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Effect of antioxidants on histamine receptor activation and sustained post-exercise vasodilatation in humans

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Abstract

An acute bout of aerobic exercise elicits a sustained post-exercise vasodilatation that is mediated by histamine H_1 and H_2 receptor activation. However, the upstream signaling pathway that leads to post-exercise histamine receptor activation is unknown. We tested the hypothesis that the potent antioxidant ascorbate would inhibit this histaminergic vasodilatation following exercise. Subjects performed 1 hr unilateral dynamic knee extension at 60% of peak power in three conditions: 1) control; 2) intravenous ascorbate infusion; and, 3) ascorbate infusion plus oral H_1/H_2 histamine receptor blockade. Femoral artery blood flow (Doppler ultrasound) was measured before exercise and for 2 hr post-exercise. Femoral vascular conductance was calculated as flow/pressure. Post-exercise vascular conductance was greater for control condition (3.4 ± 0.1 ml min^{-1} mmHg^{-1}) compared with ascorbate (2.7 ± 0.1 ml min^{-1} mmHg^{-1}, $P < 0.05$) and ascorbate plus H_1/H_2 blockade (2.8 ± 0.1 ml min^{-1} mmHg^{-1}, $P < 0.05$), which did not differ from one another ($P = 0.9$). Because ascorbate may catalyze the degradation of histamine *in vivo*, we conducted a follow-up study where subjects performed exercise in two conditions: 1) control and 2) intravenous N-acetylcysteine infusion. Post-exercise vascular conductance was similar for control (4.0 ± 0.1 ml min^{-1} mmHg^{-1}) and N-acetylcysteine conditions (4.0 ± 0.1 ml min^{-1} mmHg^{-1}; $P = 0.8$). Thus, the results in study 1 were due to the degradation of histamine in skeletal muscle by ascorbate, since the histaminergic vasodilatation was unaffected by N-acetylcysteine. Taken together, exercise-induced oxidative stress does not appear to contribute to sustained post-exercise vasodilatation.

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Competing Interests

The authors have no competing interests.

Author Contributions

SAR was responsible for conception and design, data collection, data analysis and interpretation, and drafting of the manuscript. MRE, DCS, MJL, and TMB were responsible for data collection, data interpretation, and editing of the manuscript. JMK was responsible for conception and design, and editing of the manuscript. AJB provided statistical analysis and interpretation. JRH was responsible for conception and design, data analysis and interpretation, and editing of the manuscript. All authors approved the final version of the manuscript.

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Keywords

Post-Exercise Hypotension; Regional Blood Flow; Exercise; Recovery; Receptors; Histamine; Dynamic Knee Extension; Oxidative Stress

INTRODUCTION

Blood flow to the previously active skeletal muscle remains elevated for several hours following an acute bout of aerobic exercise (Laughlin *et al.*, 2012; Halliwill *et al.*, 2013, 2014). This sustained post-exercise vasodilatation occurs following both whole-body exercise (i.e. cycling) (Patil *et al.*, 1993; Halliwill *et al.*, 1996*c*, 1996a) and isolated small muscle-mass exercise (i.e. unilateral knee extension exercise) (Barrett-O'Keefe *et al.*, 2013). In humans, following 60 min of cycle ergometry, sustained post-exercise vasodilatation results from a combination of histamine H_1 and H_2 receptor activation (Lockwood *et al.*, 2005a; McCord & Halliwill, 2006a; McCord *et al.*, 2006a), alterations in the central neural control of sympathetic outflow (Halliwill *et al.*, 1996b), and a reduced ability to transduce sympathetic nerve activation into vasoconstriction (Halliwill *et al.*, 2003). Following unilateral dynamic knee extension exercise, H_1 and H_2 receptor blockade abolishes sustained post-exercise vasodilatation, suggesting that this phenomenon is primarily histaminergic in origin and may occur independent of the neural mechanisms in this model of exercise (Barrett-O'Keefe *et al.*, 2013). The upstream signaling that drives sustained post-exercise histaminergic vasodilatation remains undetermined, but presumably relies on the release or formation of histamine within the skeletal muscle tissue. Histamine may be released by mast cells via degranulation. Alternatively, local histamine formation may be increased post-exercise through *de novo* synthesis in non-mast cells by the inducible enzyme histidine decarboxylase. Exercise-related factors such as increased intramuscular temperature and oxidative stress have been proposed to stimulate local histamine release or formation within the previously active skeletal muscle (Halliwill *et al.*, 2013), but these theories are untested. Notably, oxidative stress has been linked to mast cell degranulation using both *in vitro* and *in vivo* experimental models (Ohmori *et al.*, 1978, 1979, 1980), and has also been linked to induction of histidine decarboxylase in vitro (Höcker *et al.*, 1998a).

By common definition, oxidative stress is a condition in which pro-oxidants overwhelm antioxidant defense mechanisms resulting in macromolecular damage and altered redox signaling and control (Sie & Jones, 2007; Jones, 2008). In humans, Dillard and colleagues showed that acute whole-body aerobic exercise increases oxidative stress (Dillard *et al.*, 1978). This was first demonstrated indirectly by increased lipid peroxidation, and subsequently confirmed in rats (Davies *et al.*, 1982) and later in humans (Ashton *et al.*, 1998) using electron paramagnetic spectroscopy. Involvement of whole body exercise is not a prerequisite for oxidative stress, as unilateral dynamic knee extension exercise similarly induces oxidative stress within the exercising muscle group (Bailey *et al.*, 2003, 2007).

Thus, we propose that there may be a distinct mechanistic link between exercise-induced oxidative stress and sustained post-exercise histaminergic vasodilatation. Therefore, the purpose of this study was to determine if acute exercise-induced oxidative stress is an

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important part of the upstream signaling that leads to sustained post-exercise vasodilatation via activation of histamine receptors. In study 1, we tested the hypothesis that the infusion of ascorbate, a potent antioxidant, would inhibit sustained post-exercise vasodilatation, and that this inhibition would be similar in magnitude to that observed with histamine receptor antagonism in prior studies. Further, we hypothesized that H_1 and H_2 blockade in combination with ascorbate would not further reduce the vasodilatation beyond the effect of ascorbate alone. Given the potential for ascorbate to interact directly with histamine, we conducted a follow-up study using an alternative antioxidant, N-acetylcysteine. The purpose of the follow-up study was to determine if the findings from study 1 were due to the blunting of exercise-induced oxidative stress, and thus inactivation of a histaminergic signaling pathway, or due instead to the direct degradative action of ascorbate on histamine. We tested the hypothesis that the infusion of the potent antioxidant N-acetylcysteine would, similar to ascorbate, inhibit sustained post-exercise vasodilatation.

METHODS

Subjects

Nineteen young healthy subjects were invited to participate in this study. Seven men and 4 women participated in study 1 whereas 5 men and 3 women participated in the follow-up study. Written informed consent was obtained from all subjects subsequent to a verbal and written briefing of all experimental procedures. Subjects were deemed healthy following a standard health history questionnaire. Based on the subjects' exercise habits over the previous 12 months and self-reported physical activity levels on two questionnaires (Baecke *et al.*, 1982; Kohl *et al.*, 1988), they were classified as recreationally active based on studies conducted previously in our laboratory comparing sustained post-exercise vasodilation in untrained and trained subjects (McCord & Halliwill, 2006a). In our laboratories experience, physical activity levels in trained subjects are upwards of 170 MET hr^{-1} $week^{-1}$. All subjects were required to abstain from caffeine, alcohol, and exercise for 24h prior to the study. Additionally, subjects reported to the laboratory after an overnight fast. No subjects were using any over-the-counter (including oral antioxidants) 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. This study was approved by the Institutional Review Board at the University of Oregon and was performed in accordance to the principles outlined by the Declaration of Helsinki.

Screening Visit

The screening visit was used to determine peak power output during a unilateral dynamic knee extension exercise test performed to volitional fatigue. Dynamic knee-extension exercise during all visits was performed using a custom-built knee extension ergometer based on a computer-controlled step-motor that provided resistance against the subject's lower leg. Based on real-time measures of angular velocity and torque, power was calculated and a feedback loop maintained measured power at the assigned level. Subjects were seated with their back at 60° upright and knee-extension exercise was performed with the right leg over a 45° range of motion, starting with the leg hanging at ~90° of flexion. Subjects were

asked to maintain a cadence of 45 kicks min^{-1} while being provided with visual feedback of both kicking cadence and range of motion. Workload was ramped incrementally at a rate of 3 watts min^{-1}. During the dynamic knee extension exercise test subjects reached peak power in 9.9 ± 0.7 min (range 7 - 13 min).

Experimental Approach

Study 1—At least 7 days following the screening visit, subjects were required to visit the laboratory on three separate occasions and were studied within three different conditions: 1) control 2) ascorbate infusion and 3) ascorbate infusion plus H_1/H_2 receptor blockade. Conditions were randomized, counterbalanced, and were separated by at least 7 days. Leg haemodynamics were assessed during each visit before and after 60 min of unilateral dynamic knee extension exercise performed at 60% of peak power and a cadence of 45 kicks min^{-1}. Power was ramped at the onset of exercise to 60% peak power over the first 15 min. Power output was recorded continuously throughout 60 min dynamic knee extension exercise.

Ascorbate (Bioniche Pharma, Morgantown, WV, USA) was diluted in saline and infused intravenously for 20 min prior to exercise (primer dose, 0.5 g kg^{-1} body weight) and during 60 min of dynamic knee extension exercise (maintenance dose, 0.017 g kg^{-1} body weight). The primer dose of ascorbate was diluted with saline to a final volume of 60 ml and infused at a rate of 3.0 ml min^{-1} while the maintenance dose was diluted with saline to a final volume of 30 ml and infused at a rate of 0.5 ml min^{-1}. Both doses of ascorbate were infused along with a continuous saline drip (60 ml hr^{-1}). This pharmacological approach has been used in humans to significantly increase plasma ascorbate concentrations (Bell *et al.*, 2003; Eskurza *et al.*, 2004; Jablonski *et al.*, 2007a). Furthermore, ascorbate has been used successfully to reduce both direct and indirect markers of oxidative stress following exhaustive aerobic exercise in humans (Ashton *et al.*, 1999). In healthy young adults, ascorbate does not appear to have any direct cardiovascular effects under resting conditions (Bell *et al.*, 2003; Eskurza *et al.*, 2004; Jablonski *et al.*, 2007b) or during exercise (Bell *et al.*, 2005; Kirby *et al.*, 2009). Ascorbate is a water-soluble scavenger of reactive species such as superoxide and peroxynitrite (Halliwell & Gutteridge, 2007), and has a half-life of 30 min when administered intravenously (Padayatty *et al.*, 2004; Duconge *et al.*, 2008).

For study 1, histamine H_1 and H_2 receptors were blocked using 540 mg fexofenadine and 300 mg ranitidine. This combination of fexofenadine and ranitidine reduces sustained post-exercise vasodilatation by ~86% following unilateral dynamic knee extension exercise (Barrett-O'Keefe *et al.*, 2013). This dosage of oral fexofenadine has been shown to selectively block H_1 receptors (time to peak concentration 1.15 h and half-life 12 h), while the dose of oral ranitidine has been shown to selectively block H_2 receptors (time to peak plasma concentration 2.2 h and 2.6 h half-life) (Garg *et al.*, 1985; Russell *et al.*, 1998). Responses are 90% inhibited within 1 h and remain inhibited 6 h after administration (Garg *et al.*, 1985; Brunton *et al.*, 2011). Fexofenadine and ranitidine do not appear to cross into the central nervous system or possess sedative actions (Brunton *et al.*, 2011). Furthermore, these drugs do not have any direct cardiovascular effects in the absence of histamine receptor stimulation (i.e., when given under normal resting conditions, these drugs do not

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elicit any changes in heart rate, blood pressure, or smooth muscle tone) (McCord & Halliwill, 2006b). Subjects ingested the histamine receptor antagonists with water 1 hr prior to exercise. On the control day, saline was infused in lieu of ascorbate and subjects did not receive fexofenadine or ranitidine (no placebo).

Follow-up Study—At least 7 days following the screening visit, subjects were required to visit the laboratory on two separate occasions and were studied within two different conditions: 1) control 2) N-acetylcysteine infusion. We chose to not add a third condition combining N-acetylcysteine and H_1/H_2 receptor blockade as the follow-up study was conducted in order to determine if the findings from study 1 were related to the antioxidant effect of ascorbate or were due to a direct interaction with histamine. Conditions were randomized, counterbalanced, and were separated by at least 7 days. Leg haemodynamics were assessed during each visit before and after 60 min of unilateral dynamic knee extension exercise performed at 60% of peak power and a cadence of 45 kicks min^{-1}. Power output was ramped at the onset of exercise to 60% peak power over the first 15 min. Power output was recorded continuously throughout 60 min dynamic knee extension exercise. N-acetylcysteine (Acetadote ®, Cumberland Pharmaceuticals Inc., Nashville, TN, USA) was diluted in saline and infused intravenously for 15 min prior to exercise (primer dose, 125 mg kg^{-1} hr^{-1}) and during 60 min of dynamic knee extension exercise (maintenance dose, 25 mg kg^{-1} hr^{-1}). The primer dose of N-acetylcysteine was diluted with saline to a final volume of 45 ml and infused at a rate of 3.0 ml min^{-1} while the maintenance dose was diluted with saline to a final volume of 60 ml and infused at a rate of 1.0 ml min^{-1}. Both doses of N-acetylcysteine were infused along with a continuous saline drip (60 ml hr^{-1}). This pharmacological approach has been used in humans to significantly increase plasma N-acetylcysteine concentrations prior to and during exercise (Medved *et al.*, 2003, 2004; Brown *et al.*, 2004). Furthermore, N-acetylcysteine has been used successfully to alter blood redox status in both short-term high intensity and prolonged cycle exercise in humans (Medved *et al.*, 2003, 2004). In healthy young adults, N-acetylcysteine does not appear to have any direct cardiovascular effects under resting conditions or during exercise (Nyberg *et al.*, 2012). N-acetylcysteine is potent water-soluble pharmacological antioxidant with a half-life of ~5 hrs. It can directly scavenge reactive species such as hydrogen peroxide and hydroxyl radical and can also indirectly reduce reactive species by augmenting glutathione production via intracellular cysteine formation (Cotgreave, 1997; Halliwell & Gutteridge, 2007; Samuni *et al.*, 2013). Histamine receptor blockade was not used in this study.

Measurements

All resting measurements were made pre- and post-exercise with the subjects in the supine position. Subjects were asked to remain quiet and relaxed during all haemodynamic measurements. Room temperature remained thermoneutral (~23°C) throughout the study. All measurements were identical between study 1 and the follow-up study with the exception of venous blood sampling.

Heart rate and blood pressure—Arterial blood pressure was measured in the right arm using an automated sphygmomanometer (Tango+, SunTech Medical, Raleigh, NC, USA). Heart rate was monitored continuously using a three lead electrocardiograph (Datex-

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Ohmeda Cardiocap/5, GE Healthcare, Tewksbury, MA, USA) for study 1 and was monitored using a three lead electrocardiograph in the follow-up study (Tango+, SunTech Medical, Raleigh, NC, USA). Pre- and post-exercise heart rate was measured as a rolling 2 min average in study 1 and taken as a single measure in conjunction with blood pressure using the automated sphygmomanometer in the follow-up study. Blood pressure was taken as a single measure during each femoral blood flow measurement. Additionally, heart rate and blood pressure were also measured every 10 min during steady-state dynamic knee extension exercise using the automated sphygmomanometer.

Femoral blood flow—A pressure cuff (Hokanson E20 Rapid Cuff Inflator, D. E. Hokanson, Inc., Bellevue, WA. USA) was placed on both legs, immediately distal to the patella and inflated to 250 mmHg to ensure that blood flow measured in the femoral artery was indicative of blood flow to the thigh region. Femoral artery blood flow velocity was measured via duplex ultrasonography. A linear-array vascular ultrasound probe (10 MHz, GE Vingmed System 5, Horton, Norway) and an insonation angle of 60° was used to measure blood flow in the common femoral artery, approximately 2-3 cm proximal to the bifurcation. The Doppler ultrasound machine was interfaced with a computer running custom audio recording software. Velocity measurements were determined using an intensity-weighted algorithm (custom software), subsequent to demodulation of forward and reverse Doppler frequencies. For study 1, velocity measurements were made at an average depth of 1.63 ± 0.01 cm and were thin-beam corrected, based on a known beam-width of 2.21 mm which resulted in an average correction factor of 0.766 ± 0.001, as outlined by Buck and colleagues (Buck *et al.*, 2014). For the follow-up study, velocity measurements were made at an average depth of 1.72 ± 0.01 cm and were thin-beam corrected which resulted in an average correction factor of 0.765 ± 0.0001. Femoral diameter was measured in triplicate during diastole following velocity measurements. The average femoral artery diameter was 8.89 ± 0.03 mm for study 1 and 9.09 ± 0.04 mm for the follow-up study. Leg blood flow was calculated as cross-sectional area multiplied by femoral mean blood velocity and reported in ml per min. Femoral vascular conductance was calculated by dividing femoral blood flow by mean arterial pressures and expressed as ml per min per mmHg.

Skin blood flow—We measured cutaneous vascular conductance to ensure that any alteration in post-exercise femoral vascular conductance due to ascorbate, H_1/H_2 blockade, or N-acetylcysteine administration was not mediated by changes in the cutaneous vasculature. Laser-Doppler flowmetry (DRT4, Moor Instruments LTD, Devon, UK) was used to determine red blood cell flux, an index of skin blood flow. Laser-Doppler probes were placed anteriorly, on the midline, and halfway between the inguinal ligament and patella on both the active and inactive leg. Cutaneous vascular conductance was calculated by dividing laser Doppler flux by mean arterial pressure and normalized to maximal flux values measured during local heating to 43°C to account for the variability between laser Doppler sites (Kellogg *et al.*, 1998; Wilkins *et al.*, 2004).

Blood sampling and analysis—A 22 gauge intravenous catheter was placed in the subjects left arm and was used for infusions and blood sampling. Venous blood was collected and analyzed for only study 1. Venous blood was sampled before exercise

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preceding all infusions and immediately post-exercise. Venous blood was collected into a vacutainer® and centrifuged at 1,300 RCF within five minutes. Blood plasma (500 µl) was then aliquoted into cryogenic vials, immediately snap frozen in liquid nitrogen, and stored at −80°C. Butylated hydroxytoluene (0.005%) was added to each 500µl plasma sample prior to snap freezing to prevent *ex vivo* oxidation. Total plasma F_2-isoprostanes were assayed using gas chromatography/negative ion chemical ionization mass spectrometry (GC/NICI-MS) using stable isotope dilution with $[^2H_4]$-15-F_{2t}-Isoprostane as the internal standard (Vanderbilt University Eicosanoid Core Laboratory). Plasma F_2-isoprostanes were used as an indirect biomarker of exercise-induced oxidative stress.

Electromyography—Surface electromyography (Z03 EMG preamplifiers, Motion Lab Systems, Baton Rouge, La., USA) was used to ensure the subjects right quadriceps and posterior thigh muscles were not activated during knee flexion and to ensure that the muscles in the non-exercised leg remained inactive during exercise. Electromyography probes were placed on the anterolateral aspect of the thigh 8 − 10 cm above the patella and posteriorly one-third of the distance between the popliteal fossa and ischial tuberosity on both the active legs and inactive legs. Electromyography probes were placed in parallel with the pennation angle of the skeletal muscle fibers. The electromyography probes were integrated with custom built software in order to provide visual feedback to investigators during performance of knee extension exercise.

Statistical Analyses

The statistical analysis was identical for both studies. Initial statistical modeling incorporated terms for sex and sex-interactions with drug and time. Since no sex-interactions were significant (only sex main effects existed, such as one would expect from scaling effects), sex-related terms were dropped from the final modeling. Thus, the primary outcome variables were not assessed by sex and all analyses were performed after grouping data for both men and women. Baseline differences between conditions were analyzed using a one-way mixed model analysis of variance with repeated measures. Exercise responses were also analyzed using a one-way mixed model analysis of variance with repeated measures. Our primary outcome variables during the recovery from exercise were analyzed between conditions within the active and inactive leg using a stepwise regression and carried out with SAS Proc GLMSELECT (SAS version 9.2; SAS Institute Inc. Cary, NC, USA). As opposed to the traditional approach which would use ANOVA to test for differences between conditions at discrete time points during the recovery from exercise, our stepwise approach allows the examination of both linear and quadratic relationships across time and tests whether or not these relationships differ between conditions. Independent variables remained in the model if a minimum P-value threshold was met ($P < 0.15$). Significance was set at $P < 0.05$. Data are reported as mean ± SEM unless stated otherwise (e.g., SD is used in Table 1 to indicate the variability in the subject pool).

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RESULTS

Subject Characteristics

Subject physical characteristics and data obtained during the screening visit are shown in **Table 1**. Subject characteristics are similar to those obtained previously in our laboratory in young healthy subjects and consistent with recreationally active individuals.

Study 1

Pre-exercise Haemodynamics—Pre-exercise heart rate and mean arterial blood pressure are shown in **Table 2**. Both heart rate (P = 0.3) and mean arterial pressure (P = 0.9) did not differ across the three conditions of control, ascorbate, and ascorbate plus H_1/H_2 blockade. As shown in **Table 3**, cutaneous vascular conductance within both the active leg and inactive leg did not differ across the three conditions (P = 0.9). Pre-exercise femoral blood flow and femoral vascular conductance are shown in **Figure 1**. Within the active leg, femoral blood flow did not differ (P = 0.6) across the three conditions. Likewise, within the inactive leg, femoral blood flow did not differ across the three conditions (P = 0.6). Within the active leg, femoral vascular conductance did not differ across the three conditions (P = 0.6). Likewise, within the inactive leg, femoral vascular conductance did not differ across the three conditions (P = 0.6).

Exercise Responses—Power output during steady state dynamic knee extension exercise was similar for the three conditions of control (20.8 ± 1.3 W), ascorbate (20.0 ± 1.1 W), and ascorbate plus H_1/H_2 blockade (19.9 ± 1.4 W) (P = 0.1). Power output for each condition was within 2 W of the estimated 60% workload (21 W). Compared with the control condition (96.8 ± 1.0 mmHg), mean arterial blood pressure was higher for the ascorbate condition (100.0 ± 1.3 mmHg, $P < 0.05$), but was not different with the ascorbate plus H_1/H_2 blockade condition (98.2 ± 1.3 mmHg, P = 0.3). Mean arterial pressure did not differ for ascorbate and ascorbate plus H_1/H_2 blockade conditions (P = 0.2). Heart rate was did not differ for the three conditions of control (90.6 ± 3.3 beats min^{-1}), ascorbate (95.5 ± 4.2 beats min^{-1}), and ascorbate plus H_1/H_2 blockade (90.9 ± 3.8 beats min^{-1}) (P = 0.1).

Oxidative Stress.—Pre-exercise F_2-isoprostane concentration did not differ across the three conditions (control 87.1 ± 8.7 pg ml^{-1}; ascorbate 88.7 ± 8.2 pg ml^{-1}; ascorbate plus H_1/H_2 blockade 84.7 ± 7.2 pg ml^{-1}; P = 0.4). Compared with pre-exercise, F_2-isoprostane concentration was not elevated immediately following dynamic knee extension exercise for all three conditions (control 84.0 ± 7.7 pg ml^{-1}; ascorbate 94.3 ± 11.0 pg ml^{-1}; ascorbate plus H_1/H_2 blockade 88.7 ± 10.1 pg ml^{-1};P = 0.4). Likewise, post-exercise F_2-isoprostane concentrations did not differ across the three conditions; P = 0.4).

Post-exercise Haemodynamics—Table 2 shows heart rate and mean arterial blood pressure during recovery from exercise. Compared with the control condition, mean arterial blood pressure was similar for the ascorbate condition (P = 0.7) and the ascorbate plus H_1/H_2 blockade condition (P = 0.8). Mean arterial pressure was similar between ascorbate and ascorbate plus H_1/H_2 blockade conditions (P = 0.6). Compared with the control condition, heart rate did not differ for the ascorbate condition (P = 0.4) and the ascorbate

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plus H_1/H_2 blockade condition (P = 0.8). Heart rate was not different between ascorbate and ascorbate plus H_1/H_2 blockade conditions (P = 0.5).

As shown in **Table 3**, cutaneous vascular conductance in the active leg did not differ across all three conditions during the recovery from exercise (P > 0.15). However, in the inactive leg, cutaneous vascular conductance was lower with ascorbate plus H_1/H_2 blockade than either the control or the ascorbate conditions (P < 0.05).

Figure 1 shows femoral blood flow and femoral vascular conductance during the recovery from exercise. In the active leg, the increase from pre-exercise in femoral blood flow at 1 h post-exercise was reduced for both the ascorbate (13 ± 7 %, P < 0.05) and ascorbate plus H_1/H_2 blockade (15 ± 7%, P < 0.05) conditions compared with the control condition (37 ± 7 %). The change in femoral blood flow did not differ between ascorbate and ascorbate plus H_1/H_2 blockade conditions (P = 0.8). Likewise, the increase from pre-exercise in femoral vascular conductance at 1 h post-exercise was reduced for the both the ascorbate (14 ± 8 %, P < 0.05) and ascorbate plus H_1/H_2 blockade (18 ± 7%, P < 0.05) conditions compared with the control condition (43 ± 9 %). The change in femoral vascular conductance did not differ between ascorbate and ascorbate plus H_1/H_2 blockade conditions (P = 0.7).

In the inactive leg, the femoral blood flow change from pre-exercise to 1h post-exercise did not differ between the ascorbate (−1 ± 5 %, P = 0.3) and control (6 ± 2 %) conditions, and also between the ascorbate plus H_1/H_2 blockade (4 ± 5 %, P = 0.8) and control conditions. The change in femoral blood flow did not differ between ascorbate and ascorbate plus H_1/H_2 blockade conditions (P = 0.4). Likewise, the femoral vascular conductance change from pre-exercise to 1h post-exercise did not differ between the ascorbate (−1 ± 5 %, P = 0.2) and control (7 ± 4 %) conditions, and also between the ascorbate plus H_1/H_2 blockade (8 ± 6 %, P = 0.9) and control conditions. The change in femoral vascular conductance did not differ between ascorbate and ascorbate plus H_1/H_2 blockade conditions (P = 0.2).

Follow-up Study

Pre-exercise Haemodynamics—Pre-exercise heart rate and mean arterial blood pressure are shown in **Table 4**. Compared with the control condition heart rate was greater for the N-acetylcysteine condition (P < 0.05). Mean arterial pressure did not differ between the two conditions (P = 0.9). As shown in **Table 5**, cutaneous vascular conductance within both the active leg and inactive leg did not differ between the two conditions (P = 0.9). Pre-exercise femoral blood flow and femoral vascular conductance are shown in **Figure 2**. Within the active leg, femoral blood flow did not differ (P = 0.9) between the two conditions. Likewise, within the inactive leg, femoral blood flow did not differ between the two conditions (P = 0.9). Within the active leg, femoral vascular conductance did not differ between the two conditions (P = 0.9). Likewise, within the inactive leg, femoral vascular conductance did not differ between the two conditions (P = 0.9).

Exercise Responses—Power output during steady state dynamic knee extension exercise was similar for the control (21.2 ± 0.6 W) and N-acetylcysteine conditions (21.1 ± 0.6 W, P = 0.3). Power output for each condition matched the estimated 60% workload (21 W). Mean arterial blood pressure was similar between control (100.8 ± 1.6 mmHg) and N-

acetylcysteine conditions (101.0 ± 1.9 mmHg, $P = 0.8$). Compared with the control condition (92.5 ± 1.6 beats min^{-1}), heart rate was higher for N-acetylcysteine condition (101.5 ± 1.6 beats min^{-1}, $P < 0.05$).

Post-exercise Haemodynamics—Table 4 shows heart rate and mean arterial blood pressure during recovery from exercise. Mean arterial blood pressure did not differ between control and N-acetylcysteine conditions ($P = 0.8$). Compared with the control condition, heart rate was higher for the N-acetylcysteine condition throughout the recovery from exercise ($P < 0.05$).

Cutaneous vascular conductance during the recovery from exercise is shown in **Table 5**. Compared with the control condition, cutaneous vascular conductance in the active leg was higher for the N-acetylcysteine condition through the first 60 min of exercise recovery ($P < 0.05$). In the inactive leg, cutaneous vascular conductance did not differ between control and N-acetylcysteine conditions throughout the recovery from exercise ($P = 0.6$).

Figure 2 shows femoral blood flow and femoral vascular conductance during the recovery from exercise. In the active leg, the increase from pre-exercise in femoral blood flow at 1 h post-exercise did not differ between control (22 ± 4 %) and N-acetylcysteine (18 ± 7%, $P = 0.4$) conditions. Likewise, the increase from pre-exercise in femoral vascular conductance at 1 h post-exercise did not differ between control (22 ± 4 %) and N-acetylcysteine (16 ± 8 %, $P = 0.4$) conditions.

In the inactive leg, the femoral blood flow change from pre-exercise to 1h post-exercise did not differ between control (1 ± 4 %) and N-acetylcysteine (−6 ± 4 %, $P = 0.1$) conditions. Likewise, the femoral vascular conductance change from pre-exercise to 1h post-exercise did not differ between control (−0.4 ± 5 %) and N-acetylcysteine (−10 ± 4 %, $P = 0.1$) conditions.

DISCUSSION

The purpose of this study was to determine if exercise-induced oxidative stress contributes to sustained post-exercise vasodilatation. In agreement with our hypothesis for study 1, we observed that the infusion of ascorbate, a potent antioxidant, inhibited sustained post-exercise vasodilatation. The degree of inhibition we observed with ascorbate was similar to what we have found in our previous work utilizing H_1/H_2 receptor blockade. Furthermore, the inhibition produced by ascorbate was not augmented with the addition of H_1/H_2 receptor blockade. In disagreement with our hypothesis for the follow-up study, we observed that sustained post-exercise vasodilatation was unaffected by the infusion of the potent antioxidant N-acetylcysteine. Taken together, these results suggest that the inhibition of sustained post-exercise vasodilatation by ascorbate was due to a direct degradative effect on histamine and not due to the blunting of exercise-induced oxidative stress, as N-acetylcysteine, an antioxidant with no known histaminergic interaction, had no effect on sustained post-exercise vasodilatation.

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Sustained Post-exercise Vasodilatation and Exercise-Induced Oxidative Stress

Our laboratory has demonstrated in several studies that sustained post-exercise vasodilatation is largely inhibited by H_1/H_2 receptor blockade following either large muscle mass exercise such as cycling (Lockwood *et al.*, 2005b; McCord & Halliwill, 2006b; McCord *et al.*, 2006b), or more recently following unilateral dynamic knee-extension (Barrett-O'Keefe *et al.*, 2013). There are several advantages to the use of the single-leg model, including the availability of a "control" non-exercising limb. We therefore chose to utilize the dynamic knee-extension exercise model in order to isolate the histaminergic component of sustained post-exercise vasodilatation and to directly assess the role of exercise-induced oxidative stress.

It is well understood that acute aerobic exercise increases oxidative stress that results predominantly from reactive oxygen and nitrogen species formation within contracting skeletal muscle (Powers *et al.*, 2011). Because the local formation of oxidative stress is augmented within active skeletal muscle during dynamic knee extension exercise (Bailey *et al.*, 2003, 2007) and given the relationship between oxidative stress and histamine formation in other settings (Höcker *et al.*, 1998b; Son *et al.*, 2006), we proposed that exercise-induced oxidative stress may contribute to sustained post-exercise vasodilatation.

Given independently or in combination, ascorbate and H_1/H_2 histamine receptor blockade each inhibit sustained post-exercise vasodilatation to a similar extent, suggesting that under normal exercise conditions oxidative stress could activate a histaminergic signaling mechanism located upstream of histamine receptors. However, this interpretation is complicated due the ability of ascorbate to catalyze the degradation of histamine. Several early studies provided evidence of the histamine degradative potential of ascorbate (Imanaga, 1955a, 1955b; Nandi *et al.*, 1974; Chatterjee & Gupta, 1975; Chatterjee *et al.*, 1975; Subramanian, 1978). Uchida and colleagues later demonstrated that ascorbate degrades histamine through a selective and direct interaction at the imidazole ring (Uchida *et al.*, 1989). Ascorbate may also reduce histamine through inhibition of histidine decarboxylase, the enzyme that catalyzes the formation of histamine from the amino acid L-histidine (Oh & Nakano, 1988; Dwivedi *et al.*, 1993). Several investigations have also provided evidence of the histamine-reducing effect of ascorbate in humans. Clemetson was the first to demonstrate that three days of oral administration of 1g ascorbate reduced blood histamine concentrations (Clemetson, 1980). Similar results were found later using chronic ascorbate administration (Johnston *et al.*, 1992a, 1992b). Importantly, a recent study demonstrated that the acute intravenous administration of high dose ascorbate, an approach similar to that used in our study, reduced serum histamine concentrations in patients with allergic and infectious diseases (Hagel *et al.*, 2013). These studies provide evidence that ascorbate may reduce histamine concentrations *in vivo*. It is important to note that the combination of ascorbate and H_1/H_2 receptor blockade does not further reduce sustained post-exercise vasodilatation which supports our interpretation that these interventions block a single common pathway for vasodilatation, with ascorbate either 1) inhibiting the formation of histamine by blocking upstream reactive oxygen species, or 2) inactivating histamine, and with H_1/H_2 receptor antagonists, blocking the activation of histamine receptors.

We conducted the follow-up study to determine if the inhibition of sustained post-exercise vasodilatation observed in study 1 was the result of blunted exercise-induced oxidative stress or the result of histamine degradation by ascorbate. We designed the study to pharmaco-dissect the parallel pathways affected by ascorbate. We isolated the oxidative stress pathway by intravenously infusing N-acetylcysteine, a potent antioxidant with no known histaminergic interactions. In contrast to the findings in study 1 using ascorbate, blood flow to the previously active skeletal muscle remained elevated throughout recovery and was nearly identical to that observed in the control condition. These findings support the view that ascorbate inhibits sustained post-exercise vasodilatation through histamine degradation and not through oxidative stress related signaling mechanisms. It is worth noting that N-acetylcysteine has a longer half-life than ascorbate (5 hrs versus 30 min), and as both infusions were discontinued at the end of exercise, the lasting effect of ascorbate on conductance provides further evidence that loss of drug effectiveness during the 2-hr time-course of recovery from exercise was not an issue.

Emerging Mechanisms of Sustained Post-exercise Vasodilatation

Significant progress has been made recently in identifying the mechanisms that mediate sustained post-exercise vasodilatation. While both neural and local vascular mechanisms contribute to sustained post-exercise vasodilatation following whole-body exercise, the local vascular mechanism (i.e. histamine receptor activation) predominates following small muscle mass exercise. By using the dynamic knee extension exercise model to isolate the histaminergic component of sustained post-exercise vasodilatation, we are now able to better dissect the signaling mechanisms that drive local histamine formation and to eventually identify the source of histamine. New and colleagues (New *et al.*, 2013) recently reported that lipid hydroperoxides, an indirect biomarker of oxidative stress, and systemic vascular conductance were elevated in parallel throughout 120 min of exercise recovery. The authors proposed that sustained post-exercise vasodilatation and associated hypotension may be linked to exercise-induced oxidative stress. Our findings indicate that exercise-induced oxidative stress has no effect and that another upstream exercise-related factor is driving this response.

Histamine is notoriously difficult to measure when released or produced locally because it is metabolized quickly by histamine-N-methyltransferase and/or diamine oxidase or taken up by basophils in the bloodstream. Several studies in our lab have reported that histamine measured in both plasma and whole-blood is unchanged during the recovery from exercise despite a clear histamine receptor-mediated vasodilatation (Lockwood *et al.*, 2005b; McCord & Halliwill, 2006b; McCord *et al.*, 2006b). While the results from this study failed to provide a mechanistic link between oxidative stress and sustained post-exercise vasodilatation, they do provide evidence that histamine is indeed the ligand activating histamine receptors during the recovery from exercise. One can test a proposed physiological mechanism by inhibiting or augmenting one or more of the critical components that mediate the response. In this case, blockade of the histamine pathway at the receptor level inhibits sustained post-exercise vasodilatation following dynamic knee extension exercise (Barrett-O'Keefe *et al.*, 2013). Likewise, either inhibiting local histamine formation or degrading it with ascorbate also inhibits sustained post-exercise vasodilatation

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to a similar degree. This provides additional evidence that this physiological response is indeed histamine dependent and due to an elevation in histamine concentrations, rather than the alternative hypothesis, that histamine is not elevated following exercise, and that vasodilatation is caused by increased histamine receptor sensitivity.

Cutaneous Circulation

Sustained post-exercise histaminergic vasodilatation is not related to changes in cutaneous vascular conductance following large-muscle mass exercise (Wilkins *et al.*, 2004; Lockwood *et al.*, 2005b; McCord & Halliwill, 2006b; McCord *et al.*, 2006b). However, we measured cutaneous vascular conductance to ensure that any alteration in post-exercise femoral vascular conductance due to ascorbate, H_1/H_2 blockade, or N-acetylcysteine administration was not mediated by changes in the cutaneous vasculature. Importantly, the administration of ascorbate, H_1/H_2 blockade, or N-acetylcysteine had no effect on cutaneous vasculature in the active leg. However, within the inactive leg, cutaneous vascular conductance was reduced slightly in the ascorbate plus H_1/H_2 blockade condition. This is interesting given that systemic H_1 and/or H_2 receptor blockade has no effect on thigh cutaneous vascular conductance following whole-body exercise (Lockwood *et al.*, 2005b; McCord & Halliwill, 2006b; McCord *et al.*, 2006b). It is not completely clear why cutaneous vascular conductance in the inactive leg is reduced with ascorbate plus H_1/H_2 receptor blockade in the present study. It is possible that histamine receptor activation in the cutaneous circulation varies between the type of exercise (whole-body vs. small-muscle mass exercise) and the location measured (active vs. inactive leg). Regardless, the small reduction in cutaneous vascular conductance observed in the ascorbate plus H_1/H_2 blockade condition did not influence regional blood flow as femoral vascular conductance was similar to control and ascorbate conditions during recovery from exercise. In the follow-up study we found that administration of N-acetylcysteine did not influence cutaneous vascular conductance in the inactive leg, but elevated cutaneous vascular conductance in the active leg through the first 60 min of exercise recovery. Thus, it is possible that N-acetylcysteine may augment cutaneous vascular conductance which may obscure a true lowering of regional blood flow. However, given the minor difference in cutaneous vascular conductance between control and N-acetylcysteine conditions and given the observation that femoral blood flow is similar between conditions through 120 min of exercise recovery, we believe this difference does not impact regional blood flow to an appreciable effect.

Considerations

Several experimental considerations warrant discussion. First, we chose to intravenously infuse supraphysiological doses of ascorbate and N-acetylcysteine to reduce exercise-induced oxidative stress. These experimental approaches have been used successfully in humans to pharmaco-dissect the interactions between oxidative stress and physiological regulatory mechanisms (Bell *et al.*, 2003; Medved *et al.*, 2003, 2004; Eskurza *et al.*, 2004; Brown *et al.*, 2004; Jablonski *et al.*, 2007b; Petersen *et al.*, 2012). Both are potent antioxidants that non-specifically scavenge reactive species and also have the ability to indirectly scavenge reactive species by catalyzing the formation of other antioxidants (Halliwell & Gutteridge, 2007). While there is much overlap in the reactive species scavenged by ascorbate and N-acetylcysteine (e.g. OH^-); each antioxidant can specifically

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target reactive species that the other does not. Thus, a possibility remains that the differential effects that we observed with N-acetylcysteine were due to the scavenging of a specific reactive species that is not targeted by ascorbate. In general, this is a challenge to any human studies using antioxidants. Further, it should be noted that plasma F_2-isoprostanes were measured in only study 1 and were not increased with exercise and were also unaffected by ascorbate administration. Prior studies have shown that unilateral dynamic knee extension exercise augments oxidative stress measured using electron paramagnetic resonance spectroscopy in the venous blood draining the active skeletal muscle (Bailey *et al.*, 2003). Moreover, acute oral antioxidant administration at doses well below those used in our study have been shown to reduce oxidative stress, measured via electron paramagnetic spectroscopy, induced by moderate intensity dynamic knee extension exercise (Wray *et al.*, 2009). Therefore, we do not believe that our inability to detect an increase in F_2-isoprostanes reflects a lack of exercise-induced oxidative stress and the inability of ascorbate to scavenge reactive species. Our results more likely reflect the use of remote venous blood sampling to assess oxidative stress induced by moderate intensity small muscle-mass exercise.

Second, it is always prudent to consider whether such interventions might alter central or local vascular regulatory mechanisms, thus confounding the interpretation of an experiment. Along these lines, it is unlikely that ascorbate administration affected central control of the vasculature as ascorbate is unable to freely traverse the blood-brain barrier. This is further supported by Bell *et al*. who documented that muscle sympathetic nerve activity, an index of central sympathetic outflow, is unchanged following high dose ascorbate administration (Bell *et al.*, 2003). Furthermore, baseline blood flow and femoral vascular conductance did not differ across our three conditions indicating both the central and local vascular regulatory mechanisms were unaffected with ascorbate administration. The infusion of N-acetylcysteine is used less often in human studies investigating the influence of oxidative stress on cardiovascular regulatory mechanisms. However, a recent study using an infusion protocol similar to ours reported that both arterial blood pressure and femoral vascular conductance are unaffected with high dose intravenous N-acetylcysteine (Nyberg *et al.*, 2012). We reported similar findings, thus suggesting N-acetylcysteine does not have a direct effect on cardiovascular regulatory mechanisms, although we acknowledge that, unlike ascorbate, it likely crosses the blood brain barrier.

Finally, the effect of H_1/H_2 receptor blockade alone on sustained post-exercise vasodilatation was not examined in this current investigation. We felt there was no added benefit to include this condition as we have previously established the efficacy of H_1/H_2 receptor blockade to inhibit sustained post-exercise vasodilatation following unilateral dynamic knee extension exercise (Barrett-O'Keefe *et al.*, 2013). While these considerations are valid points of discussion, our experimental approaches represent the best methods available to test our hypothesis in humans and we remain confident that these considerations do not limit or undermine our interpretation.

Perspectives

Pharmaco-dissecting the control systems and pathways that regulate the human cardiovascular system is inherently complex. Mechanistic studies provide an added level of

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complexity. We are often limited by the invasiveness of the experimental protocol or the limited availability of pharmacological agents that are available for use in humans. Most importantly, the pharmacological agents used in human investigations may affect physiological systems other than that targeted for investigation. Thus, conclusions drawn from these studies may be inadvertently incorrect or misleading. Understanding the pharmacology and potential side-effects of the chosen drug is critical, and employing alterative drugs, to re-test an idea when more than one mechanism may be affected, is prudent. The number of human studies investigating the link between oxidative stress and pathophysiological conditions has increased tremendously within the last decade. Additionally, recent studies have also provided unique insight between acute and chronic cardiovascular and metabolic changes that occur as a result of exercise-induced oxidative stress. These studies often use antioxidants of various forms to pharmaco-dissect these interactions, raising the question of whether histamine degradation by ascorbate may be common confound in this field of study. Understanding the pharmacology of the antioxidants and the multiple systems and pathways they may affect is essential in human investigations.

Summary

Acute aerobic exercise promotes a sustained post-exercise histaminergic vasodilatation within the vasculature perfusing the previously active skeletal muscle. This study contributes further to the understanding of the mechanisms that drive this response. The findings from this study demonstrate that exercise-induced oxidative stress does not contribute to sustained post-exercise vasodilatation. While the systemic infusion of N-acetylcysteine did not affect sustained post-exercise vasodilatation, ascorbate, an antioxidant with the unique ability to catalyze the degradation of histamine, inhibited the vasodilatation to a similar degree of that observed in our previous work utilizing H_1/H_2 receptor blockade. This observation provides another line of evidence that histamine is released locally within the previously exercised muscle and where it can then activate H_1/H_2 receptors leading to sustained post-exercise vasodilatation.

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REFERENCES

Ashton T, Rowlands CC, Jones E, Young IS, Jackson SK, Davies B, Peters JR. Electron spin resonance spectroscopic detection of oxygen-centred radicals in human serum following exhaustive exercise. Eur J Appl Physiol Occup Physiol. 1998; 77:498–502. [PubMed: 9650733]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Ashton T, Young IS, Peters JR, Jone E, Jackson SK, Davies B, Rowlands CC. Electron spin resonance spectroscopy, exercise, and oxidative stress: an ascorbic acid intervention study. J Appl Physiol. 1999; 87:2032–2036. [PubMed: 10601146]

Baecke JAH, Burema J, Frijters JER. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr. 1982; 36:936–942. [PubMed: 7137077]

Bailey DM, Davies B, Young IS, Jackson MJ, Davison GW, Isaacson R, Richardson RS. EPR spectroscopic detection of free radical outflow from an isolated muscle bed in exercising humans. J Appl Physiol. 2003; 94:1714–1718. [PubMed: 12626489]

Bailey DM, Lawrenson L, McEneny J, Young IS, James PE, Jackson SK, Henry RR, Mathieu-Costello O, McCord JM, Richardson RS. Electron paramagnetic spectroscopic evidence of exercise-induced free radical accumulation in human skeletal muscle. Free Radic Res. 2007; 41:182–190. [PubMed: 17364944]

Barrett-O'Keefe Z, Kaplon RE, Halliwill JR. Sustained post-exercise vasodilatation and histamine-receptor activation following small muscle-mass exercise in humans. Exp Physiol. 2013; 98:268–277. [PubMed: 22848080]

Bell C, Carson JM, Motte NW, Seals DR. Ascorbic acid does not affect the age-associated reduction in maximal cardiac output and oxygen consumption in healthy adults. J Appl Physiol. 2005; 98:845–849. [PubMed: 15501926]

Bell C, Jones PP, Seals DR. Oxidative stress does not modulate metabolic rate or skeletal muscle sympathetic activity with primary aging in adult humans. J Clin Endocrinol Metab. 2003; 88:4950–4954. [PubMed: 14557479]

Brown M, Bjorksten A, Medved I, McKenna M. Pharmacokinetics of intravenous N-acetylcysteine in men at rest and during exercise. Eur J Clin Pharmacol. 2004; 60:717–723. [PubMed: 15619135]

Brunton, L.; Chabner, B.; Knollman, B. Goodman & Gilman's Pharmocological Basis of Therapeutics. 12th. McGraw-Hill; 2011.

Buck TM, Sieck DC, Halliwill JR. Thin-beam ultrasound overestimation of blood flow: How wide is your beam? J Appl Physiol. 2014; 116:1096–1104. [PubMed: 24557801]

Chatterjee IB, Gupta SD. Effect of ascorbic acid on histamine metabolism in scorbutic guinea-pigs. J Physiol. 1975; 251:271–279. [PubMed: 52707]

Chatterjee IB, Majumder AK, Nandi BK, Subramanian N. Synthesis and some major functions of vitamin C in animals. Ann N Y Acad Sci. 1975; 258:24–47. [PubMed: 1106297]

Clemetson CA. Histamine and ascorbic acid in human blood. J Nutr. 1980; 110:662–668. [PubMed: 7365537]

Cotgreave IA. N-acetylcysteine: pharmacological considerations and experimental and clinical applications. Adv Pharmacol. 1997; 38:205–227. [PubMed: 8895810]

Davies KJA, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun. 1982; 107:1198–1205. [PubMed: 6291524]

Dillard CJ, Litov RE, Savin WM, Dumelin EE, Tappel AL. Effects of exercise, vitamin E, and ozone on pulmonary function and lipid peroxidation. J Appl Physiol. 1978; 45:927–932. [PubMed: 730598]

Duconge J, Miranda-Massari JR, Gonzalez MJ, Jackson JA, Warnock W, Riordan NH. Pharmacokinetics of vitamin C: insights into the oral and intravenous administration of ascorbate. P R Health Sci J. 2008; 27:7–19. [PubMed: 18450228]

Dwivedi N, Das M, Joshi A, Singh GB, Khanna SK. Modulation by ascorbic acid of the cutaneous and hepatic biochemical effects induced by topically applied benzanthrone in mice. Food Chem Toxicol. 1993; 31:503–508. [PubMed: 8340029]

Eskurza I, Monahan KD, Robinson JA, Seals DR. Effect of acute and chronic ascorbic acid on flow-mediated dilatation with sedentary and physically active human ageing. J Physiol. 2004; 556:315–324. [PubMed: 14754992]

Garg DC, Eshelman FN, Weidler DJ. Pharmacokinetics of ranitidine following oral administration with ascending doses and with multiple-fixed doses. J Clin Pharmacol. 1985; 25:437–443. [PubMed: 4056078]

Hagel AF, Layritz CM, Hagel WH, Hagel H-J, Hagel E, Dauth W, Kressel J, Regnet T, Rosenberg A, Neurath MF, Molderings GJ, Raithel M. Intravenous infusion of ascorbic acid decreases serum

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histamine concentrations in patients with allergic and non-allergic diseases. Naunyn Schmiedebergs Arch Pharmacol. 2013; 386:789–793. [PubMed: 23666445]

Halliwell, B.; Gutteridge, JM. Free Radicals in Biology and Medicine. 4th. Oxford University Press; 2007.

Halliwill JR, Buck TM, Lacewell AN, Romero SA. Postexercise hypotension and sustained postexercise vasodilation: What happens after we exercise? Exp Physiol. 2013; 98:7–18. [PubMed: 22872658]

Halliwill JR, Dinenno FA, Dietz NM. Alpha-adrenergic vascular responsiveness during postexercise hypotension in humans. J Physiol. 2003; 550:279–286. [PubMed: 12766237]

Halliwill JR, Sieck DC, Romero SA, Buck TM, Ely MR. Blood pressure regulation X: What happens when the muscle pump is lost? Post-exercise hypotension and syncope. Eur J Appl Physiol. 2014; 114:561–578. [PubMed: 24197081]

Halliwill JR, Taylor JA, Eckberg DL. Impaired sympathetic vascular regulation in humans after acute dynamic exercise. J Physiol. 1996a; 495:279–288. [PubMed: 8866370]

Halliwill JR, Taylor JA, Eckberg DL. Impaired sympathetic vascular regulation in humans after acute dynamic exercise. J Physiol. 1996b; 495:279–288. [PubMed: 8866370]

Halliwill JR, Taylor JA, Hartwig TD, Eckberg DL. Augmented baroreflex heart rate gain after moderate-intensity, dynamic exercise. Am J Physiol Regul Integr Comp Physiol. 1996c; 270:R420–R426.

Höcker M, Rosenberg I, Xavier R, Henihan RJ, Wiedenmann B, Rosewicz S, Podolsky DK, Wang TC. Oxidative Stress Activates the Human Histidine Decarboxylase Promoter in AGS Gastric Cancer Cells. J Biol Chem. 1998a; 273:23046–23054. [PubMed: 9722530]

Höcker M, Rosenberg I, Xavier R, Henihan RJ, Wiedenmann B, Rosewicz S, Podolsky DK, Wang TC. Oxidative Stress Activates the Human Histidine Decarboxylase Promoter in AGS Gastric Cancer Cells. J Biol Chem. 1998b; 273:23046–23054. [PubMed: 9722530]

Imanaga Y. Auto-oxidation of L-ascorbic acide and imidazole nucleus I. The effects of imidazole derivatives on the auto-oxidation of L-ascorbic acid. J Biochem. 1955a; 42:657–667.

Imanaga Y. Auto-oxidation of L-ascorbic acid and imadazole nucleus II. the decomposition products of imadazole derivaties present in the auto-oxidation mixture. J Biochem. 1955b; 42:669–676.

Jablonski KL, Seals DR, Eskurza I, Monahan KD, Donato a. J. High-dose ascorbic acid infusion abolishes chronic vasoconstriction and restores resting leg blood flow in healthy older men. J Appl Physiol. 2007a; 103:1715–1721. [PubMed: 17965239]

Jablonski KL, Seals DR, Eskurza I, Monahan KD, Donato a. J. High-dose ascorbic acid infusion abolishes chronic vasoconstriction and restores resting leg blood flow in healthy older men. J Appl Physiol. 2007b; 103:1715–1721. [PubMed: 17965239]

Johnston CS, Martin LJ, Cai X. Antihistamine effect of supplemental ascorbic acid and neutrophil chemotaxis. J Am Coll Nutr. 1992a; 11:172–176. [PubMed: 1578094]

Johnston CS, Retrum KR, Srilakshmi JC. Antihistamine effects and complications of supplemental vitamin C. J Am Diet Assoc. 1992b; 92:988–989. [PubMed: 1640045]

Jones DP. Radical-free biology of oxidative stress. Am J Physiol Cell Physiol. 2008; 295:849–868.

Kellogg DL, Crandall CG, Liu Y, Charkoudian N, Johnson JM. Nitric oxide and cutaneous active vasodilation during heat stress in humans. J Appl Physiol. 1998; 85:824–829. [PubMed: 9729553]

Kirby BS, Voyles WF, Simpson CB, Carlson RE, Schrage WG, Dinenno FA. Endothelium-dependent vasodilatation and exercise hyperaemia in ageing humans: impact of acute ascorbic acid administration. J Physiol. 2009; 9:1989–2003. [PubMed: 19307300]

Kohl HW, Blair SN, Paffenbarger RS, Macera CA, Kronenfeld JJ, Paffenbarger RS Jr. A mail survey of physical activity habits as related to measured physical fitness. Am J Epidemiol. 1988; 127:1228–1239. [PubMed: 3369421]

Laughlin MH, Davis MJ, Secher NH, van Lieshout JJ, Arce-esquivel AA, Simmons GH, Bender SB, Padilla J, Bache RJ, Merkus D, Duncker DJ. Peripheral Circulation. Compr Physiol. 2012; 2:321–447. [PubMed: 23728977]

Lockwood JM, Wilkins BW, Halliwill JR. H1 receptor-mediated vasodilatation contributes to postexercise hypotension. J Physiol. 2005a; 563:633–642. [PubMed: 15618271]

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Lockwood JM, Wilkins BW, Halliwill JR. H1 receptor-mediated vasodilatation contributes to postexercise hypotension. J Physiol. 2005b; 563:633–642. [PubMed: 15618271]

McCord JL, Beasley JM, Halliwill JR. H2-receptor-mediated vasodilation contributes to postexercise hypotension. J Appl Physiol. 2006a; 100:67–75. [PubMed: 16141376]

McCord JL, Beasley JM, Halliwill JR. H2-receptor-mediated vasodilation contributes to postexercise hypotension. J Appl Physiol. 2006b; 100:67–75. [PubMed: 16141376]

McCord JL, Halliwill JR. H1 and H2 receptors mediate postexercise hyperemia in sedentary and endurance exercise-trained men and women. J Appl Physiol. 2006a; 101:1693–1701. [PubMed: 16888049]

McCord JL, Halliwill JR. H1 and H2 receptors mediate postexercise hyperemia in sedentary and endurance exercise-trained men and women. J Appl Physiol. 2006b; 101:1693–1701. [PubMed: 16888049]

Medved I, Brown MJ, Bjorksten a R, Murphy KT, Petersen AC, Sostaric S, Gong X, McKenna MJ. N-acetylcysteine enhances muscle cysteine and glutathione availability and attenuates fatigue during prolonged exercise in endurance-trained individuals. J Appl Physiol. 2004; 97:1477–1485. [PubMed: 15194675]

Medved I, Brown MJ, Bjorksten AR, Leppik JA, Sostaric S, Mckenna MJ. N-acetylcysteine infusion alters blood redox status but not time to fatigue during intense exercise in humans. J Appl Physiol. 2003; 94:1572–1582. [PubMed: 12496140]

Nandi BK, Subramanian N, Majumder AK, Chatterjee IB. Effect of ascorbic acid on detoxification of histamine under stress conditions. Biochem Pharmacol. 1974; 23:643–647. [PubMed: 4132605]

New KJ, Reilly ME, Templeton K, Ellis G, James PE, Mceneny J, Penney M, Hooper J, Hullin D, Davies B, Bailey DM. Free radical-mediated lipid peroxidation and systemic nitric oxide bioavailability: implications for postexercise hemodynamics. Am J Hyperten. 2013; 26:126–134.

Nyberg M, Blackwell JR, Damsgaard R, Jones AM, Hellsten Y, Mortensen SP. Lifelong physical activity prevents an age-related reduction in arterial and skeletal muscle nitric oxide bioavailability in humans. J Physiol. 2012; 590:5361–5370. [PubMed: 22890714]

Oh C, Nakano K. Reversal by ascorbic acid of suppression by endogenous histamine of rat lymphocyte blastogenesis. J Nutr. 1988; 118:639–644. [PubMed: 3367243]

Ohmori H, Komoriya K, Azuma A, Hashimoto Y, Kurozumi S. Xanthine oxidase-induced foot-edema in rats: involement of oxygen radicals. Biochem Pharmacol. 1978; 27:1397–1400. [PubMed: 697944]

Ohmori H, Komoriya K, Azuma A, Kurozumi S, Hashimoto Y. Xanthine oxidase-induced histamine release from isolated rat peritoneal mast cells: involvement of hydrogen peroxide. Biochem Pharmacol. 1979; 28:333–334. [PubMed: 85451]

Ohmori H, Yamamoto I, Akagi M, Tasaka K. Properties of hydrogen peroxide-induced histamine release from rat mast cells. Biochem Pharmacol. 1980; 29:741–745. [PubMed: 20227950]

Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, Katz A, Wesley RA, Levine M. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med. 2004; 140:533–537. [PubMed: 15068981]

Patil RD, DiCarlo SE, Collins HL. Acute exercise enhances nitric oxide modulation of vascular response to phenylephrine. Am J Physiol Heart Circ Physiol. 1993; 265:H1184–H1188.

Petersen AC, McKenna MJ, Medved I, Murphy KT, Brown MJ, Della Gatta P, Cameron-Smith D. Infusion with the antioxidant N-acetylcysteine attenuates early adaptive responses to exercise in human skeletal muscle. Acta Physiol. 2012; 204:382–392.

Powers SK, Ji LL, Kavazis AN, Jacksonm MJ. Reactive Oxygen Species: Impact on Skeletal Muscle. Compr Physiol. 2011; 1:941–969. [PubMed: 23737208]

Russell T, Stoltz M, Weir S. Pharmacokinetics, pharmacodynamics, and tolerance of single- and multiple-dose fexofenadine hydrochloride in healthy male volunteers. Clin Pharmacol Ther. 1998; 64:612–621. [PubMed: 9871426]

Samuni Y, Goldstein S, Dean OM, Berk M. The chemistry and biological activities of N-acetylcysteine. Biochim Biophys Acta. 2013; 1830:4117–4129. [PubMed: 23618697]

Sies H, Jones D. Oxidative Stress. Encyclopedia of Stress (2nd). 2007:45.

Son A, Nakamura H, Kondo N, Matsuo Y, Liu W, Oka S, Ishii Y, Yodoi J. Redox regulation of mast cell histamine release in thioredoxin-1 (TRX) transgenic mice. Cell research. 2006; 16:230–239. [PubMed: 16474438]

Subramanian N. Histamine degradative potential of ascorbic acid: considerations and evaluations. Agents Actions. 1978; 8:484–487. [PubMed: 717181]

Uchida K, Mitsui M, Kawakishi S. Monooxygenation of N-acetylhistamine mediated by L ascorbate. Biochim Biophys Acta. 1989; 991:377–379. [PubMed: 2719978]

Wilkins BW, Minson CT, Halliwill JR. Regional hemodynamics during postexercise hypotension. II. Cutaneous circulation. J Appl Physiol. 2004; 97:2071–2076. [PubMed: 15322061]

Wray DW, Uberoi A, Lawrenson L, Bailey DM, Richardson RS. Oral antioxidants and cardiovascular health in the exercise-trained and untrained elderly: a radically different outcome. Clin Sci (Lond). 2009; 116:433–441. [PubMed: 18795893]

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NEW FINDINGS

- What is the central question of this study?

  Is exercise-induced oxidative stress the upstream exercise-related signaling mechanism that leads to sustained post-exercise vasodilation via activation of H_1 and H_2 histamine receptors?

- What is the main finding and its importance?

  Systemic administration of the antioxidant ascorbate inhibits sustained post-exercise vasodilatation to the same extent as previously seen with H_1 and H_2 histamine receptor blockade following small muscle-mass exercise. However, ascorbate has a unique ability to catalyze the degradation of histamine. Therefore, we conducted a follow-up study and found that systemically infusing the antioxidant N-acetylcysteine had no effect on sustained post-exercise vasodilatation suggesting that exercise-induced oxidative stress does not contribute to sustained post-exercise vasodilatation.

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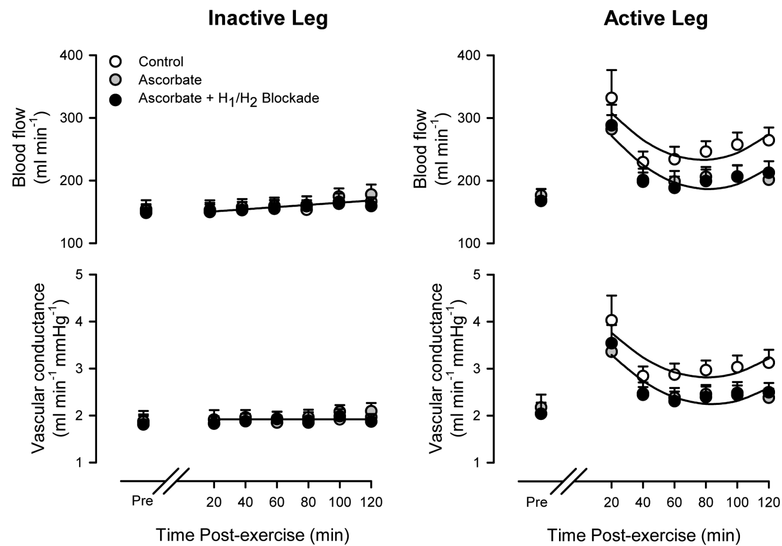

Figure 1.

Femoral blood flow and femoral vascular conductance are shown for study 1 in both the inactive (left panels) and active (right panels) leg prior to exercise and throughout two hours of recovery. Open circles, control condition; Gray circles, ascorbate condition; Closed circles, ascorbate plus H_1/H_2 blockade. Parallel regression lines for femoral blood flow and femoral vascular conductance (right panels) indicate a significant drug effect ($P < 0.05$) for ascorbate and ascorbate plus H_1/H_2 blockade conditions versus control condition during the recovery from exercise (flow $r^2 = 0.19$; conductance $r^2 = 0.20$). Solitary regression lines (left panels) indicate the absence of main effects or interactions for the control, ascorbate, and ascorbate plus H_1/H_2 blockade conditions during the recovery from exercise (flow $r^2 = 0.02$; conductance $r^2 < 0.01$).

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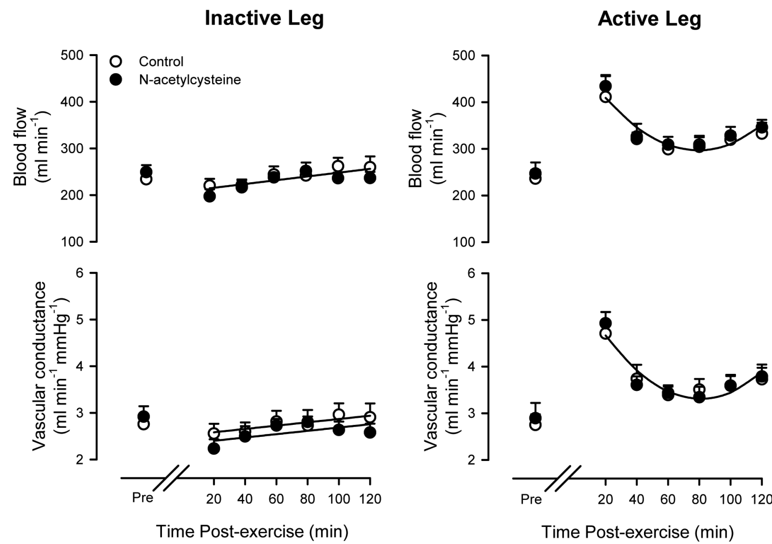

Figure 2.

Femoral blood flow and femoral vascular conductance are shown for the follow-up study in both the inactive (left panels and active (right panels) leg prior to exercise and throughout two hours of recovery. Open circles, control condition. Closed circles, N-acetylcysteine condition. Parallel regression lines for femoral vascular conductance (bottom, left panel) indicate a significant drug effect ($P < 0.05$) for N-acetylcysteine condition versus control condition during the recovery from exercise ($r^2 = 0.30$). Solitary regression line for femoral blood flow (upper, left panel) indicates the absence of main effects or interactions for the control and N-acetylcysteine conditions during the recovery from exercise ($r^2 = 0.27$). Solitary regression lines (right panels) indicate the absence of main effects or interactions for the control and N-acetylcysteine conditions during the recovery from exercise (flow $r^2 = 0.10$; conductance $r^2 = 0.06$).

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Table 1

Subject Characteristics

	Study 1	Follow-up Study
n	11	8
Age (yrs)	22.2 ± 1.9	20.6 ± 2.3
Height (cm)	173 ± 9	178 ± 8
Weight (kg)	72.8 ± 11.4	75.0 ± 14.0
Body mass index (kg m^{-2})	24.0 ± 2.8	23.5 ± 3.4
Baecke sport index (arbitrary units)	3.0 ± 0.7	3.3 ± 0.7
Physical activity index (MET hr^{-1} $week^{-1}$)	79 ± 48	39 ± 17
Peak power output (W)	35.2 ± 6.4	34.8 ± 6.9

Values are mean ± SD. MET, metabolic equivalents.

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Table 2

Central Haemodynamics

	Heart Rate (beats min $^{-1}$)			Mean Arterial Pressure (mmHg)		
Time Point	**Control**	**Ascorbate**	**Ascorbate + H_1/H_2 Blockade**	**Control**	**Ascorbate**	**Ascorbate + H_1/H_2 Blockade**
Pre-exercise	57.1 ± 0.5	60.1 ± 0.7	56.7 ± 0.7	83.0 ± 0.6	82.9 ± 0.8	82.6 ± 0.6
Time post-exercise						
20 min	60.8 ± 1.0*	61.6 ± 1.4	61.6 ± 1.2*	81.4 ± 0.7	83.1 ± 1.2	81.6 ± 0.7
40 min	57.8 ± 0.9	59.5 ± 1.2	58.6 ± 1.0*	80.8 ± 0.7*	81.7 ± 1.2	81.7 ± 0.7
60 min	56.6 ± 0.8	58.4 ± 1.1	57.7 ± 1.2	82.3 ± 1.1	84.3 ± 1.3	81.1 ± 0.7
80 min	56.7 ± 1.0	57.3 ± 1.1*	56.8 ± 1.2	83.2 ± 0.9	83.7 ± 1.2	83.4 ± 0.6
100 min	56.2 ± 0.8	57.4 ± 0.8*	57.2 ± 1.0	85.3 ± 0.8*	84.0 ± 1.1	83.9 ± 0.7
120 min	56.4 ± 0.9	57.4 ± 0.8*	56.5 ± 0.9	85.3 ± 0.9*	85.3 ± 1.1	85.0 ± 0.7*

Values are mean ± SEM,

* $P < 0.05$ vs. pre-exercise.

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Table 3

Cutaneous Vascular Conductance

	Inactive Leg			Active Leg		
Time Point	**Control**	**Ascorbate**	**Ascorbate + H_1/H_2 Blockade**	**Control**	**Ascorbate**	**Ascorbate + H_1/H_2 Blockade**
Pre-exercise	8.3 ± 1.6	10.8 ± 2.5	7.8 ± 1.1	10.0 ± 2.4	14.6 ± 4.5	9.9 ± 1.9
Time post-exercise						
20 min	10.7 ± 2.2	10.0 ± 1.1	8.0 ± 1.4*	20.3 ± 3.3	25.1 ± 4.6	21.3 ± 4.2
40 min	10.0 ± 2.4	8.5 ± 1.0	7.2 ± 1.2*	18.6 ± 3.9	20.3 ± 3.4	17.4 ± 3.2
60 min	8.4 ± 1.4	7.8 ± 1.0	7.2 ± 1.1*	15.9 ± 3.5	16.2 ± 2.7	15.7 ± 3.2
80 min	8.5 ± 1.4	7.8 ± 1.1	6.6 ± 1.0*	14.0 ± 3.1	14.1 ± 2.2	13.9 ± 2.8
100 min	7.5 ± 1.1	7.7 ± 1.2	6.6 ± 0.9*	11.7 ± 2.6	13.0 ± 2.4	13.1 ± 2.9
120 min	7.8 ± 1.4	7.1 ± 0.9	6.9 ± 0.9*	10.8 ± 2.5	12.4 ± 2.2	12.3 ± 2.7

Data are presented as percentage of maximal cutaneous vascular conductance. Values are mean ± SEM.

* $P < 0.05$ vs. control and ascorbate conditions.

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Table 4

Central Haemodynamics

	Heart Rate (beats min^{-1})		Mean Arterial Pressure (mmHg)	
Time Point	**Control**	**N-acetylcysteine**	**Control**	**N-acetylcysteine**
Pre-exercise	59.7 ± 0.9	63.8 ± 1.0†	86.0 ± 0.7	86.9 ± 1.2
Time post-exercise				
20 min	61.5 ± 1.3	70.4 ± 1.9*†	87.1 ± 1.0	88.6 ± 0.9
40 min	59.3 ± 1.3	66.6 ± 1.5†	86.8 ± 0.9	88.6 ± 1.0
60 min	56.9 ± 1.2	64.5 ± 1.4†	87.8 ± 0.7	89.0 ± 1.1
80 min	56.0 ± 1.4*	63.8 ± 1.4†	88.8 ± 0.8	91.0 ± 0.9*
100 min	57.1 ± 1.4	64.4 ± 1.4†	89.2 ± 0.7*	91.3 ± 1.1*
120 min	57.2 ± 1.6	63.7 ± 1.4†	90.2 ± 0.9*	92.1 ± 1.0*

Values are mean ± SEM.

* *P* < 0.05 vs. pre-exercise;

† *P* < 0.05 vs. control

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Table 5

Cutaneous Vascular Conductance

	Inactive Leg		Active Leg	
Time Point	Control	N-acetylcysteine	Control	N-acetylcysteine
Pre-exercise	7.5 ± 0.4	7.1 ± 0.5	8.2 ± 0.7	8.8 ± 0.7
Time post-exercise				
20 min	7.1 ± 0.6	7.3 ± 0.5	11.7 ± 1.6	19.7 ± 1.9*†
40 min	6.5 ± 0.5	7.0 ± 0.6	10.0 ± 1.3	16.3 ± 1.1*†
60 min	5.9 ± 0.5	6.8 ± 0.6	9.5 ± 1.2	15.2 ± 1.2*†
80 min	5.7 ± 0.5	6.2 ± 0.7	9.4 ± 1.4	13.1 ± 0.9*
100 min	5.6 ± 0.5	6.7 ± 0.6	9.0 ± 1.3	11.9 ± 1.2
120 min	5.7 ± 0.5	6.9 ± 0.7	8.8 ± 1.4	12.6 ± 1.3

Data are presented as percentage of maximal cutaneous vascular conductance. Values are mean ± SEM.

* $P < 0.05$ vs. pre-exercise;

† $P < 0.05$ vs. control