.627	0.739
	Blockade	7.7±2.0	9.0±3.0	8.1±2.5	7.6±2.0	7.3±1.6	6.4±2.0	6.7±1.9			
IFNa2 (pg/ml)	Placebo	5.3±1.6	5.9±1.8	5.5±1.4	5.1±1.6	5.1±1.3	5.5±1.3	5.1±1.5	0.699	0.635	0.987
	Blockade	5.7±1.7	6.0±2.3	5.8±1.9	5.2±1.6	5.0±1.4	5.3±1.6	5.0±1.6			
IFN-y (pg/ml)	Placebo	3.9±1.0	4.0±1.1	3.7±0.8	3.7±1.0	3.6±0.7	3.7±0.8	3.5±0.9	0.809	0.623	0.959
	Blockade	4.0±0.9	4.1±1.4	4.0±1.2	3.5±0.9	3.3±0.8	3.8±0.9	3.7±1.0			
TNF-a (Pg/ml)	Placebo	8.6±2.8	9.5±2.8	8.3±2.8	6.2±1.7	8.0±2.3	7.9±2.0	6.5±1.7	0.508	0.415	0.946
	Blockade	8.1±2.2	8.3±2.6	7.8±2.2	7.0±2.0	6.7±1.7	6.7±1.8	6.2±1.7			
MCP-1 (pg/ml)	Placebo	47.7±6.9	42.0±5.6	91.7±20.5*	68.5±10.3*	42.9±6.8	43.8±8.2	37.2±4.5	0.812	<0.05	0.965
	Blockade	48.2±7.0	41.7±5.6	80.5±12.5*	71.8±11.6*	44.0±7.2	38.2±6.5	36.7±4.3			
IL-6 (pg/ml)	Placebo	5.7±1.5	5.8±1.5	5.8±1.3	5.7±1.4	5.6±1.0	5.5±0.9	5.5±1.2	0.807	0.422	0.329
	Blockade	6.0±1.4	7.7±3.5	6.9±2.5	5.2±1.1	4.7±0.9	5.0±1.3	4.8±1.2			
IL-8 (pg/ml)	Placebo	5.1±1.6	5.6±1.5	5.3±1.3	5.0±1.6	5.6±1.7	6.7±2.6	5.2±1.5	0.394	0.879	0.116
	Blockade	5.7±1.4	4.7±1.3	4.5±1.1	3.9±0.8	4.0±0.7	7.5±3.7	4.0±0.9			
IL-10 (pg/ml)	Placebo	4.8±1.6	5.4±2.2	4.7±1.4	4.6±1.6	4.3±0.9	4.5±0.9	4.4±1.3	0.730	0.546	0.732
	Blockade	4.8±1.7	6.5±3.6	5.8±2.8	4.1±1.1	4.1±1.2	4.5±1.3	4.3±1.7			
IL-17A (pg/ml)	Placebo	6.2±1.3	6.3±1.4	6.6±1.3	5.5±1.2	6.3±1.2	6.6±1.4	5.4±1.2	0.721	0.459	0.726
	Blockade	7.5±2.5	6.8±1.7	6.8±1.7	6.1±1.4	6.2±1.4	5.7±1.4	5.5±1.2			
IL-18 (pg/ml)	Placebo	53.4±15.2	55.3±16.0	53.4±11.7	50.6±14.0	50.4±14.0	58.1±16.6	49.2±15.2	0.892	0.565	0.711
	Blockade	52.2±14.1	57.0±14.6	54.9±15.4	54.6±18.3	53.7±15.0	52.1±16.6	48.5±13.6			
IL-23 (pg/ml)	Placebo	4.8±1.4	4.8±1.4	4.8±1.3	4.5±1.5	4.5±1.2	4.4±1.2	4.2±1.3	0.627	0.335	0.593
	Blockade	4.7±1.4	6.1±2.7	5.8±2.2	4.3±1.2	4.1±1.2	4.2±1.3	4.1±1.3			
Il-33 (pg/ml)	Placebo	10.1±3.1	10.7±3.3	10.4±2.7	9.7±3.1	9.7±2.5	9.9±2.5	8.7±2.8	0.350	.0574	0.860
	Blockade	11.1±3.5	13.9±6.3	12.4±4.5	10.5±3.6	9.5±3.0	10.0±3.5	9.7±3.6			

Interleukin-1B (IL-1B), Interferon alpha 2 (IFNa2), Interferon-gamma (IFN-y), Tumor Necrosis Factor alpha (TNFa), Monocyte Chemoattractant Protein-1 (MCP-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-17a (IL-17a), Interleukin-18 (IL-18), Interleukin-23 (IL-23), Interleukin-33 (IL-33). * = elevated over time points, Values are means ± SEM

DISCUSSION

The main finding of the present investigation was that taking combined histamine H_1 and H_2 histamine receptor antagonists had no effect on the systemic inflammatory response following muscle-damaging exercise. Additionally, these results suggest, at least at the systemic level, that antihistamine therapy prior to exercise does not affect the functional recovery of the muscle in the ensuing 72 hours. This study does not address local repair and remodeling processes within the muscle tissue, where histamine is released during exercise.

Exercise Inflammatory Response

It is now generally accepted that a tightly regulated inflammatory response is necessary for muscle repair and regeneration following strenuous exercise (231, 307). Multiple inflammatory cell types including mast cells, neutrophils, and monocytes aid in this response (173, 174, 183, 231, 308, 309). Following exercise, there is an immediate and robust increase in circulating leukocytes that wanes in the hours to days that follow (39, 51, 111, 210, 230, 280). Within an hour after exercise neutrophils are observed in the extracellular space of skeletal muscle tissue and monocytes/macrophages in hours to days that follow (39, 66, 173, 231). There are also large increases in circulating cytokines, many released from the contracting muscle, that are part of a paracrine and endocrine communication for the coordination of the inflammatory response (229, 232). Once inside the muscle tissue, the neutrophils and monocytes/macrophages breakdown damaged tissue and begin the repair process. Although the leukocytes and cytokines aid in inflammatory response after exercise, many aspects in the regulation of repair are unclear and involve unknown molecules (231).

Histamine

Histamine is a molecule that not only has associations to immune and inflammatory responses, but is also released and produced within the contracting skeletal muscle. Intramuscular histamine concentrations increase ~3 fold during moderate intensity endurance exercise (255). It is possible that the exercise-induced rise in intramuscular histamine is involved with the exercise inflammatory response in order to deliver and direct the circulating leukocytes to the muscle tissue. For example, in immune and inflammatory responses, histamine aids in the diapedesis of leukocytes from circulation into damaged/infected tissue. The diapedesis occurs through a histamine initiated margination of capillary endothelial and pericyte cells, an increased capillary permeability, the expression of adhesion molecules on endothelial cells, and in acting as a chemoattractant to leukocytes (22, 146, 224). Histamine may also be involved with the delivery of immune cells to the muscle as there is a histamine mediated dilation of resistance arterioles that results in an elevation of local blood flow that can last upward of 120 min within the previously active muscle (187, 188, 255). Further evidence that histamine is involved with the exercise inflammatory response comes from a recent study that documented a down regulation of approximately 800 protein-coding genes within muscle tissue with a systemic blocking of histamine actions. Many of the protein-coding genes were related to inflammation and cell maintenance (254). Therefore, histamine may be the link between exercise and the associated inflammatory response.

Research Design

This is the first known investigation to examine the link between histamine and the systemic inflammatory response following exercise. Important to the interpretation of the results of this study was the selection of an exercise protocol that elicited a rise in circulating blood cells, an increase in intramuscular histamine concentrations, and one that could be repeated within the same subject to test for changes that occur with a drug intervention. Subjects performed 300 unilateral eccentric contractions of the knee-extensor muscles. The contractions were performed in sets of 30 with little rest between sets in an attempt to mimic the mode, type and, duration of endurance type exercise that is known to cause an elevation in intramuscular histamine and result in a histamine mediate post-exercise dilation (110, 187, 188). Additionally, previous studies using 300 unilateral eccentric knee-extension contractions have demonstrated a systemic leukocytosis and an increase in white blood cells within the muscle 24 hours after exercise (173, 207). Importantly, the isolation of single muscle groups on both sides of the body were used for comparison between drug conditions.

Using the unilateral eccentric knee-extension model, the subjects performed a similar amount of work between Placebo and Blockade conditions (Figure 19), had similar reduction in peak torque (Figure 21), and elevation in plasma creatine kinase (Figure 20) in the 72-hrs after exercise. Analysis of evoked twitch potentials and central activation indicate that minimal reductions in strength were due to decreased central activation (Figure 22, Figure 23). Importantly, these central and peripheral evaluations of strength loss did not differ between Placebo and Blockade conditions (Figure 22, Figure

23). Therefore, it was assumed that the exercise protocol would initiate a similar inflammatory response in both testing sessions.

Muscle Pain and Discomfort.

Pain is interpreted from signals from Group III/IV afferent neurons (151, 217). Histamine has the ability to directly sensitize the afferent neurons (151) and may also contribute to the upregulation of neurotrophic factors (78, 254) involved with extended sensitization and potentiation of the same neurons (237, 298). A previous study found that blocking histamine during exercise attenuated the rise muscle pain and the associated delayed soreness experienced after muscle-damaging exercise (downhill running) (78). The current study, while finding a sharp increase in the subjective assessment of quadricep muscle pain and reduction in the pain pressure threshold (more pain) after knee-extension exercise did not find a difference in pain sensations between the Placebo and Blockade conditions a (Figure 23, Figure 24). A potential explanation for the differing outcomes could be attributed to the individual subjective assessment of pain. The downhill running study in comparison to the present results compared two separate groups of individuals (78) and the difference between groups confounded the results of the drug condition. Another possible explanation could relate to the smaller amount of muscle mass (quadriceps) exercised in single-leg knee extension exercise compared to the whole body exercise of downhill running (78). The subjects in the present study experienced greater soreness overall (5/10) than the downhill running (3.5/10). It is possible that greater quadricep muscle damage occurred during the 300 eccentric contractions compared to the downhill running and factors other than histamine contributed to the activation/sensitization of Group III/IV afferents, which negated the

effects of the histamine receptor antagonists. These muscle pain measurements also serve as another indicator that the exercise protocol produced a similar amount of damage between Placebo and Blockade conditions.

Inflammatory Response

Similar to previous reports of muscle damaging exercise, there was a transient elevation in circulating leukocytes that peak increase 6 hs after exercise and returned to baseline levels approximately 24 hrs after exercise (173, 230, 231) (Figure 26). It was hypothesized that exercise-associated elevation of histamine would aid in the migration of leukocytes out of circulation and into the previously active skeletal muscle. This hypothesis was based on the functions of histamine during inflammation, characterized by the vasodilation of blood vessels, increased capillary permeability, and expression of endothelial adhesion molecules. Therefore, blocking histaminergic actions during and following exercise would result in an extended elevation in leukocyte concentrations. Contrary to the hypothesis, blocking histamine's actions did not affect the systemic elevation or the time course leukocytes concentrations in the blood (Figure 26). The time course in the rise of leukocytes and the return to baseline between Blockade and Placebo conditions were not different. However, it is unknown if the skeletal muscle infiltration of the leukocytes was the same between Placebo and Blockade conditions. It is possible that the entry of leukocytes into the skeletal muscle was impeded with blockade and a separate tissue bed took up the leukocytes.

Immediately after exercise, there is an increased concentration of circulating cytokines such as IL-6, TNFa, IL-1B, MCP-1, IL-8, IL-1ra, and IL-10 (47, 85, 210, 232, 291). Additionally, studies have documented an association in the rise of cytokines and

inflammatory cells with increased creatine kinase levels following muscle damaging exercise (66, 202, 210). In the present study there were no detectable changes in any cytokine over the 72-hr recovery period or between Blockade and Placebo conditions (Table 12). There was a rise in monocyte chemoattractant protein-1 at 6 and 12 hr but the rise was not affected by Blockade. Monocyte chemoattractant protein is involved with monocyte recruitment into tissues and is produced by endothelial as well as skeletal muscle myocytes during contraction (51, 62). Given that that this protein increased similarly in both the Blockade as well as the placebo conditions, it is not surprising that the leukocytosis profile was also similar between conditions (Figure 26).

Studies indicate that the leukocytosis after exercise is not limited to one cell type and many cell types may increase in different proportions (122). In addition, cell culture experiments with polymorphic mononuclear cells suggest that histamine has the ability to shift the expression of interleukin-10 and interleukin-12 (147, 224). These two interleukins control the expression cell surface markers and function of monocyte sub-populations (147, 224). Therefore, it was assumed that blocking histamine during exercise may also alter the percentage of monocyte sub-populations and in the leukocyte response. Contrary to this hypothesis, neither the percentage of monocytes nor the sub-population of monocytes were altered over the 72 hrs of recovery from muscle damaging-exercise in the Placebo or Blockade conditions (Table 11). Interleukin-12 was not detected at any time point in the blood and interleukin-10 showed no changes over the 72- hr recovery period (Table 12), so it is possible that the interleukin stimulus to shift monocyte sub-populations was not altered.

Limitations

The present study was limited in that it only examined the systemic inflammatory response and did not examine the skeletal muscle directly. Histamine concentrations are increased within the skeletal muscle during repeated contractions (255) and are reduced by rapid breakdown through membrane bound and cytosolic enzymes (130). Due to the rapid breakdown of histamine, it has a short half-life of approximately 100 seconds and any physiological actions of histamine are likely in close proximity to where it is released/produced. If histamine is participating in the exercise associated inflammatory response; it is at the level of the endothelial, pericyte, mast, and other associated cells within the muscle tissue. If this is a reasonable interpretation of the current data and known functions of histamine, than the skeletal muscle tissue should be the focus of follow up examinations.

This study also used an oral dose of histamine H_1 and H_2 receptor antagonists. The antagonists were distributed systemically and do not specifically block histamine receptors only within the muscle. It is probable that the antagonists effects were concentrated within the active skeletal muscle as it has been previously demonstrated that histamine concentrations do not increase within inactive muscle (255). However, it is acknowledged that the systemic administration of histamine receptor antagonists may have affected other unknown pathways.

Lastly, it is possible that the small volume of muscle exercised and resultant exercise-induced damaged did not provide enough of a stimulus to induce measureable and alterable systemic inflammatory response. Although there was a rise in circulating total leukocytes (74±18%) and neutrophils (29±5%), this rise is less than that seen with

whole body exercise, such as downhill running where cell counts and cytokines increase 300-500% (62, 229–231).

Importance

Histamine is a molecule most associated with inflammatory and immune responses. The discovery that histamine concentrations are increased within skeletal muscle during endurance exercise has led researchers to decipher this odd association. The idea that exercise itself initiates a pro-inflammatory state sets the stage to bridge the gap between exercise-associated increases in histamine and the exercise associated inflammatory response. Although there was no detectable difference in leukocytosis or the production of cytokines from muscle-damaging exercise when histaminergic signaling was blocked, this study suggests that histamine is exerting a local influence within the skeletal muscle and not systemically. Future studies focusing specifically on the skeletal muscle, histamine, myokines, and infiltration of leukocytes will provide information concerning the regulation of muscle degradation and repair (37). This will also shed light on the movement of leukocytes after exercise and as the number of circulating leukocytes represent only 1-10% of the total cell population, small increases/decreases are of clinical significance (280). Additionally, if disrupting the inflammatory process occurs with common "antihistamines" this may delay or prevent the repair process, as has been noted with reduced circulating neutrophils and inflammatory cytokines, as well as common anti-inflammatory therapies (ibuprofen, acetaminophen) (64, 171, 235, 304, 314). Finally, uncovering histamines role in inflammation and the recovery from exercise is important as very little is known about

the cross talk between muscle and other organs that could underlie the effects of exercise on systemic health (185, 205).

Conclusion

This study addresses the issue of pharmacotherapy in the interaction between commonly used medications (histamine-receptor antagonists) and exercise-induced muscle damage and aids in the identification of histamine's role as a molecular transducer of exercise responses (205). Additionally, the present findings address important issues identified by The National Institutes of Health (NIH) on the interaction of physical activity, inflammation, and immune function (205). This study demonstrated that blocking histaminergic signaling on H_1 and H_2 receptors did not affect the systemic inflammatory response of increased white blood cells or cytokines following muscle-damaging exercise. Additionally, blocking histaminergic signaling on H_1 and H_2 receptors did not affect the functional recovery of muscle from muscle-damaging exercise. This study is important to guide the scientific understanding of the exercise-induced rise in skeletal muscle histamine and its association with inflammation resulting from muscle damage.

CHAPTER VII

SUMMARY AND FUTURE DIRECTIONS

"*It is not without an inner feeling of disappointment that we look upon the possible role played by histamine in normal physiology. Here we have a powerful, endogenous, active agent, present in almost every organ or tissue of the body in amounts that can produce not only physiological but also drastic pathological effects, and yet it appears devoid of physiological significance*."

–Mauricio Oscar da Rocha e Silva, 1955 (286)

Dr. Silva was one of the original members of the "Histamine Club", a group of scientists studying the presence and role of histamine in the human body. Much of the work presented in this dissertation is built upon the pioneering work of these scientists. Now, 64 years after Dr. Silva's statement, we have a better understanding of histamine's role in body processes concerning inflammation, digestion, and as a neurotransmitter, but a void still exists in the understanding of its function in the exercise response.

It is now well established that histamine concentrations increase within skeletal muscle tissue during repeated contractions. The rise of intramuscular histamine is at least 3 fold greater than resting levels during 60 min of exercise (255). The increase in histamine concentration comes from degranulation of mast cells and from *de novo* production via the enzyme histidine decarboxylase (255). Although the exact stimulus leading to the increased histamine concentration is unknown, *in situ* and *in vitro* work suggest that multiple stimuli arising from exercise can independently cause mast cell degranulation and increased enzymatic activity. The stimuli include hypoxia, hyperosmolality, oxidative stress, chemokines, increased temperature, vibration, decreased pH, shear stress, substance P, bradykinin, and nerve growth factor (61, 97, 110, 124, 125, 141, 186, 190, 213, 267, 268, 271, 279, 294). Collectively, the influence of even small changes in some or all of these factors must provide a strong stimulus to

increase intramuscular histamine concentrations. Additionally, as exercise duration increases, the production and activity of the histidine decarboxylase enzyme increase (13, 14, 320). These findings suggest that histamine is an inherent component of the exercise response and it may serve a more prominent role in long duration endurance exercise.

Previously documented animal and human performance tests mirror the suggestion that histamine is important during endurance exercise but not necessarily short duration exercise. Studies performed in humans showed that blocking histamine's actions on H_1 and H_2 receptors had no effect on short-duration high-intensity exercise (≤10 min) such as maximal strength tests and sprint performance (194–196). Murine studies demonstrated reductions in endurance capacity from hours long gnawing and walking tasks with H_1 and/or H_2 receptor antagonism, in mice bred without H_1 receptors, and in mice bred without histidine decarboxylase (12, 84, 212, 328). The results of the study presented in Chapter IV, "Histamine-receptor antagonists slow 10-km cycling performance in competitive cyclists", is the first known study to examine the effect of blocking histamine's actions on endurance exercise performance in humans. This study showed that blocking histamine's actions slowed performance in competitive cyclists. The slowing was ~2% in magnitude of each subject's completion time. On the surface, a 2% slowing of performance may not seem like much but it is of similar magnitude of performance enhancement seen with caffeine supplementation and altitude training (91, 140). Although the blockade of histamine actions decreased performance, there were no changes in variables known to affect exercise performance such as blood glucose, lactate, or muscle strength from before to after the time trial. Additionally, there were no effects of blockade on systemic measures relating to hemodynamics (e.g. cardiac output, blood

pressure, total peripheral resistance), metabolism (e.g. oxygen consumption, carbon dioxide production, blood lactate or glucose), or muscle strength during a 120 min steady state cycling exercise. Therefore, while blocking histamine's actions resulted in a reduced endurance performance, it appears to have a localized effect, as systemic values remained consistent.

It is not entirely surprising that systemic hemodynamic, metabolic, and strength measures were unaffected by histamine receptor antagonists during exercise. Histamine is produced in high amounts within the muscle during contractions. This rise in histamine is limited to the contracting muscle, as inactive muscle does not demonstrate an increase in histamine (i.e. arms during leg exercise)(109). Also, histamine has a short half-life (~100 s) as is rapidly degraded by cytosolic and membrane bound enzymes or reabsorbed by mast cells, (22, 130, 204). Given that histamine rises locally within exercising muscle and is degraded rapidly, it likely exerts an autocrine and paracrine action within skeletal muscle during activity (212, 328). The local effects of blocking histamine's actions are likely buffered by other tissues to maintain systemic homeostasis.

Histamine, when it is produced or released within tissues has a strong vasodilator influence which leads to an increased local blood flow. A histamine-related increase in blood flow has been demonstrated in inflammatory and immune responses, during reactive hyperemia, in the skin during heat stress, in sustained post-exercise-vasodilation, and in tumor growth (9, 10, 187, 188, 226, 243, 325). In skeletal muscle, mast cells are located in close proximity to arterioles and HDC is present in high concentrations within arterial endothelial cells as well as skeletal muscle myocytes (13, 270, 312). Therefore, it was a reasonable premise to believe that the exercise-induced histamine release and

production might act as a vasodilator during exercise and contribute to exercise hyperemia. This premise, if true, would explain the reduced endurance exercise performance with blocking histamine. Adequate blood flow to skeletal muscle during endurance exercise is necessary for nutrient delivery and metabolite removal. Blocking histamine's actions may have reduced perfusion, limited nutrient delivery and metabolite removal resulting in decreased performance.

Contrary to this assumption, leg blood flow was elevated during exercise at multiple exercise intensities and over a long duration steady state exercise bout when histaminergic signaling was blocked during exercise (Chapter V, "Histamine receptor antagonism increases leg blood flow during exercise"). This is the first known study to examine systemic blockade of histamine H_1 and H_2 receptors and limb blood flow during exercise. Specifically, the results suggest that femoral artery blood flow, while not affected during resting conditions with histamine receptor antagonism, was elevated during short exercise bouts (3 min) at low (20% peak) to relatively high (80% peak) exercise intensities and during sustained moderate-intensity exercise (60 min at 60% peak) compared to placebo conditions. Again, similar to the findings from steady state cycling in Chapter IV, there were no effects of histamine receptor blockade on systemic measures of blood pressure, ventilation, oxygen consumption, or cardiac output. Interestingly, measures of intramuscular pH using near-infrared spectroscopy recorded lower pH values as exercise intensity increased in blockade compared to placebo conditions. This implies that there was an alteration in either hydrogen ion production or removal from the skeletal muscle. It is possible that this increased acidosis stimulated

more blood flow, but histamine's role in capillary permeability did not allow for the transfer of nutrients/metabolites between the tissue and blood.

It is possible that histamine production in during exercise is related to the exercise recovery process. The recovery from exercise is synonymous with an inflammatory response and histamine has many associations with inflammatory and immune responses. For example, histamine directly and indirectly assists in directing leukocytes to areas of tissue damage through capillary cell margination, the expression of adhesion molecules, and diapedesis of leukocytes (22, 24, 169, 176, 203, 300). Endurance exercise itself initiates an inflammatory like state in which there is an increase in circulating white blood cells that peak in concentrations 6-12 hours after exercise (39, 51, 210, 230, 280). Following arduous exercise that may result in extensive tissue damage, the increase in circulating white blood cells is accompanied by muscle pain and soreness. The pain and soreness are part of the inflammatory response and it is theorized that perceptions of pain are part of a protective mechanism to limit volitional force output to protect from further damaged and aid in repair. Therefore, histamine release during exercise was hypothesized be a component of the immune response to repair damaged muscle tissue.

In the study detailed in Chapter VI, "The effect of histamine receptor antagonism on the inflammatory response from muscle damaging exercise" there were no differences in rise or return to baseline of circulatory leukocytes including sub-populations of monocytes and neutrophils, nor of cytokines associated with inflammation (IL-6, I-10, IL-12, MCP1). Additionally, in contrast to previous reports H_1 and H_2 receptor antagonism did not affect muscle soreness or leg muscle pain in the hours after exercise (78). Importantly, H_1 and H_2 receptor antagonism did not affect the recovery of muscle

function following muscle-damaging exercise. This study may have been limited in detecting a robust inflammatory response due to the small mass of muscle exercised. Both the rise in leukocyte count and the rise in creatine kinase (a marker of muscle damage) were approximately 1/4 that reported in the literature resulting from downhill running, eccentric cycling, and whole body weight lifting (62, 229–231).

In total and in keeping with Darwin's Theory of Evolution, that traits remain within a species if they serve a purpose leading to survival across generations, the increase of histamine within skeletal muscle during exercise must serve a purpose. It is evident that histamine is important for endurance exercise, is involved with exercising blood flow regulation, and is involved with inflammation. Although, the exact function or functions of histamine in the exercise response still require further investigation and clarification. A limitation of this work is the use of systemic blockade of H_1 and H_2 receptors rather than a tissue-specific blockade. It is assumed that major function of histaminergic signaling is occurring within the exercising skeletal muscle, the only tissue with known elevations in histamine during contractions. However, it is acknowledged that the systemic blockade could be affecting other unknown pathways during exercise as well.

It is my hope that the readers walk away with a view completely opposite of Dr. Silva that histamine is not "devoid of physiological significance", but in fact, histamine plays vital roles in many physiological pathways associated with the stress of exercise. Additionally, given the importance of histamine in the inflammatory response, in conjunction with the emerging ideas of good and bad inflammation on health, that this little 111 dalton molecule deserves further investigation.

Future Directions

Based on the outcomes of these studies and previous research, histamine's actions during and following exercise are contained within the tissue that it is produced (muscle). The direction forward should shift focus toward a localized examination of histamine within the muscle tissue and away from a systemic evaluation.

Broadly, within the following categories, histamine's role in the exercise response should be further assessed.

1) Endurance exercise performance tasks should be replicated.
    a. Intramuscular pH probes can be used for a direct assessment during exercise as pH is implicated in muscular fatigue.
    b. Muscle biopsies should be obtained prior to and following exercise to asses intramuscular glycogen concentration changes. A low intramuscular glycogen content is associated with muscle fatigue.
    c. Post-performance glucose tolerance test followed by muscle biopsy to test for glucose delivery and intramuscular glycogen replenishment.

2) Muscle blood flows should be re-assessed but include a study design where rest periods between exercise intensities are removed. This may remove variability in blood flow measures and may remove any return to intramuscular homeostasis that may occur during the rest period.

3) Examine the changes of inflammatory cells within the muscle (biopsy) in conjunction with the systemic concentrations (circulatory). Additionally,

labeled white blood cells can be injected into circulation prior to exercise and can be quantified within the muscle following exercise to assess histamine's involvement in leukocyte recruitment into the muscle (see MacIntyre 1996)(173).

4) Assess capillary permeability using the methods of Dr. John Gamble (20, 21, 35, 90), who is an expert in assessing capillary permeability using step-changes in venous occlusion and strain gauges (venous occlusion plethesmography) to calculate capillary filtration constants (CFC). The methods refined by Gamble remove "rest" periods between occlusion pressures and allow for truncated assessments of filtration that can then be used for multiple assessments during recovery from exercise.
    a. If histamine affects capillary permeability this might be an effective countermeasure to acute mountain sickness (AMS) as it is thought that altitude increases the permeability of capillaries and that AMS might be, in part, due to a reduction in central volume (8, 114, 295).
    b. If histamine affects capillary permeability this might also affect plasma volume expansion with heat acclimation. Albumin may leave central circulation and accumulate in the interstitial area bringing fluid with it. This would stimulate an increase in thirst and protein production to replace central volume, therefore increasing total body water (114).

5) Examine histamine's potential role in the repeated bout effect. There is little information available on the peripheral adaptation process associated with repeated eccentric training (87). This effect is believed to be a reorganization of the contractile myofibrils initiated by the inflammatory process (87). If histamine disrupts the normal repair and adaptation from exercise, than blocking histamine may attenuate the protection afforded by the "repeated bout effect".

6) Studies should be carried out on individuals with exercise induced urticaria and asthma (and possibly exercise induced anaphylaxis) as plasma histamine concentrations have been reported to reach 26 ng/ml 25-min after starting exercise (24, 149). These individuals have excessive histamine release during exercise. Examination of systemic inflammatory responses (leukocytosis) in these individuals can provide an avenue to study a pathological condition in which excessive histamine is released.

7) The pathway associated with chronic pain associated with muscle damage is not well characterized and may be associated with NGF/GDNF effects on neuronal sodium channels (NaV1.8). The administration of Ambroxol (A-803467) via microdialysis blocks potentiation of NaV1.8 currents (137) and would be a novel way to study DOMS.

APPENDIX A

Leukocyte isolation and preparation for flow cytometry

___Take a vial of PFA from the freezer to thaw (may need to put in a beaker of water to accelerate)
___Make dilution of 10x RBC lysis buffer (brown bottle in fridge) - 3 ml + 27 ml H2O in a green lid tube
___Add 2.0 ml fresh whole blood to RBC lysis buffer. ___ Incubate **10 min** at RT
___Wipe down shaft of pipet with disinfectant towel to remove blood splatter
___Spin tube at 500 x G for 10 min (program 1)
___Pour off supernatant into beaker with a splash of bleach in hood.
___Resuspend in 30 mL 1x PBS from fridge. Pipet to break up cell pellet.
___Pass through 40 micron filter into fresh tube.
___Spin 10 min @ 500G, discard super into bleach beaker
___Resuspend in 3-5 ml PBS :_______ml **(R)**
___In a tiny tube, mix 10 ul trypan blue with 10 ul cell suspension. ___Load 10ul to each slide well
___Insert slide into countess and zoom to adjust focus (cells with blue edge with white center)
___Count both sides of slide, record cells/ml alive and %alive. Average two sides.

Alive: _________ , ___________ Average ____________cells/ml **(A)**

Initials:_________

% alive: ________ , __________ Average __________%

___Divide 1,000,000 by **A** cells/ml = ______________ml for 1,000,000 cells **(B)**
___(One or two unstained samples total per subject-week is enough)Remove **B** amount of suspension to microcentrifuge tube to save as unstained. Store in fridge until fixing step
___Remaining cells → **R** – **B** = ______________ml x **A** cells/ml= _______________cells **(C)**

*Only subtract **B** if you have removed a sample for "unstained"*

___Dilute cells to 1 x 10^6 cells/ml with cold PBS (if **C** is 4.2x10^6 cells, bring volume up to 4.2 ml) **(D)**
___If there's no reconstituted violet dye less than 2 weeks old in the freezer, make a fresh tube by adding 50 ul DMSO to lyophilized stain (both DMSO and stain are in Invitrogen pouch in freezer. Label stain with date after reconstituting).
___Add 1 ul violet stain per ml of cell suspension (same as 1 ul per million cells – volume **D** but **in ul.** Swirl and incubate in the dark at RT for **30 min.**
___Retrieve unstained cells from fridge (if unstained cells were collected at this time point).
___Fill violet stained cells up to 30 ml with PBS to dilute stain before spinning
___Spin tubes 10 min @ 500G, discard super
___ To each tube, add 1 ml 4% PFA per million cells (volume **D**). Pipet to break up cell clumps.

___Incubate at RT in dark for **10 min**
___Top off cell suspension to 30 ml w/ PBS to dilute PFA**.**
___Spin and discard super into **Formaldehyde Waste Bottle.**
___Resuspend cells in 2.0 mL PBS. Transfer to 2mL tube with label.
___Store in the dark in the fridge until ready for staining & cytometering (up to one week)
___Make sure remaining PFA and violet dye solutions are returned to freezer
___Blood beaker with 10% bleach can be washed down the sink 20 minutes after the last addition

Subject ID_________ Time point_______________
Date_____________________Initials:__________
Alive: _________________ , _____________________ Average (A):____________________ cells/mL
% alive:______________ , ________________ Average:_________________%
R:________mL
B:_________________mL C:___________________cells D:____________mL (added ____________mL)
1,000,000/avg live cells avg live cells x remaining vol C/10^6 mL

Leukocyte Staining for Flow Cytometry

Tube	Channel	Antibody	Stock solution (ug/ul)	Antibody volume (ul)	Stain Buffer (ul)	Master cocktail
1	FL1-10	Unstained		0	100	
2-9	FL1	CD**62E** 0.75ug	.100	7.5		60
	FL2	CD**86** 1.56ug	.200	7.8		62.4
	FL3	CD**309** 0.125ug	.025	5		40
	FL4	CD**34** 5ug	.200	25		200
	FL5	CD**192** 0.156ug	.200	.78		6.24
	FL6	CD**66B** 0.5ug	.200	2.5		20
	FL7	CD**14** 0.625ug	.200	3.13		25
	FL8	CD**16** 0.156ug	.200	.78		6.24
	FL9	CD**3** 0.156ug	.200	.78		6.24
		CD**19** 0.0625ug	.050	1.25		10
		CD**56** 0.156ug	.200	.78		6.24
	FL10	CD**45** 0.75ug	.100	7.5		60

Staining protocol

- Take cell suspensions from fridge and swirl to make sure cells are well suspended. Transfer 1.5 x 10^6 cells to individual 2.0 ml microcentrifuge tubes for each staining sample plus the 10^6 unstained cells . Pellet cells from suspensions 500G x 5 min.

- Re-suspend cell pellet in 100 ul of staining buffer (PBS + 1% BSA + 10% FBS = Biolegend 420201 + 50 mL FBS -Gibco 26140-129, heat deactivated at 56° for 45 min) plus 10 ul mouse IgG (Jackson Immuno – ChromPure 015-000-003). Incubate for 30 min in dark at RT. In the meantime, make up the staining cocktail.

- Wash with 1 mL staining buffer – centrifuge and discard supernatant

- Add 100 µL antibody staining cocktail to appropriate tubes (chart above). Incubate 30 min in dark at RT

- Add 1mL staining buffer to each tube. Centrifuge/discard supernatant

- Re-suspend in 500uL staining buffer. Read in flow cytometer.

The methods of leukocyte isolation and staining in this lab were made with the expertise of Karen Needham.

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