THE EFFECT OF HISTAMINE ON POST-EXERCISE CAPILLARY PERMEABILITY

by

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A THESIS

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Title: Effect of Histamine on Post-Exercise Capillary Permeability

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Dr. John Halliwill

Histamine, an endogenously released molecule in immune and inflammatory responses increases local vasodilation, blood flow, and capillary permeability. During exercise, histamine is produced within exercising muscle and contributes to an elevated post-exercise blood flow. The histamine-induced post-exercise vasodilation is contained within previously exercised muscle. It is unknown if intramuscular histamine also contributes to elevate capillary permeability following exercise. This study compared capillary permeability of the leg before and after prolonged unilateral knee-extension exercise under normal conditions and when histaminergic signaling was blocked. It was hypothesized that H_1/H_2 receptor antagonists would decrease capillary permeability following exercise in an exercised leg but not in a resting leg. Ten (2F) volunteers performed 60 min of unilateral knee-extension exercise at 60% of peak power after consuming either Placebo or histamine (H_1/H_2) receptor antagonists (Blockade). A capillary filtration coefficient (CFC) reflecting the rate of change in limb girth per rise in venous pressure was calculated using venous occlusion plethysmography. CFC was

calculated prior to and following exercise in both the Exercise Leg and the Rest Leg. Data were analyzed with a 3-way RM ANOVA and presented as Means±SEM.

Under Placebo conditions, there was no difference in pre-exercise CFC in the Exercise Leg or the Rest Leg (1.4±0.4 vs. 1.3±0.3 $\mu g \cdot 100g^{-1} \cdot min^{-1} \cdot mmHg^{-1}$). Post-exercise CFC was greater in the Exercise Leg than the Rest Leg, $P<0.05$ (2.7±0.7 vs 1.4±0.3 $\mu g \cdot 100g^{-1} \cdot min^{-1} \cdot mmHg^{-1}$). CFC increased in the Exercise Leg from pre to post-exercise by 120±25% ($P<0.05$) but did not increase in the Rest Leg ($P=0.732$). Under Blockade conditions, there was no difference in pre-exercise CFC in the Exercise Leg or the Rest Leg (1.7±0.5 vs. 1.3±0.5 $\mu g \cdot 100g^{-1} \cdot min^{-1} \cdot mmHg^{-1}$). Post-exercise CFC did not differ in the Exercise Leg compared to the Rest Leg (1.8±0.5 vs. 1.1±0.4 $\mu g \cdot 100g^{-1} \cdot min^{-1} \cdot mmHg^{-1}$). CFC did not increase in the Exercise Leg ($P=0.608$) or the Rest Leg ($P=0.295$) from pre to post-exercise.

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Introduction

Post-Exercise Recovery Period

The Exercise and Environmental Physiology (EEP) Lab at the University of Oregon, led by Dr. John Halliwill, places special emphasis on what is known as the post-exercise recovery period. This refers to the physiological state that occurs immediately following exercise but before the body has returned to a resting state. Post-exercise recovery is physiologically distinct from both exercise and rest and is characterized by unique neural, metabolic, and hormonal changes that can last up to several hours (Romero, Minson, & Halliwill, 2017). This state is not merely a transition phase between exercise and rest, but also a dynamic period with specialized signaling pathways and systems that may be necessary for long-term adaptations to exercise training and have broader applications to vascular health.

The post-exercise recovery period is characterized by changes in the cardiovascular system that affect blood distribution in the body. The first is vasodilation. Following a bout of moderate-intensity endurance exercise, resistance arterioles within skeletal muscles widen to allow more blood to travel to previously active skeletal muscle in a process known as vasodilation. This excess of blood, or hyperemia, can be maintained for upwards of 100 minutes post-exercise (Lockwood, Wilkins, & Halliwill, 2005). Vasodilation and concurrent hyperemia in skeletal muscle leads to a decrease in blood pressure. John Halliwill and colleagues have uncovered the primary mediator of these features of post-exercise recovery: the signaling molecule histamine (Lockwood et al., 2005).

Histamine

Histamine is an organic compound that is most commonly known for its presence in the inflammatory response caused by injury, as it initiates the process of inflammation through vasodilation as well as increased blood vessel permeability. Research from the EEP Lab has substantiated that histamine is released not only in response to inflammatory stimulators, but also intramuscularly during and after exercise (Romero et al., 2017). When histamine is released, it binds to receptors on target cells triggering physiological reactions. Inhibition of histamine receptors significantly reduces sustained post-exercise vasodilation, hyperemia, and hypotension.

There are four histamine receptors in the body: H_1, H_2, H_3, and H_4. Histamine binding to any of these receptors will trigger signaling cascades that can result in various physiological effects. Sustained post-exercise hypotension and vasodilation is mediated specifically by H_1 and H_2 receptors located in the endothelium and smooth muscle of skeletal muscle capillaries. These receptors are activated at different times following exercise. H_1 receptors are most active for the first 30 minutes post-exercise, and H_2 receptors are most active 60-90 minutes post-exercise (McCord and Halliwill, 2006). Local histaminergic vasodilatation is robust at 60 min after exercise.

To investigate these pathways, researchers employ antagonists: molecules that bind to the receptor but do not trigger the signaling cascade or effects. For example, blocking histamine receptors with fexofenadine, an H_1 receptor antagonist (Allegra) and ranitidine, a H_2 receptor antagonist (Zantac), eliminates sustained post-exercise vasodilation during moderate-intensity small muscle-mass endurance exercise. By administering histamine H_1 and H_2 receptor antagonists to individuals and measuring

their physiological post-exercise response, the EEP Lab demonstrated that histamine is the primary cause of sustained vasodilation and consequent hypotension (Figure 1; Halliwill et al., 2013). Similarly, Barrett-O'Keefe et al. (2013) administered histamine blockades and then measured vascular conductance in an exercised and resting leg following a unilateral dynamic knee-extension exercise. They found that blockade only affected blood flow to the exercised leg, illustrating that histamine is released locally in previously active skeletal muscle following exercise by initiating a signaling pathway that begins with histamine binding to H_1 and H_2 receptors.

There are two potential sources of histamine release in skeletal muscle following exercise. First is de novo (new) formation of histamine through the inducible enzyme histidine decarboxylase, which catalyzes the formation of histamine by decarboxylating the amino acid L-histidine (Romero et al., 2016b; Moya-Garcia et al., 2005). Previous studies by Romero et al. (2016a) have indicated that 1 hour of aerobic exercise is sufficient to upregulate expression of histidine decarboxylase mRNA, which would subsequently raise histamine levels. The second source is mast cells. Mast cells exist within skeletal muscle that produce and store histamine, releasing it upon degranulation in response to exercise (Romero et al., 2016b). This research illustrates that histamine is produced and released locally within skeletal muscle, activating H_1 and H_2 receptors (Figure 1). The upstream signaling mechanisms for these outcomes have yet to be identified.

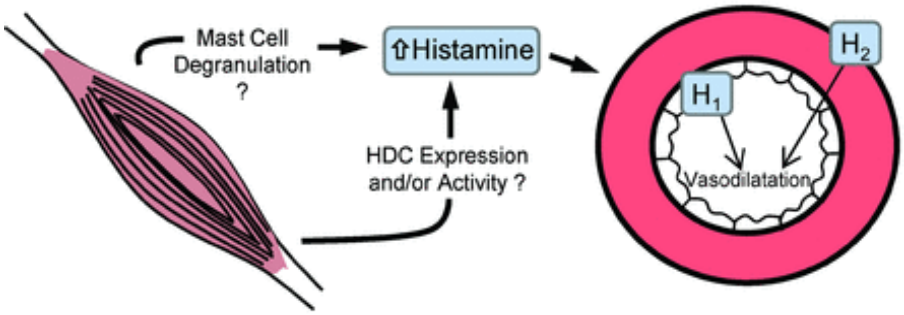

Figure 1: Histamine release and effects in skeletal muscle vasculature.

Following a bout of moderate-intensity endurance exercise, histamine is either formed through the decarboxylation of histidine by histidine decarboxylase (HDC) or released locally from mast cell degranulation. It then acts on H_1 and H_2 receptors within skeletal muscle microcirculation, triggering vasodilation and consequent increase in vascular conductance within previously active skeletal muscle (Halliwill, Buck, Lacewell, & Romero, 2013).

Capillary Permeability

Exercise-induced histamine production and release may be a primary signal for initiating an inflammatory state by increasing capillary permeability after exercise, allowing immune cells and fluid to permeate muscle and cause local edema (Majno, Shea, & Leventhal, 1969). Past studies have mapped the connection between histamine and capillary permeability. Majno and Palade (1961) noted that the increase in blood vessel permeability is likely due to increased size in submicroscopic gaps between endothelial cells and pericytes. Endothelial cells form the single-layer lining on the surface of blood vessels and act as an interface between circulating blood and the basement membrane, which is freely permeable to plasma proteins and water (Suzuki et al., 1999). On the venous side of capillary beds, pericytes begin to appear. Pericytes are known for stabilizing vessels and regulating blood flow, but like endothelial c

ells, they also respond to histamine binding (Majno et al., 1969). Submicroscopic gaps that form among and between these cells are known as fenestrations, which allow fluid to filter from systemic circulation into surrounding tissues.

These discrete intracellular gaps are formed when histamine binds to H_1 and H_2 receptors on the cell surface, triggering second messenger systems within the cell that activate the cytoskeleton (Majno et al., 1969). Andriopoulou et al. (1999) found that histamine augmented capillary permeability further by weakening the intercellular adhesions between endothelial cells and increasing fenestration size. Specifically, histamine caused dissociation of the adhesive, vascular endothelial cadherin from the actin cytoskeleton. Similarly, pericytes transition from wide, spider-like shapes into an oblong formation in response to histamine. This transformation creates even larger fenestrations and allows for enhanced filtration on the venous side of capillary beds. Some studies suggest that contraction of pericyte cells is the primary factor in the formation of intracellular gaps (Díaz-Flores, Gutiérrez, Varela, Rancel, & Valladares, 1991).

The movement of fluid to and from blood vessels is dependent on Starling forces, a term that describes hydrostatic and oncotic pressures acting on molecules within a vessel and dictate fluid movement (Figure 2). Hydrostatic pressure refers to the buildup of fluid within blood vessels and interstitial space that "push" molecules away. Oncotic pressure refers to the number of proteins within blood vessels and interstitial space that "pull" fluid in, maintaining balanced osmolarities between blood plasma within the circulatory system and fluid within tissues. Both hydrostatic and oncotic

pressures exist in capillaries and interstitial space. Together, these forces determine the net fluid movement due to filtration or absorption across capillary walls (Taylor, 1981).

In post-exercise conditions, vasodilation and hyperemia contribute to increased hydrostatic pressure within capillaries, exerting a "pushing" force. For an increase in capillary permeability that allows fluid to permeate muscle, there must be fenestrations among endothelial cells and/or pericytes as well as an increase in hydrostatic pressure within the capillary bed.

The net driving pressure is summarized by the Starling equation:

$$NFP = (P_c - P_i) - (\pi_c + \pi_i)$$

Where *NFP* is the net filtration pressure, P_c is the capillary hydrostatic pressure P_i is the interstitial hydrostatic pressure, π_c is the capillary oncotic pressure, π_i is the capillary oncotic pressure. Multiplying the net filtration pressure and CFC gives the rate of capillary filtration (Hall & Guyton, 2011). If capillary hydrostatic pressure outweighs influences of other pressures, and the capillaries are sufficiently permeable, there will be a net outward filtration.

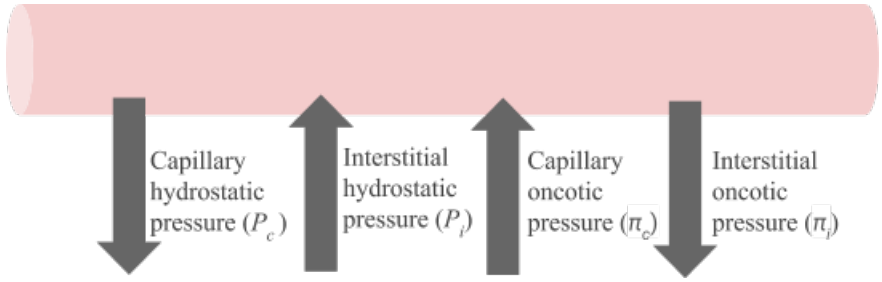

Figure 2: Starling forces.

Capillary hydrostatic pressures (P_c) and interstitial oncotic pressure (π_i) are the primary drivers of fluid filtration, while interstitial hydrostatic pressure (P_i) and capillary oncotic pressure (π_c) are the primary drivers of fluid absorption.

It is important to note that there are other potential mechanisms that could underlie increases in capillary filtration aside from increases in fenestrations caused by histaminergic signaling. An alternative possibility involves a hypothesized anatomical feature within capillaries known as precapillary sphincters. These sphincters are bands of smooth muscle that can relax and contract to modulate blood flow through certain capillaries or capillary beds. Each band can open and close to allow blood to either travel through the capillary or bypass the capillary entirely. Opening of precapillary sphincters, which may be caused by histamine or another signaling pathway, would create more surface area of contact between the bloodstream and vessel wall. This increase in surface area provides additional space for potential fluid movement. In the case of post-exercise increases in permeability, it could mean increased fluid filtration into the interstitial space. This mechanism would *not* affect capillary permeability, but would result in similar physiological responses. Interestingly, this concept is disputed. Initially discovered in the mesentery, precapillary sphincters were speculated to exist throughout the body. However, direct evidence of their existence in skeletal muscle is yet to be observed (Sakai, & Hosoyamada 2013). This study will make predictions

based on what is already known about the formation of fenestrations in response to histamine, but recognizes there are alternative or additional possibilities that may influence transcapillary fluid dynamics.

Effect of Sex Hormones

Capillary permeability was further investigated by Stachenfeld, Keefe, and Palter (2001), specifically regarding female sex hormones: estrogen and progesterone. The findings illustrated that these hormones affect the quantity of plasma volume retained in the interstitial space varied during the menstrual phases and may be due to changes in capillary permeability. Tollan et al., (1993) illustrated that from the follicular to the luteal phase of the menstrual cycle, interstitial oncotic pressure was significantly reduced, leading to a 30% increase in capillary filtration coefficient (CFC), which reflects capillary permeability. These results demonstrate instability in fluid distribution in women with premenstrual syndrome (PMS). The protocol and timing of data collection based on menstrual cycles used by Stachenfeld et al. informed this study: female subjects participated only during the follicular phase of their menstrual cycle to maintain consistent CFC values that could be compared across multiple visits and between both males and females.

Significance of Research

Regular endurance exercise is recommended to maintain health and to reduce morbidity associated with aging, however known factors only account for 50% of health benefits afforded by exercise (Romero et al., 2017). Exercise-associated histamine production is potentially a primary signal for initiating an inflammatory state by

increasing capillary permeability to allow immune cells and fluid to permeate muscle. Vascular leakage of fluid from the blood to the interstitial space may provide tissue nutrition and enhance recovery (Länne *et al.*1992). Therefore, this inflammatory state is associated with healthy adaptation to exercise and recognized as an important part of the recovery process.

Luttrell and Halliwill (2015) describe post-exercise recovery as a "window of opportunity," suggesting that we can potentially "maximize or even exploit the altered physiology of the recovery period" and improve health. Histamine plays an integral role in this post-exercise physiological state. By better understanding post-exercise histaminergic signaling pathways, researchers can capitalize on the recovery state and construct opportunities for innovative cardiovascular health interventions.

Purpose

The aim of this study is to further understand the effect of histamine in the exercise response. Current literature has established two important concepts:

1. Histamine is released in skeletal muscle tissues during and after exercise.
2. Histamine causes an increase in capillary permeability.

This study endeavors to piece these concepts together by asking the question, are endurance exercise-associated increases in skeletal muscle histamine associated with increased capillary permeability?

Hypothesis

It is hypothesized that the increased capillary permeability following endurance exercise is mediated by skeletal muscle elevations in histamine concentrations and that blocking histamine's actions will attenuate this response.

Methods

Experimental Outline

This double-blind, placebo-controlled crossover study was approved by the Institutional Review Board (IRB) at the University of Oregon. The ultimate question to be answered was whether histamine contributes to an increase in skeletal muscle capillary permeability following 60 minutes of single-leg dynamic knee-extension at 60% of maximal work rate.

Subjects

Ten healthy 18-40-year-old subjects, 8 men and 2 women, were recruited from the Eugene Oregon area and provided written, informed consent following a verbal and written briefing of experimental procedures. No subjects were using over the counter or prescription medications (except for oral contraceptives), herbal remedies, dietary supplements, or illegal or recreational drugs during the study. Subjects were also excluded for known allergies to drugs or medications. Enrolled subjects were informed that could withdraw from the study at any point. Each subject was assigned a 3-digit number to protect their privacy throughout the study.

Both estrogen and progesterone have been shown to affect capillary permeability (Stachenfeld et al., 2011; Tollan et al., 1993), as rising levels of female sex hormones correlate with an increase in capillary filtration (Wong et al., 1972). Therefore, female subjects participated during the early follicular phase of their menstrual cycle or during the placebo phase of their oral contraceptive when estrogen and progesterone are both low. Testing females during the follicular phase reduced the

potential confounding factor of sex hormones on capillary permeability. This created a similar sex hormone profile between males and females during testing and ensured similar hormone levels throughout the study.

Screening

For their first visit, subjects were familiarized with the protocol of the study, and completed a medical questionnaire to screen for medical conditions to determine their eligibility for the study. Height and body weight were measured along with skin fold percentage. All subjects were required to abstain from caffeine, alcohol, and exercise for 24 hours and food for 2 hours prior to each study visit. Additionally, pregnancy tests were administered to all female subjects upon arrival to the lab. A negative result was required for the subject to continue the study.

Eligible subjects performed a single-leg knee-extension peak test on custom built, leg extension ergometer to volitional fatigue to assess their maximal performance. The ergometer uses a computer-controlled step-motor that provides resistance against the subject's lower leg that increases incrementally at a rate of 3 W/min. The subjects performed a repeated kicking exercise, moving the leg from 90 degrees of flexion to 45 degrees of flexion at a rate of 45 kicks per minute. The computer screen relayed visual feedback for the subject to maintain the appropriate cadence and power.

Experimental Protocol

In the second and third visits, subjects consumed a randomly assigned oral dose of Placebo or histamine (H_1/H_2) receptor antagonist (Blockade) pills with 3 ounces of water one hour before assessment. The Blockade effectively blocked both H_1 and H_2

receptors. Each subject rested for 60 minutes prior to data collection to allow the Blockade or Placebo to take effect. Then, capillary permeability was measured in both legs, followed by an hour of single-leg dynamic knee-extension exercise with the subjects' right leg, or Exercise Leg, performing at 60% of their measured maximal performance while the left leg, or Rest Leg, remained in place. Then, capillary permeability of the Exercise and Rest Leg was assessed again (Figure 3).

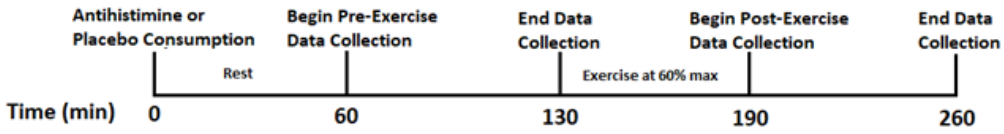

Figure 3: A single visit protocol.

The subject begins by consuming either a Blockade or a Placebo and resting for 60 minutes. Then, pre-exercise CFC assessment begins using venous occlusion plethysmography. Following the CFC assessment, single-leg dynamic knee-extension exercise is performed at 60% maximum for 60 minutes. Lastly, post-exercise CFC assessment occurs following the same protocol as pre-exercise.

Capillary permeability was assessed by calculation of CFC, which relates the change in limb circumference to changes in venous occlusion pressures. CFC was measured using venous occlusion plethysmography, which elevates venous pressure and records the corresponding change in limb girth.

To measure CFC, subjects were instructed to lie supine on a padded table, and pneumatic congestion cuffs were placed around the upper thighs to occlude venous blood flow. The cuffs were inflated to three pressure steps (20, 30, and 40 mmHg) above venous pressure but below arterial pressure. This permits blood in capillaries to be diverted to the muscle via arteries but prevents it from returning to the heart through

the veins. The increase in pressure causes fluid to “leak” out of circulation from the veins and into surrounding tissues. The rate of increase in limb circumference depends on the permeability of local vasculature. Limb volume changes were measured using Mercury in Silastic strain gauges placed around the mid thighs of both the Exercise and Rest Leg (Figure 4). Each pressure level was repeated once, with five-minute breaks between pressure levels. The cuffs were inflated for 7 minutes, with change in limb girth measurements occurring during the final three minutes (Figure 5). It was assumed that during the first 4 minutes the veins were filling up with blood to capacity, and any changes in limb circumference during the final three minutes were due to filtration of intravascular fluid into the interstitial space (Figure 6; Katz, 1977; Stachenfeld et al., 2001). Change in limb circumference was measured at each pressure step twice before and after exercise.

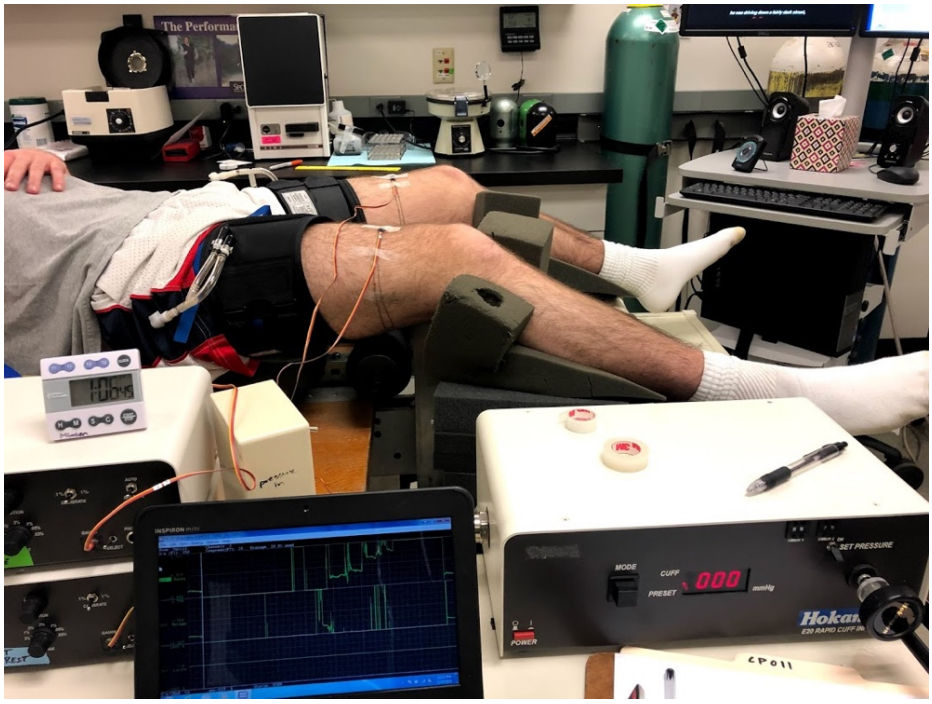

Figure 4: Subject during CFC assessment.

The Mercury in Silastic strain gauges were placed mid-way between the congestion cuff on the upper thigh and the patella. As the congestion cuffs filled to the determined pressures, the strain gauges provided feedback on changes in limb circumference.

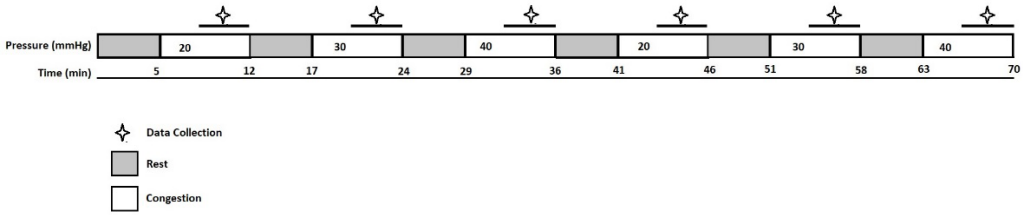

Figure 5: Schematic for pressure cuff inflation and measuring changes in limb circumference.

Following a 5-minute rest, the pressure cuff was inflated at 20 mmHg for 7 minutes (grey), and changes in limb circumference were recorded for the final three minutes of congestion. This process was repeated five additional times, with the pressure cuff increasing incrementally to 20, 30, and 40 mmHg twice. There was a 5-minute rest between each pressure level (white).

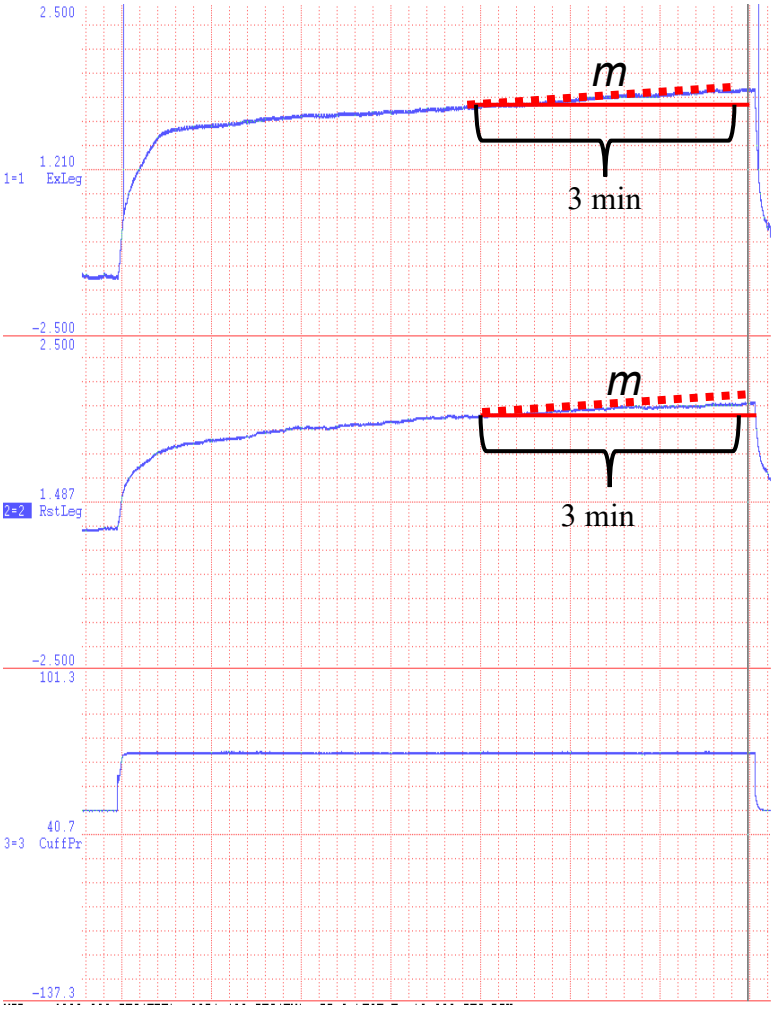

Figure 6: Representative tracing of the rate of change (m) in *limb circumference* vs *time.*

These tracings reflect change in circumference in the Exercise Leg (top), Rest Leg (center), and cuff pressure (bottom) over time at 40 mmHg and is an index of changes in CFC.

Histamine's influence on CFC was assessed under the influence of Blockade versus Placebo, pre vs post-exercise, and in the Exercise vs Rest Leg.

Any uncharacteristic subject movements observed during CFC assessment (muscle twitches, etc.) were recorded so that corresponding measurements could be analyzed with additional scrutiny.

Calculations

CFC represents the slope of the increase in leg girth during the final 3 minutes of occlusion (Figure 7). At this point we assume the veins have filed with blood, so any other increases were due to the transfer of fluid to the surrounding tissues (Stachenfeld et al., 2011). The following equation was used to calculate CFC:

$$\mathrm{CFC} = \left(\frac{2 \times 100 \times 1000}{\text{limb girth} \times \frac{1}{1+\psi}} \right) \left(\frac{\Delta\, limb\ girth}{time \times \Delta\, venous\ pressure} \right)$$

In this equation, ψ represents the ratio of post to pre-capillary resistance, constant 0.16 and Δ represents change (Haskell et al., 1997). The units for CFC are microliters per 100 g per minute per mmHg ($\mu g \cdot 100 g^{-1} \cdot min^{-1} \cdot mmHg^{-1}$).

A 3-way repeated measures analysis of variance was used to compare leg (left: Rest vs right: Exercise), drug (Placebo vs Blockade), and time (Before vs After Exercise) changes in capillary permeability. Data were analyzed with a 3-way repeated-measures ANOVA and presented as Means±SEM

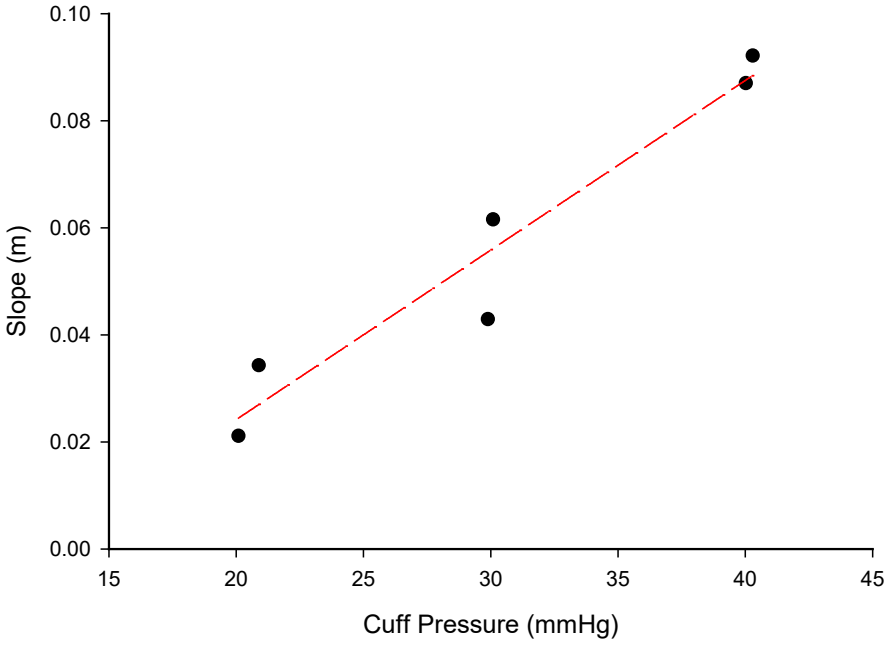

Figure 7: Representative tracing of the *rate of change* (m) *in limb circumference* vs *cuff pressure.*

The slope of change in limb circumference is used in the calculation of CFC.

Results

Subjects

The results reflect data from 10 (2F, 8M) subjects that participated in the study. The physical characteristic data taken during the screening visit are displayed in Table 1. These measures reflect healthy, young individuals consistent with those that typically participate in studies in the EEP Lab.

n	10 (2F, 8M)
Age (yrs)	23 ± 4
Height (cm)	180.0 ± 8.9
Weight (kg)	77.8 ± 14.5
Body Mass Index ($kg \cdot m^2$)	23.9 ± 3.6
Knee-Extension Peak Power (Watts)	37 ± 10
60% Workload (Watts)	22 ± 6

Values are means ± SD.

Table 1: Subject Characteristics

One male subject was not included in the analysis due to inaccurate measures of CFC due to frequent subject movement. Accurate data collection required subjects to remain perfectly still. Data reflecting unexpected movements that caused an uncharacteristic change in CFC were excluded from the overall results. This included unintentional muscle twitching and any adjustments that affected blood flow to the legs.

Another subject was not able to complete the exercise protocol in full due to fatigue. At 42 minutes, wattage was decreased from 60% max to 47% max. This was repeated for both visits.

Capillary Filtration Coefficient

Placebo Conditions

1. *Pre-exercise:* CFC did not differ between the Exercise Leg and the Rest Leg (1.4±0.4 vs. 1.3±0.3 μg·100g-1·min-1·mmHg-1)
2. *Post-exercise:* CFC increased in the Exercise Leg, but not in the Rest Leg (2.7±0.7 vs 1.4±0.3 μg·100g-1·min-1·mmHg-1)

There was an increase in CFC from pre to post-exercise in the Exercise Leg ($P<0.05$), however there was no increase in CFC from pre to post-exercise in the Rest Leg ($P=0.732$) under Placebo conditions. There was a greater CFC between the pre and post-exercise measurement in the Exercise Leg compared to the Rest Leg (Figure 8). On average, CFC increased 120±25% in the Exercise Leg and 17.2±35% in the Rest Leg (Figure 9).

Blockade Conditions

1. *Pre-exercise:* CFC did not differ between the Exercise Leg and the Rest Leg (1.7±0.5 vs. 1.3±0.5 $\mu g \cdot 100g^{-1} \cdot min^{-1} \cdot mmHg^{-1}$)
2. *Post-exercise* CFC did not increase in the Exercise Leg or the Rest Leg (1.8±0.5 vs. 1.1±0.4 $\mu g \cdot 100g^{-1} \cdot min^{-1} \cdot mmHg^{-1}$)

CFC did not increase from pre to post-exercise in the Exercise Leg ($P=0.608$) or in the Rest Leg ($P=0.295$). There was no difference in CFC between the pre and post-exercise measurement in the Exercise Leg compared to the Rest Leg (Figure 8). On

average, Blockade attenuated the exercise-induced rise in CFC in the Exercise Leg by 27±31% and in the Rest Leg by -34% (Figure 9).

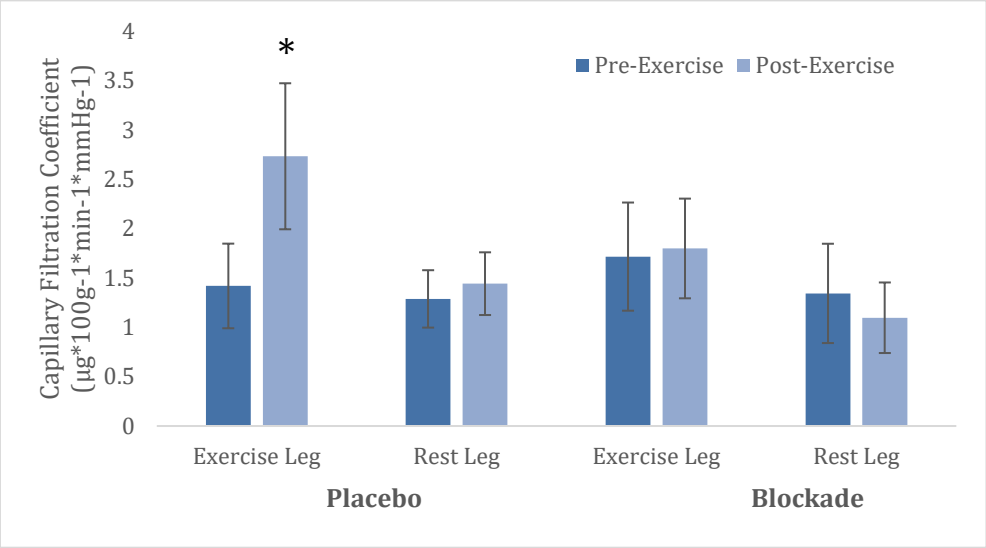

Figure 8. Change in CFC *pre* and *post-exercise* in the *Exercise* and *Rest Leg* under *Placebo* and *Blockade* conditions.

* = Significant difference pre to post-exercise

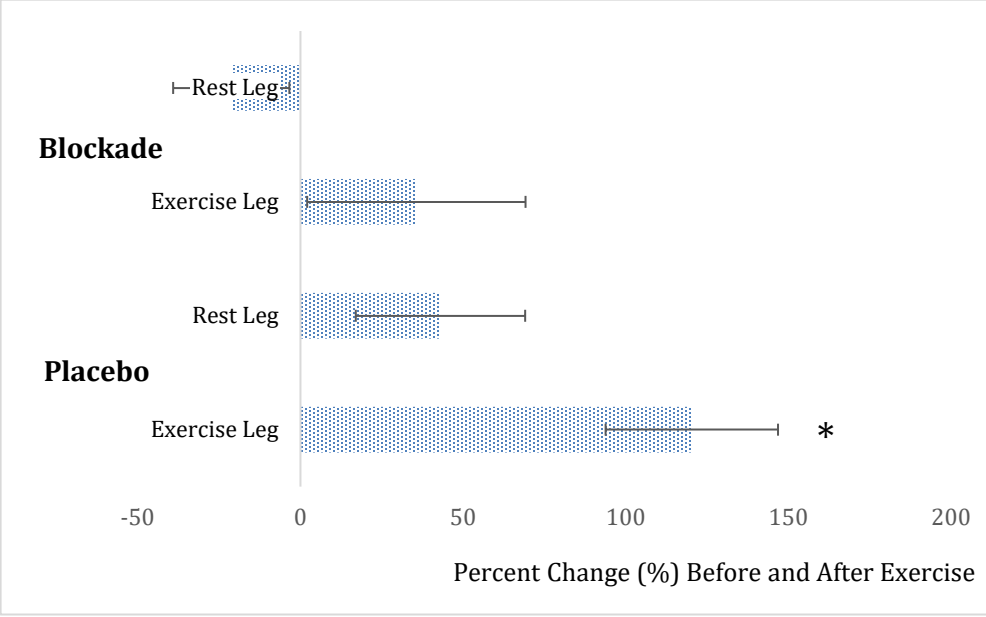

Figure 9: Percent change in CFC *pre* and *post-exercise* under *Placebo* and *Blockade* conditions.

* = Significant difference pre to post-exercise

Discussion

Past studies have indicated that histamine released intramuscularly following exercise binds to H_1 and H_2 receptors on capillaries (Romero et al., 2017; Halliwill et al., 2013; McCord & Halliwill, 2006; Lockwood et al., 2005). Other research has shown that histamine injected into the bloodstream leads to the formation of submicroscopic gaps that allow fluid movement from the bloodstream into skeletal muscle tissue (Andriopoulou et al, 1999; Majno, & Palade, 1961). These findings suggest that post-exercise histamine release may contribute to increased capillary permeability, however until now this concept had not been directly explored.

The findings of this study align with existing research and indicate that that post-exercise histamine release may contribute to an increase in capillary permeability. This is illustrated in the Exercise Leg under Placebo conditions: histamine released locally in exercised muscle led to a 120±25% increase in CFC, suggesting an increase in fluid filtration. This is most likely due to a histaminergic signaling pathway forming fenestrations in capillaries and consequently increasing their permeability to fluid. When histamine receptor antagonists blocked signaling and these pathways were not utilized, capillary permeability did not change. This was illustrated under Blockade conditions, where there was no change in CFC in the Exercise Leg from pre to post-exercise. There was no change in the Rest Leg CFC before or after exercise under both Placebo and Blockade conditions. This corroborates past research that has illustrated that histamine is released locally following small-muscle mass exercise (Barrett-O'Keefe et al., 2013).

Limitations

There were some challenges with data collection in this study. Subjects were required to remain perfectly still as changes in limb circumference were assessed for each 7-minute when the pressure cuff was inflated. Even small movements influenced blood flow enough to significantly affect limb circumference and thus CFC. For example, sometimes muscle contractions caused a redistribution on blood that reflected a decrease in limb circumference, which is theoretically improbable since the congestion cuff is above venous pressure and should not allow more blood to leave the leg through the veins than is being delivered by the arteries. These uncharacteristic movements, which were noted during data collection, led to unreliable values that were omitted from final data analysis.

It is important to recognize that the measures of CFC reflect changes in limb circumference, and do not visualize changes in the capillaries. Based on prior research, it is reasonable to speculate that this local edema in the leg is caused by the presence of fenestrations triggered by histaminergic signaling. However, because this study was non-invasive and did not investigate or image histamine's effect on a microvascular level, these theories cannot be confirmed.

There are alternative mechanisms that could potentially lead to increased capillary permeability. Precapillary sphincters could obstruct some capillaries within the skeletal muscle microvasculature, limiting surface area for fluid filtration. The specific signaling pathways that trigger the opening and closing of these sphincters is currently unknown, and may or may not be related to histamine. Of course, the existence of precapillary sphincters remains in dispute, so further research would be needed to

confirm whether this scenario is possible. Additionally, post-exercise vasodilation could theoretically contribute to increased capillary permeability. Like the precapillary sphincter theory, dilation of arterioles provides a greater surface area for perfusion. With more contact area between the bloodstream, capillaries, and interstitial space, fluid could travel more easily into muscle tissue following exercise. This possibility also aligns with our results; however, it is unclear by what magnitude vasodilation would influence capillary permeability.

Past research on the effect of histamine on capillary permeability relied primary on intravenous injections of histamine (Andriopoulou et al, 1999; Majno et al., 1967; Suzuki et al., 1999). It is possible that direct injections of histamine to the bloodstream had different effects than post-exercise histamine, which is released from within muscle tissue. Histamine may act differently on the walls of capillaries from inside of the vessels compared to outside in the interstitial space. This could be investigated this by performing investigating capillary permeability directly on a microvascular level following exercise. Future research is necessary to confirm the specific effects of post-exercise histamine on endothelial and pericytes within capillaries.

Perspectives

Deepening our understanding of underlying mechanisms and possible adaptations to exercise may illuminate methods to capitalize on the post-exercise recovery period and improve health outcomes. If histamine mediates a beneficial fluid transfer of plasma proteins from the blood into muscle tissue that aid in recovery, then histamine blockades may inhibit long-term muscular adaptations to exercise. This research is relevant to athletes that commonly take histamine blockades as allergy

medication, depending on the severity of histamine blockade on long-term adaptations to exercise. Additionally, this research explores physiological mechanisms that underlie the advantageous cardiovascular adaptations that are the focus of the EEP Lab and adds detail to our collective understanding of the physiological effects of histamine release in the body. We can apply this knowledge base to capitalize on the recovery period, perhaps by identifying physiological interventions that target the histaminergic signaling pathway to improve cardiovascular health.

Conclusions

The objective of this study was to compare capillary permeability of the leg before and after prolonged unilateral knee-extension exercise under Placebo conditions and when histaminergic signaling is blocked. It was hypothesized that H_1 and H_2 receptor antagonists would decrease capillary permeability following exercise. Previous research has illustrated that histamine is released locally within previously active skeletal muscle following a bout of moderate-intensity endurance exercise (Romero et al., 2017; Halliwill et al., 2013; McCord & Halliwill, 2006; Lockwood et al., 2005). Other studies have demonstrated a marked effect of histamine on capillary permeability (Andriopoulou et al, 1999; Majno, & Palade, 1961). In the context of ongoing research, this study endeavored to illustrate that post-exercise histamine release and increased fluid perfusion are related. Our findings indicate the exercise associated increases in intramuscular histamine may contribute to changes in capillary permeability after exercise.

Although the precise mechanisms for increased capillary permeability are still up for debate, these findings have helped elucidate physiology of post-exercise recovery. Future research could examine the precise mechanisms of histamine or other signaling pathways within the microvasculature. Regardless, the fluid transfer between the bloodstream and muscle tissues could prove to be important to long-term muscular and cardiovascular adaptations to exercise. By better understanding the role of histamine in post-exercise recovery, researchers will be more equipped to further study exercise and cardiovascular health.

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