EXERCISE-INDUCED ACIDOSIS AND HISTAMINE RELEASE IN SKELETAL MUSCLE

by
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A THESIS

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Title: Exercise-Induced Acidosis and Histamine Release in Skeletal Muscle

Approved: _____*Dr. John Halliwill*_____
Primary Thesis Advisor

Histamine is a molecular transducer of physical activity responses in humans, namely endurance exercise. Research shows that H1/H2-receptors mediate exercise responses, such as post-exercise vasodilation and hypotension. Histamine is produced and released within skeletal muscle in response to exercise; however, the intramuscular trigger of histamine release that mediates this response to dynamic exercise has not been identified. Exercise factors, such as an acidic pH, could favor increased histadine decarboxylase enzyme activity and mast cell degranulation and thus heightened *de novo* histamine formation as a response to exercise. Using sodium bicarbonate (SB) to buffer the acidosis in the skeletal muscle may help clarify the relationship, if any, between histamine release and blood pH. The overall goal of the project was to determine if histamine-mediated vasodilation in skeletal muscle is mediated by a decrease in pH that occurs in response to exercise.

This was a double-blind placebo-controlled study to access the concentration of histamine release before and after an exercise bout. The experiment included three subjects participated in three separate sessions. An hour prior to exercise, the subject blindly ingested either 0.3g/kg body mass of NaCl (control) or 0.3 g/kg body mass of

sodium bicarbonate to prevent acidosis. The subject then performed 60-minutes of one-legged knee extension exercise at 60% of their maximum resistance determined prior. Blood flow velocity and diameter in the femoral artery were bilaterally measured via ultrasound at the end of the rest period, and at minutes 0, 30 and 60 of the post-exercise period. I hypothesized that bicarbonate would decrease the post-exercise histamine-mediated vasodilation.

Results demonstrated femoral blood flow at minute 0 post-exercise was significantly increased compared to resting conditions within both placebo and bicarbonate conditions. There was a significant increase in vascular conductance as well as blood flow within the active leg from pre-exercise to minute 0 post-exercise compared to resting in both placebo and bicarbonate groups. The acute increase in blood flow and vascular conductance in the active leg were unexpectedly not significantly sustained at minutes 30 and 60 post-exercise. There were no group differences in femoral blood flow and vascular conductance within the active or inactive leg, suggesting that the given concentration of sodium bicarbonate may not have a significant effect on post-exercise vasodilation. Further studies should be done with an appropriate tool to ascertain the efficacy of sodium bicarbonate as a buffer, such as a near-infrared spectroscopy tool.

Acknowledgements

Firstly, I would like to thank my primary advisor Dr. John Halliwill, my second reader Josh Mangum and my CHC representative Professor David Frank for the significant amount of time they've spent teaching me how to view the world through science, debate and literature. Creating this thesis, although arduous, was greatly fulfilling and a satisfying reflection of how I have grown over my four years at University of Oregon.

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Thank you for my parents, little brother and grandparents for their relentless faith in me, even when I lost faith in myself during hard times in college. You are and will always be my reason why.

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Introduction

Recovery from Exercise & Post-Exercise Hypotension

Exercise physiology has long been focused on the physiological adaptations and changes in the body that occur during an exercise period; however, the physiology of recovery from exercise is a more recent realm of study. “Recovery” from exercise is temporally defined by Dr. Halliwill and Dr. Luttrell as the time to return your body back to resting levels, which they explain can be minutes of lowering your elevated heart to normal or it can be weeks to heal damaged skeletal muscle from prior activation. Physiologically, “recovery” can describe the biological processes taken to transition from exercise to the resting state (Luttrell & Halliwill 2015). The body has cardiovascular changes during a resting state, during exercise and during recovery, which is attributed to alterations in distributionsof blood flow to vascular beds. After an exercise period, blood flow to previously-active skeletal muscle is elevated due to vessel dilation increasing its diameter. This increase in blood flow decreases vascular resistance and ultimately leads to hypotension (lowered blood pressure) that can last several hours to days. A 30 to 60-minute exercise bout of moderate-intensity dynamic exercise in the supine position has been shown to decrease post-exercise blood pressure approximately 5–10 mm Hg in the supine position, which can be sustained for several hours in young, healthy and normotensive subjects. In those with hypertension, post-exercise vasodilation is more notably seen with blood pressures decreasing as low as 20 mm Hg that can be sustained for up to 12 hours (Hagberg *et al*. 1987; Kenny & Seals 1993; Forjaz *et al.* 2000).

Dr. Halliwill describes the post-exercise period as a “window of opportunity” that may be clinically relevant because the physiology of recovery may be altered or strategically utilized to improve resting or exercise conditions. Exercise is already well-depended on by health and athletic professionals for chronic disease intervention, including those associated withhypertension, elevated lipid levels and age-related sarcopenia (Nagi 2006; Waters 2010). Further studies of the histaminergic signals that lead to vasodilation during the recovery period may illuminate a clearer physiological environment for possible exercise and pharmacological dual intervention for patients with chronic vascular disease.

Histamine Found to Locally Mediate Post-Exercise Vasodilation

As noted, blood flow increases to active skeletal muscle in response to exercise. Immediate vasodilation after exercise is due to multiple local and systemic factors. Reduced sympathetic activation decreases vascular resistance, leading to hyperemia that may last up to 90 minutes (Halliwill *et al.* 1996). Increased blood flow can also be locally achieved by multiple vasodilators, namely nitric oxide (NO) and prostacyclin (PGI2), which were previously thought to be the major contributors of sustained vasodilation after exercise. More recent research has demonstrated other vasodilatory substances may be involved, namely histamine, which has been associated with NO and PGI2 release (Lockwood *et al.* 2005; Halliwill *et al.* 2001; Halliwill *et al.* 2000; Hellsten *et al.* 2012; Li *et al.* 2003; Baenziger *et al.* 1980). This molecule has successfully been shown to have a significantly sustained or long-term vasodilatory effect on previously-active skeletal muscle, in which its trigger during exercise remains unknown and well-researched currently.

Histamine is a molecular transducer of physical activity responses in humans, namely endurance exercise (Luttrell & Halliwill 2017). The compound is commonly known for its role in immune and inflammatory reactions, but research shows that activated histamine receptors, namely H_1 and H_2, also mediate exercise responses after exercise, including post-exercise vasodilation (Pellinger et. al, 2013). As seen in Figure 1, histamine is produced by the enzyme histidine decarboxylase (HDC), and can also be released via mast cell degranulation (Hegyesi *et al.* 1999; Romero et. al 2017). Mast cells are located within the connective tissue layer that surrounds skeletal muscle as well as near blood vessels, both within proximity to release histamine locally to specifically active skeletal muscle vessels (Metcalfe *et al.* 1997). Histamine can bind to type H_1 and H_2receptors within vessels, leading to signaling cascades for physiological effects.

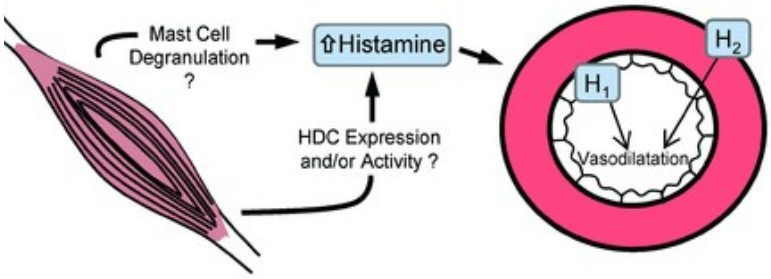

Figure 1: Histamine Release and Effects in Skeletal Muscle Vasculature.Histamine can be formed via histidine decarboxylation by histidine decarboxylase protein (HDC) or released locally by mast cell degranulation. (Halliwill, Buck, Lacewell, & Romero, 2013).

Dr. Halliwill and others have discovered that at specific times after exercise, different histamine receptors are activated – H_1 receptors are active at 30 minutes after and H_2 receptors are active at 60-90 minutes (McCord and Halliwill, 2006). Post-exercise vasodilation of the previously-active skeletal muscle results in about a 50% increase in femoral artery blood flow above resting levels. This vasodilatory effect can be reduced by introducing histamine receptor antagonists, or molecules that bind to the

receptor without activating it. Antagonists used include fexofenadine (Allegra, a selective H_1-receptor blockade)(Lockwood, Wilkins& Halliwill 2005) or ranitidine (Zantac, a selective H_2-receptor blockade) (McCord et. al. 2006). Researchers investigated these signaling pathways by utilizing both Allegra and Zantac to block activation of both H_1 and H_2 receptors to thus prevent approximately 80% of the post-exercise vasodilation during recovery from moderate-duration whole-body exercise. This response indicates a significant role for histaminergic signaling in the post-exercise vasodilatory response.

Physiological measures, such as femoral vascular conductance and mean arterial pressure (MAP), indicate vascular tone. Vascular conductance is the change in blood flow in response to the change in pressure during vessel construction or dilation. Mean arterial pressure is the average pressure within arteries throughout one cardiac cycle. Vascular conductance and MAP were found to be within normal range 30 minutes after 60 min bout of cycling at 60% of maximal oxygen consumption with the ingestion of Allegra (Lockwood, Wilkins& Halliwill 2005). Similarly, femoral and systemic vascular conductance were not elevated after 90 minutes with intake of ranitidine after the same whole-body cycling exercise. This signifies that H_1 and H_2 receptor activation has demonstrated to be the essential vascular mechanism of sustaining post-exercise vasodilation. The mechanistic processes of this are not clear to date; however, it is suspected a pH reduction that occurs from metabolic processes during exercise may be related to the release of histamine on skeletal muscle endothelium.

Exercise-Induced Acidosis and Metabolism

A brief overview of metabolism during exercise should be held regarding its relation to exercise-induced acidosis, or pH reduction. Humans store energy as adenosine triphosphate (ATP), a molecule that releases energy upon its breakdown into adenosine diphosphate (ADP), as illustrated in Figure 2. As indicated in their chemical names, ATP has three phosphate groups that carry a high amount of energy released to skeletal muscle when needed for increased physical activity, such as exercise. Ingested carbohydrates are broken down into glucose that may be formed into ATP for more immediate utilization or glycogen molecules for future energy storage. Some ATP may be attached to creatine to create phosphocreatine for immediate use, as it can be rapidly and easily broken down in the first 5-10 seconds of maximum performance (Hall 2013). After phosphocreatine is used up, the body then depends on free glucose and the stored glycogen for energy during endurance exercise. Glucose and glycogen are transformed into pyruvate, a reaction that produces two ATP molecules. From this step, pyruvate may be metabolized either anaerobically or aerobically. Anaerobic respiration is ideal for rapid and high-resistance exercise bouts, such as sprinting. It begins by turning pyruvate into lactate, resulting in 1-2 ATP molecules. Lactate can turn back into glucose and used again for energy.

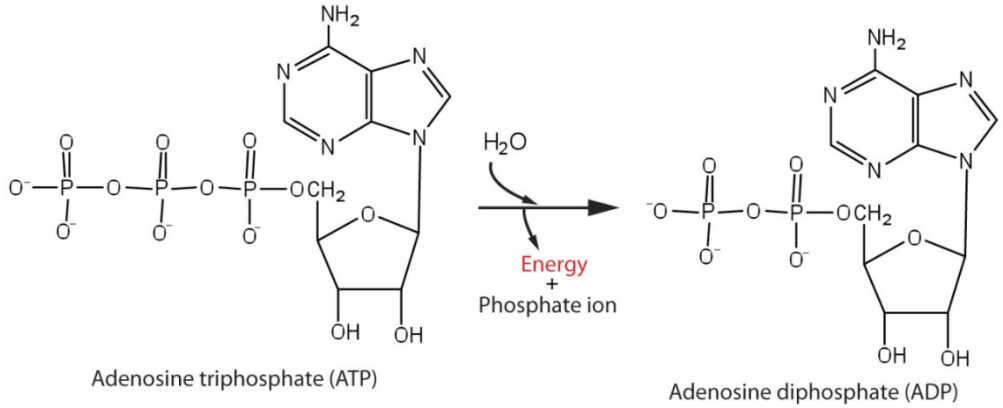

Figure 2. Adenosine triphosphate (ATP) is hydrolyzed into adenosine diphosphate.Byproducts include a phosphate ion and energy release.

The body then depends on aerobic respiration, much more suitable for steady-state endurance exercise, by turning pyruvate into acetyl-CoA derived from glucose, amino acids or lipids. Acetyl-CoA undergoes multiple reactions in a process called the Krebs cycle. Generally, the Krebs cycle results in production of NADH and $FADH_2$ molecules that sustain an ATP-producing process called the electron transport chain. Although the process involves more reactions and takes more time than anaerobic respiration, aerobic processes are much more efficient and appropriate for constant endurance exercise versus short-term high-performance exercise bouts.

The metabolic processes described are relevant to the pH conditions of skeletal muscle. A slight decrease in blood pH in active skeletal muscle, or acidosis, occurs due to an increased concentrationof hydrogen ions stimulated by metabolic changes during exercise.The phenomenon of acidosis had previously been attributed to increased production of the byproduct lactic acid during anaerobic respiration, which results in a proton (hydrogen ion) release (Robergs et al., 2004; Hall 2013); however, it is now known that aerobic respiration produces hydrogen ions in the previously-described Krebs cycle and electron transport chain (Robergs et al., 2004; Hall 2013). A proton is released when an ATP is broken down into ADP + phosphate group within the mitochondria, the part of a cell that biochemically processes energy production using oxygen. At the point of what is named the steady-state of exercise, protons are utilized in the mitochondria at a pace that does not result in proton accumulation due to the demand of ATP-utilization matching the rate of mitochondrial metabolic reactions. If exercise intensity increases above steady-state, then metabolism depends more on anaerobic metabolic processes of the glycolytic and phosphocreatine systems, as noted

before. The use of these faster non-oxidative systems consequently leads to proton accumulation and thus acidosis of active skeletal muscle. Overall, it is well-known that blood pH decreases during exercise conditions due to non-oxidative metabolism. The bigger question is, can post-exercise histamine release be related to this exercise-induced acidosis?

Importance of Project

Exercise-induced acidosis when isolated may be a primary trigger to initiate the signaling cascade leading to post-exercise vasodilation. Histamine is produced and released within skeletal muscle in response to exercise; however, the intramuscular trigger of histamine release that mediates this response to dynamic exercise has not been identified (Romero et. al, 2017). It has been suggested that physical stimuli may increase histamine levels, such as vibration and heat that the exercise condition elevates (Atkinson TP, White MV, and Kaliner MA, 1992) as well as reactive oxygen species (Son et al. 2006). Exercise-induced acidosis is another exercise factor that could favor increased HDC enzyme activity and mast cell degranulation and thus increased *de novo* histamine formation as a response to exercise.

Using sodium bicarbonate (SB), or $NaHCO_3$,to buffer the acidosisin the skeletal muscle may help clarify the relationship, if any, between histamine release and blood pH. Many studies have investigated the effects of SB ingestion during endurance and acute exercise conditions. There is limited evidence suggesting that bicarbonate can increase performance, namely time to exhaustion (TTE), exercise tolerance, RPE and work (Gough 2017; Freis 2016; Deb et al. 2018). This is important due to the popularity of antihistamines used as common allergy drugs to reduce allergic reactions. Furthermore, there is a possibility that bicarbonate may improve athletic performance of endurance events by preventing a reduction in pH. The question is, does using SB to inhibit an acidic pH alter the histamine mediated post-exercise vasodilatory response? Using a single-leg dynamic knee extension (“leg kicking”) exercise model can reproduce vascular responses from whole-body exercise, which can be used to explore

potential pathways involved in H_1 and H_2 activation. Vasodilation can be measured via ultrasound (US) on the femoral artery, a vessel appropriately sized to indicate physiological changes in diameter.

Determining Sodium Bicarbonate Dose for Acidosis Attenuation

What had to be determined prior to the experiment was the optimal concentration of sodium bicarbonate to successfully prevent the typical exercise-induced reduction in blood pH. Previous studies reported a positive effect of sodium bicarbonate under resistance and endurance exercise conditions at concentration of 0.3 g/kg body mass. Six trained male subjects ingested 0.3 g/kg body mass 105 minutes before completing four sets of 12 repetitions, followed by a fifth set to fatigue on a Universal leg press machine set to approximately 70% of the subjects' individual maximum resistance. SB ingestion was shown to increase resting pH and bicarbonate levels, and the SB condition was found significantly more basic than the placebo group after each exercise set (Webster et. al, 1993).Similar to this study, a 2002 project had subjects ingest the same concentration of SB two hours prior to completing 30-min cycling exercise at 77 ± 1% peak oxygen consumption ($\dot{V}O_{2peak}$) followed by completion of 469 ± 21 kJ as quickly as possible (∼30 min, ∼80% $\dot{V}O_{2peak}$). Plasma and muscle proton concentrations were significantly lower in SB group before and during exercise conditions, demonstrating acidosis was attenuated (Stephens et.al, 2002).In a later study conducted in 2017, pH levels and sodium bicarbonate concentrations were significantly higher 1.5 hours before, during and after a performance of two exhaustive graded exercise tests and two constant load tests (30 min at 95% individual anaerobic threshold followed by 110% IAT until exhaustion) following ingestion of SB or 4 g placebo

(sodium chloride dissolved in 0.7 l water) (Freis et.al, 2017).These studies suggest that the ingested concentration 0.3 g/kg body mass of SB was successful in attenuating the exercise-induced pH decrease in high-intensity endurance and resistance exercise.This makes this an effective and optimal dose for attenuating acidosis during exercise for this project.

Purpose

The specific purpose of this study was to compare the effect of 0.3 g/kg body mass sodium bicarbonate vs placebo on post-exercise vasodilation.

Hypothesis

It was hypothesized that 0.3 g/kg body mass of sodium bicarbonate ingestion will reduce the histamine-mediated post-exercise vasodilatory response following performance of 60 minutes of single-leg dynamic knee-extension at 60% of maximal work rate.

Methods

This study was approved by the Institutional Review Board of the University of Oregon. Each subject gave informed and written consent before participation in the study.

Experimental Design

This project was a double-blind, placebo-controlled crossover study approved by the Institutional National Review Board (IRB) at the University of Oregon. The main question this project aimed to answer is if the histamine-mediated post-exercise vasodilation within previously-active skeletal muscle can be attributed to an exercise-induced decrease in pH (acidosis) following 60 minutes of single-leg dynamic knee-extension at 60% of maximal work rate.

Subjects

A total of three 18 to 40-year-old men and women were recruited from Eugene, OR. They were informed verbally and through written consent forms of our experimental procedures. Ideal subjects were chosen if they had no known allergies to drugs or medication, as well as if they refrained from using over-the-counter or prescription medications (except for oral contraceptives), herbal remedies, dietary supplements, or illegal or recreational drugs during the study. Enrolled subjects were informed that they could withdraw from the study at any point. Each subject was assigned a 3-digit number to protect their privacy throughout the study.

Endothelium-dependent vascular relaxation, the endothelium being the inner-most layer of skeletal muscle where histamine receptors reside, has been shown to be induced by both estrogen and progesterone. The grade of endothelium-dependent

vascular relaxation is greater in female than male hypertensive rats (Kauser & Rubanyi, 1995). It is possible hormones could cause vascular relaxation by modifying or synthesizing certain relaxing factors released by the endothelium; thus, female subjects participated during their menstrual cycle's early follicular phase or during the placebo phase of their oral contraceptive when estrogen and progesterone are both low. Ensuring females were tested during the early follicular phase decreases confounding factor of sex hormones on vasodilation, and attempted to set up comparable hormone levels between men and women through the project.

Screening

The initial visit with subjects consistent of familiarization with the experimental protocol and completion of a medical questionnaire screening for medical conditions, which determined their eligibility for the experiment. Once determined eligible, subjects' height and body weight were measured, and they were informed they must avoid caffeine, alcohol and exercise for 24 hours and food for 2 hours prior to each study visit. Female subjects underwent a pregnancy test prior to beginning each study visit as well, in which a negative result was required to continue.

Next, to assess individual maximal performance, the eligible subject performed a single-leg knee extension peak test on a right-leg extension ergometer to fatigue to assess their maximal performance. They performed a seated repeated kicking exercise to exercise the quad muscles at a rate of 60 kicks/minute. The ergometer is computer-controlled for resistance and incrementally increased 3 W/min against the subject's lower leg. The computer screen demonstrated visual feedback for the subject to maintain the appropriate cadence and power, as supervised by the investigator.

Experimental Protocol

Eligible subjects arrived for a second and third study visit, and were set up with electrocardiogram electrodes and a blood pressure cuff to record heart rate (HR) and blood pressure (BP) every ten minutes through the entire study visit. An hour before beginning exercise, subject would intake a randomly assigned sodium bicarbonate (0.3 g/kg body mass) or a placebo (3g/kg body mass of sodium chloride)mixed in 30 oz water, which would take effect during an hour of rest. At the 60-minute mark of rest, blood flow velocity and diameter in the femoral artery were bilaterally measured via ultrasound. Then subjects underwent 60 minutes of single leg extension knee exercise at 60% maximum performance. At 60-minute mark of exercise period, blood flow velocity and diameter were again measured. Subject then had a 10-minute cooldown at wattage reduced to 10W. This was followed by 60 minutes of rest laid in a supine position, with blood flow velocity and diameter measured at the 30 and 60-minute marks.

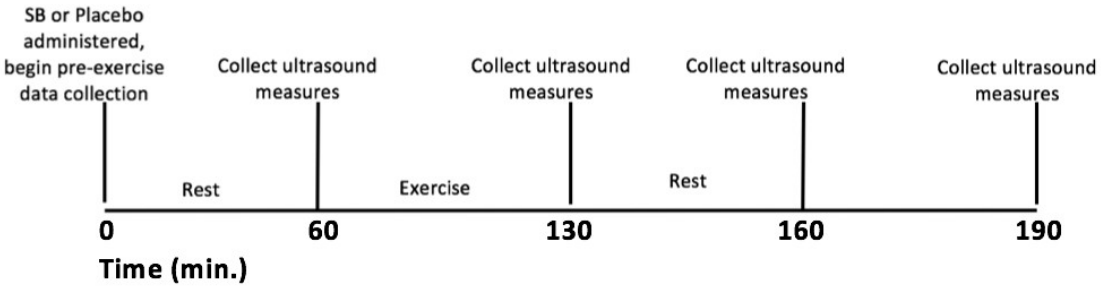

Figure 3: Study visit experimental design.The subject initially is administered a beverage with sodium bicarbonate or sodium chloride, followed by 60 minutes of rest. Heart rate and blood pressure are taken every ten minutes. After the rest period, US-guided measurements are collected and followed by a 60-minute performance of a single leg knee extension ergometer at 60% maximum performance. Post-exercise US measurements are taken again in 30-minute intervals at 120, 150 and 180-minute marks.

Measurements

*Heart Rate and Blood Pressure.*HR and BP were monitored and collected every ten minutes during every procedure. A five-lead electrocardiogram (Q710, Quinton Instruments, Bothell, WA, USA) was utilized to measure HR. A blood-pressure cuff was used on the arm to measure BP.

Leg Blood Flow and Conductance. Pressure cuffs (Hokanson E20 Rapid Cuff Inflator; D. E. Hokanson, Inc., Bellevue, WA, United States) around the ankle of each leg were inflated to 250 mmHg to occlude the foot circulation 1 min prior to and during measurements, to avoid interference from blood flowing in arteriovenous anastomoses of the feet. Femoral artery blood flow velocity was determined 2–3 cm proximal to the common femoral artery bifurcation via duplex ultrasonography using a linear-array ultrasound transducer (L9-3 probe, Philips iE33, Andover, MA, United States) with an insonation angle of 60°. Custom ultrasound software was used to capture the forward and reverse Doppler-shifted signals from the ultrasound system. Recordings were subsequently analyzed with an intensity-weighted algorithm to determine mean blood velocity following standard methods for quantification (Buck et al., 2014). Velocity measurements were assessed at an average depth of 1.91 ± 0.48 cm and were corrected for beam-width of 3.13 ± 0.24 mm, which resulted in an average correction factor of 0.790 ± 0.011 (Buck et al., 2014). Average femoral artery diameter was determined by automated edge-detection software (Vascular Research Tools 5 – Version 6.1.1. Medical Imaging Application LLC, Coralville, IA, United States). Leg blood flow (ml min^{-1}) was calculated as the product of femoral artery cross-sectional area and mean

femoral blood velocity. Leg vascular conductance (ml min^{-1} $mmHg^{-1}$) was calculated by dividing leg blood flow by mean arterial pressure.

Data Analysis

The individual analyzing data was blind regarding drug condition for each study day. There were no discernible differences between male and female subjects, thus data from the two sex groups were combined for statistical analysis. We used a two-way repeated-measures ANOVA for heart rate, mean arterial pressure, blood flow and vascular conductance.

Results

The results consist of data from 3 subjects that participated in this study. Time points at pre- and post-exercise periods were compared, as well as the conditions of placebo vs bicarbonate.

Femoral blood flow. In the active leg, post-exercise blood flow was significantly elevated above pre-exercise period at minute 0 in both placebo and bicarbonate conditions, seen in Figure 4. There was no difference between placebo and bicarbonate conditions in the inactive leg, seen in Figure 5.

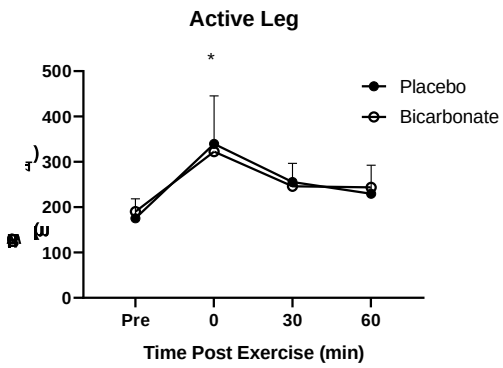

Figure 4. Change in blood flow (ml/min) within active leg throughout pre-exercise and post-exercise periods under placebo and barbonate conditions.

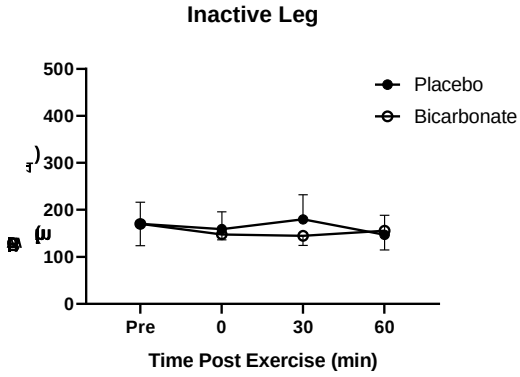

Figure 5. Change in blood flow (ml/min) within the inactive leg throughout pre-exercise and post-exercise periods under placebo and barbonate conditions.

Vascular conductance. Similar to the change in blood flow, there was a significant increase in vascular conductance from pre exercise to minute 0 in both placebo and bicarbonate conditions, seen in figure 6. There were no differences between the two conditions. Vascular conductance was unchanged in the inactive leg and no differences between placebo and bicarbonate conditions, seen in figure 7.

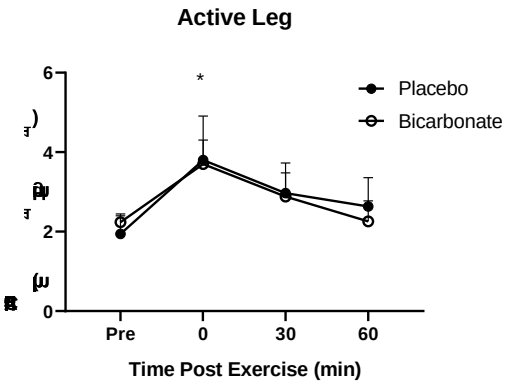

Figure 6. Change in vascular conductance (ml/min) within the inactive leg throughout pre-exercise and post-exercise periods during placebo and barbonate conditions.

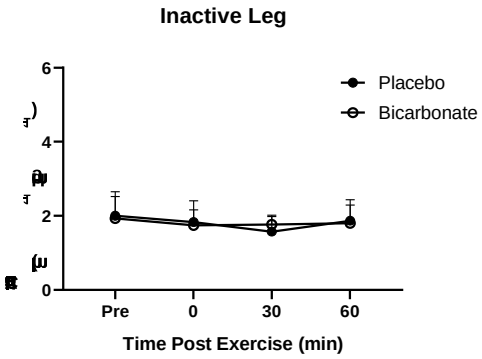

Figure 7. Change in vascular conductance (ml/min) within the inactive leg throughout pre-exercise and post-exercise periods during placebo and barbonate conditions.

Heart rate and Mean Arterial Pressure. Heart rate throughout pre- and post-exercise time points was comparatively stable in both placebo and bicarbonate conditions, demonstrated in Figure 8. Mean arterial pressure (MAP) was decreased following exercise, p=.08 at 0 minutes. There was no statistical difference at 30 or 60 minutes post-exercise compared to pre-exercise regardless of condition, seen in Figure 9. There were no statistical differences between conditions in heart rate or MAP.

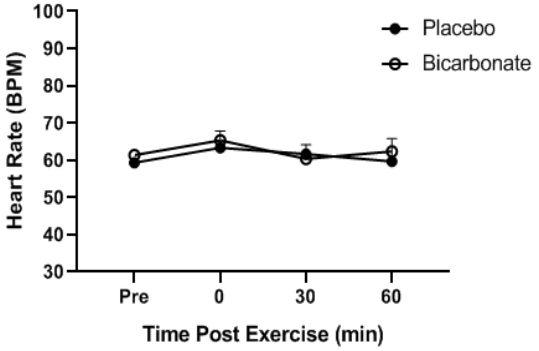

Figure 8. Heart rate (beats per minute) throughout pre-exercise and post-exercise periods during placebo and barbonate conditions.

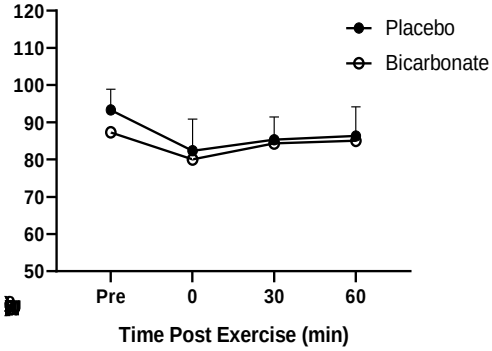

Figure 9. Mean arterial pressure (mmHg) throughout pre-exercise to post-exercise periods.

Discussion

This study ultimately endeavored to determine the effect of sodium bicarbonate on post-exercise vasodilation after 60 minutes of single leg knee extension exercise bout at 60% maximum performance. Comparisons between the pre- and post-exercise conditions within both placebo and bicarbonate conditions were made, as well as comparisons between the two conditions. As expected, femoral blood flow at minute 0 post-exercise was significantly increased compared to resting conditions within both placebo and bicarbonate conditions. Similarly, there was a significant increase in vascular conductance within the active leg from pre exercise to minute 0 post-exercise compared to resting in both placebo and bicarbonate groups. As noted, these expected acute responses are accredited to impaired sympathetic outflow and local vasodilatory factors including NO and PGI2.The acute increase in blood flow and vascular conductance in the active leg were unexpectedly not significantly sustained at minute 30 and 60, seen in figures 5 and 7. This is likely due to one of three subjects did not demonstrate the typical hypotensive response, decreasing the overall trend of blood flow and vascular conductance. There were no group differences in femoral blood flow and vascular conductance within the active or inactive leg, suggesting that the given concentration of sodium bicarbonate did not have an effect on post-exercise vasodilation.

Heart rate was not significantly changed between pre-exercise and post-exercise periods. Heart rate decreases as sympathetic activation decreases with post-exercise rest. Subjects were given a five-minute cooldown immediately after exercise at 10 W to avoid risk of syncope, which increased some time for heart rate to return to baseline.

MAP was decreased insignificantly post-exercise at minute 0, and there was no difference at the 30- and 60-minute time points compared to pre-exercise MAP. MAP is equal to the product of cardiac output (Q) and total peripheral resistance (TPR), which increases above resting conditions during exercise as an increased Q outweighs the decrease in TPR. At the onset of post-exercise period, MAP reduces below pre-exercise baseline for up to 60 minutes as vasodilation lowers TPR and the reduced sympathetic activity decreases Q (Raine 2001). The lack of suppressed MAP is likely due to one of the three subjects lacking the typical robust MAP response, which increased the overall MAP trend.

A Limitation and Possible Future Method with NIRS

The methods taken had aimed to shed light on this relationship between blood pH and post-exercise vasodilation; however, a notable limitation other than low participation (n=3)included the absence of an instrument to accurately measure blood pH alterations, which would confirm the effectiveness of 0.3 g/kg body mass SB as a buffer. Thus, it remains unknown if pH certainly has a role in histamine-mediated post-exercise vasodilation if it was indeed altered with the bicarbonate supplement. Preliminary data suggest that NIRS unit (near-infrared spectroscopy) is a reliable, uncomplicated and minimally-invasive tool in continuously monitoring both intramuscular pH and tissue oxygenation (HbO_2). Given pH and skeletal muscle oxygenation both decreases with exercise, and this effect is exacerbated with intensity,then NIRS can be a convenient tool to demonstrate the efficacy of SB as a buffer as well as indicate the primary aerobic or anaerobic metabolic pathways being utilized. (Belardinelli et. al, 1995; Martin et.al, 2009). Our protocol had originally

included the NIRS device to monitor and collect intramuscular pH and HbO_2 values via a single probe placement on the surface of the working vastus muscle. The initial protocol had subjects connected to the NIRS unit prior to consuming the SB or placebo solution, which would be ingested an hour before initiating the exercise protocol. Values would be collected during the pre-exercise period, throughoutexercise and the 60-minute post-exercise period. Due to technical issues and the outdated NIRS software, the device was not available for use. An NIRS unit with updated software with the ability to read intramuscular pH and HbO_2 in real time should be strongly considered for future related projects, which may demand an alternative buffer depending on results.

Conclusion

What was consistent in previous methodology was the use ofthe single-leg dynamic knee extension exercise to exhibit histamine-mediated post-exercise hypotension, which marks this exercise model reliable for future investigations into other potential exercise factors involved with histamine release. The seated position allows the study to focus on one exercising muscle group (the right vastus muscles) and compare this to a non-active muscle group in the left leg.Importantly, the size of the femoral artery diameter at rest relative to other smaller vessels make this an ideal and standard vascular to observe vasodilatory changes. Although mechanisms behind histamine release remains unclear, the findings steer future experiments to continue utilizing the single-knee extension exercise model as a standard. On a broader perspective, this study continues to contribute to a goal of understanding exercise-mediated vascular responses to improve the health of those with hypertensive or aging-related disease.

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