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Title: The effect of α_1 -adrenergic blockade on post-exercise brachial artery flow-mediated dilatation at sea-level and high-altitude

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Running head: Post-exercise endothelial function at sea-level and high-altitude

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Key Points Summary

- Our objective was to quantify endothelial function (via brachial artery flow-mediated dilatation) at sea-level (344m) and high-altitude (3800m) at rest and following both maximal exercise and 30-minutes of moderate-intensity cycling exercise with and without administration of an α_1 -adrenergic blockade.
- Brachial endothelial function did not differ between sea-level and high-altitude at rest, nor following maximal exercise.
- At sea-level, endothelial function decreased following 30-minutes of moderate-intensity exercise, and this decrease was abolished with α_1 -adrenergic blockade. At high-altitude, endothelial function did not decrease immediately post 30-minutes of moderate-intensity exercise, and administration of α_1 -adrenergic blockade resulted in an increase in flow mediated dilatation.
- Our data indicates that post-exercise endothelial function is modified at high-altitude (i.e. prolonged hypoxemia). The current study helps elucidate the physiological mechanisms associated with high-altitude acclimatization, and provides insight into the relationship between sympathetic nervous activity and vascular endothelial function.

Abstract

We examined the hypotheses that 1) at rest, endothelial function would be impaired at high-altitude compared to sea-level, 2) endothelial function would be reduced to a greater extent at sea-level compared to high-altitude after maximal exercise, and 3) reductions in endothelial function following moderate-intensity exercise at both sea-level and high-altitude are mediated via an α_1 -adrenergic pathway. In a double-blinded, counter-balanced, randomized and placebo-controlled design, nine healthy participants performed a maximal-exercise test, and two 30-minute sessions of semi-recumbent cycling exercise at 50% peak Watt following either placebo or α_1 -adrenergic blockade (prazosin; 0.05mg/kg). These experiments were completed at both sea-level (344m) and high-altitude (3800m). Blood pressure (finger photoplethysmography), heart rate (electrocardiogram), oxygen saturation (pulse oximetry), and brachial artery blood flow and shear rate (ultrasound) were recorded prior to, during, and following exercise. Endothelial function assessed by brachial artery flow-mediated dilatation (FMD) was measured prior to, immediately following, and 60-minutes post-exercise. Our findings were: 1) at rest, FMD remained unchanged between sea-level and high-altitude (placebo $P=0.287$; prazosin: $P=0.110$); 2) FMD remained unchanged after maximal exercise at sea-level and high-altitude ($P=0.244$); 3) the $2.9\pm 0.8\%$ ($P=0.043$) reduction in FMD immediately after moderate-intensity exercise at sea-level was abolished via α_1 -adrenergic blockade. Conversely, at high-altitude, FMD was unaltered following moderate-intensity exercise, and administration of α_1 -adrenergic blockade elevated FMD ($P=0.032$). Our results suggest endothelial function is differentially affected by exercise when exposed to hypobaric hypoxia. These findings have implications for understanding the chronic impacts of hypoxemia on exercise, and the interactions between the α_1 -adrenergic pathway and endothelial function.

Abbreviations:

CO, cardiac output

FMD, flow-mediated dilatation

HR, heart rate

MAP, mean arterial pressure

SNS, sympathetic nervous system

SpO₂, peripheral oxyhemoglobin saturation

SRAUC, shear rate area under the curve

SV, stroke volume

TPR, total peripheral resistance

Introduction

Flow-mediated dilatation (FMD) is a commonly used, non-invasive measurement of conduit artery diameter in response to an imposed change in shear rate; it is widely accepted to be an index of endothelial function (Thijssen *et al.*, 2011), and is largely nitric oxide mediated (Green *et al.*, 2014). It has been demonstrated that immediately after cycling exercise, endothelial function measured by brachial (i.e. non-exercising limb) FMD is transiently reduced (Jones *et al.*, 2010, Birk *et al.*, 2013, Atkinson *et al.*, 2015, Dawson *et al.*, 2013), and the reduction in FMD is inversely related to exercise intensity (Birk *et al.*, 2013). Although the precise mechanisms responsible for this acute effect of exercise on FMD remain unclear, several possibilities exist, including: 1) elevated oxidative stress (Goel *et al.*, 2007a, Silvestro *et al.*, 2002); 2) altered hemodynamics (i.e. shear rate, shear pattern, and blood pressure) (Johnson *et al.*, 2012a, Dawson *et al.*, 2008, Birk *et al.*, 2013, Lamping and Dole, 1987, Millgard and Lind, 1998); 3) changes in baseline artery diameter post-exercise (Padilla *et al.*, 2007, Atkinson *et al.*, 2013), and; 4) elevations in sympathetic nervous system (SNS) activity (Hijmering *et al.*, 2002, Dyson *et al.*, 2006, Atkinson *et al.*, 2015). The role of the SNS was recently examined in a study which observed that the exercise-mediated reduction in FMD following exercise was abolished after administration of an α_1 -adrenergic receptor blockade (Atkinson *et al.*, 2015). Interestingly, the relationship between increased SNS activity and post-exercise FMD has not previously been explored under conditions where resting SNS activity is chronically elevated, such as in aging, pathology (e.g. obstructive sleep apnea, heart failure), or in hypoxia (i.e. normobaric and hypobaric) (Saito *et al.*, 1988, Duplain *et al.*, 1999, Xie *et al.*, 2001, Hansen and Sander, 2003).

Exposure to hypoxia is associated with arterial stiffening, but the effects of hypoxia on endothelial function remain unclear (Lewis *et al.*, 2014a, Boos *et al.*, 2012, Rhodes *et al.*, 2011).

For example, different studies have reported that exposure to simulated high-altitude (i.e. acute normobaric hypoxia), results in no change in FMD [$\sim 4000\text{m}$, $F_{\text{I}}\text{O}_2 = 0.13$; (Iglesias *et al.*, 2015)], and decreased FMD [$\sim 5000\text{m}$, $F_{\text{I}}\text{O}_2 = 0.11$ (Lewis *et al.*, 2014a)]. Upon ascent to high-altitude between 3700m-5050m (i.e. hypobaric hypoxia), a decrease in FMD has been observed in most (Bakker *et al.*, 2015, Lewis *et al.*, 2014a), but not all studies (Bruno *et al.*, 2015, Bruno *et al.*, 2016). The disparity within this literature might be due to different methodological approaches between investigations. For example, the two studies that reported reductions in FMD involved ascent to high-altitude over 7-10 days of trekking for several hours each day (Lewis *et al.*, 2014a, Bakker *et al.*, 2015). In contrast, the study that reported no change in FMD involved participants ascending rapidly to high-altitude via cable car (Bruno *et al.*, 2015). The methodological difference between these studies raises the possibility of a moderating impact of trekking exercise at altitude, and total acclimatization time [e.g. 3-10 days of exposure (FMD reduced) vs. 1 day of exposure (no change in FMD)] on vascular responses at high-altitude.

By employing a double-blinded, counter-balanced, randomized and placebo-controlled design, the primary purposes of the current study were to investigate: 1) the effects of non-trekking (i.e., passive) ascent to high-altitude on endothelial function (via brachial FMD) and shear patterns, and 2) the effects of post-exercise related increases in SNS activity on FMD and shear patterns at both sea-level and at high-altitude (3800m). We hypothesized that: 1) at rest, FMD would be impaired at high-altitude compared to sea-level, 2) FMD would be reduced to a greater extent at sea-level compared to high-altitude after maximal exercise, and 3) reductions in endothelial function following moderate-intensity exercise at both sea-level and high-altitude are mediated via an α_1 -adrenergic pathway.

Methods and Materials

Ethical Approval. All experimental procedures and protocols were approved by the clinical research ethics board at the University of British Columbia and conformed to the Declaration of Helsinki. All participants provided written informed consent prior to participation in this study. This study was part of a larger research expedition conducted in October 2015. As such, participants took part in a number of studies conducted at the University of British Columbia (Kelowna, BC; 344m) and during two weeks at the Barcroft high-altitude research station (White Mountain, California, USA; 3800m). However, the *a priori*, primary research questions addressed in the current paper are novel and are exclusively dealt within this study alone – there is no overlap between this investigation and others completed on the research expedition.

Participants. Recruited participants (n=11; 3F) were normotensive (systolic blood pressure <140 and diastolic pressure <90 mmHg) at rest, and completed a medical history questionnaire. Two of the recruited participants were excluded from all mean data analysis; one participant due to illness (i.e. syncope, light-headedness, nausea) caused by our drug intervention (prazosin), and one participant due to illness at high-altitude. The participants (n=9; 2F) included in the data analysis were non-smokers, had no previous history of cardiovascular, cerebrovascular, or respiratory diseases, were not taking any medications during testing besides oral contraceptives (n=1). All participants arrived at the Barcroft high-altitude research facility (elevation = 3800m) on the same day, and approximately at the same time (i.e. within one hour). Nine of these participants drove to the Barcroft high-altitude research facility after staying overnight in Palm Springs, CA, USA (elevation = 146m), while one participant stayed overnight in Bishop, CA, USA (elevation = 1265m). Maximal exercise testing for this study occurred on day three, while moderate-intensity exercise experimentation occurred between days four to seven at high-altitude.

Experimental Design.

This study was conducted in two parts: sea-level and high-altitude investigations. Prior to each experiment, all participants abstained from exercise, alcohol, and caffeine for at least 12 hours. Additionally, participants were asked to consume a light meal at least two-hours prior to experimentation, and to keep their diet consistent between experimentation days. In order to determine whether our participants had normal healthy lung function, at sea-level we conducted a forced vital capacity (FVC) test to measure lung function, a vital capacity and inspiratory capacity maneuver to measure lung volumes, and a single breath carbon monoxide test to quantify diffusing capacity on each individual. All testing procedures were conducted in accordance with the American Thoracic Society and European Respiratory Society's joint guidelines (Macintyre *et al.*, 2005, Miller *et al.*, 2005). For each of these tests, participants sat within a body plethysmography box (V6200, Vmax SensorMedics, Yorba Linda, CA, USA) with a rigid upright posture and their feet flat on the ground, whilst breathing through a spirometer and bacteriological filter while wearing a nose-clip. All pulmonary function measurements were compared against population-based predictions.

Exercise testing was then conducted on three separate lab visits at both sea-level and high-altitude while participants lay in the semi recumbent position. The time of day of the testing sessions were kept the same for each participant, with a minimum of 24-hours between testing sessions.

Maximal exercise protocols. Prior to the prolonged moderate-intensity exercise testing at sea-level and high-altitude, participants were required to conduct a maximal exercise protocol in order to

obtain peak Watt. In the semi-recumbent position, participants rested during a quiet baseline period on the cycle ergometer (Lode Ergometer, Lode, Groningen, Netherlands) for 15-minutes. Immediately after the baseline period a brachial artery FMD was performed (Thijssen *et al.*, 2011). Exercise began with a two-minute warm-up period (40 watts for females, 60 watts for males), followed by staged exercise with workload increased by 20 watts every minute. This maximal exercise protocol was terminated when participants either 1) reached volitional exhaustion, or 2) cycling cadence could no longer be maintained. Immediately after the maximal exercise protocol was completed, another brachial artery FMD was performed.

Moderate-intensity exercise protocol. Following at least 24 hours after the maximal exercise test, participants performed two moderate-intensity exercise protocols on two independent days separated by at least 24 hours. Upon arrival on each of these testing sessions, participants ingested a capsule containing either oral prazosin (α_1 -adrenergic receptor blocker; 0.05 mg kg⁻¹), or Placebo (i.e. sugar pill). The order of condition (placebo and prazosin) was counter-balanced and randomized at both sea-level and high-altitude. The dosage of prazosin used has been demonstrated to provide ~80% α_1 -adrenergic blockade, and has been used in other studies by our research group (Lewis *et al.*, 2014b, Atkinson *et al.*, 2015). Seventy-five-minutes after ingestion, participants were instrumented on the semi-recumbent cycle ergometer (*see details below in section Experimental Measurements*). Participants were asked to sit in the semi-recumbent position quietly for 15-minutes, and afterwards, a baseline FMD on the left brachial artery was conducted. Immediately after the baseline FMD, participants completed 30-minutes of cycle exercise at 50% of their peak exercise workload. At sea-level, this workload was 137.0 ± 6.9 Watts, while at high-altitude the average workload was 114.3 ± 5.9 Watts. Every five-minutes during

exercise a one-minute measurement of brachial artery shear rate and blood flow was recorded (total of six recordings during the 30 minutes of exercise). After completion of the 30-minutes of moderate-intensity exercise, a brachial FMD was conducted immediately, and then following 60-minutes of post-exercise rest.

Experimental Measurements.

Cardiovascular measurements. All continuously recorded cardiovascular measurements were acquired at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments, Colorado Springs, CO, USA) interfaced with a personal computer. Commercially available software was used to analyze cardiovascular variables (LabChart V7.1, ADInstruments, Colorado Springs, CO, USA). Electrocardiogram electrodes were placed in lead II configuration (Bioamp, ML132, ADInstruments, Colorado Springs, CO, USA) to measure heart rate. Beat-by-beat arterial pressure, cardiac output, stroke volume, and total peripheral resistance was measured by finger photoplethysmography (Finometer Pro, Finapres medical systems, Amsterdam, Netherlands). Prior to baseline data collection, the Finometer was calibrated using the return-to-flow function, and blood pressure from the Finometer was confirmed with automated brachial blood pressure readings (HEM-775CAN, Omron Healthcare, Bannockburn, IL, USA). Mean, systolic, and diastolic arterial pressure were quantified from the raw Finometer recordings.

Brachial artery imaging. With the participants left arm extended perpendicular (i.e. 80 degrees) from their body while seated on the semi-recumbent cycle ergometer, an inflation/deflation cuff was placed on the participants left forearm, and their arm was fixed into position on a table. Brachial artery image acquisition was obtained using a 10 MHz multifrequency linear array probe

attached to a high-resolution ultrasound machine (15L4, Terason t3200, Burlington, MA, USA). All brachial artery images were performed by the same experienced ultrasonographer (JT), whom has a between day coefficient of variation in FMD of $8.3 \pm 2.1\%$ ($n=10$, *unpublished data*). Following optimal image acquisition, and one-minute of baseline recordings, the forearm was occluded by inflating the cuff to 220-250 mmHg for five-minutes. Recordings of diameter and velocity continued 30-seconds prior to cuff deflation and continuously for three-minutes thereafter (Thijssen *et al.*, 2011).

Data Analysis

Brachial artery diameter and blood flow analysis. Ultrasound recordings were continuously screen captured and saved for offline analysis. Blood flow analysis of the brachial artery was performed using automated edge-detection and wall tracking software, which allows for the integration of synchronous diameter and velocity measurements to continuously determine flow, shear, diameter and velocity at 30-Hz, independent of investigator bias (Woodman *et al.*, 2001). Antegrade, retrograde, and mean shear rates were calculated as four times the mean blood velocity, divided by vessel diameter. The FMD was calculated as the percent increase in vessel diameter from resting baseline diameter, where baseline and peak diameters were automatically detected from the continuous data described above.

Statistics

All statistical analyses were performed using SigmaStat V11 (Systat, Chicago, IL, USA), and were reported as mean \pm SEM. Statistical significance was assumed at $P<0.05$. When significant F-ratios were detected, post-hoc comparisons were made using Tukey's post hoc test for pair-wise

comparisons. Our study is an extension of a previous study from our laboratory (Atkinson *et al.*, 2013), which found a ~2% decrease in FMD post 30-minutes of exercise. To detect a 2% decrease in post-exercise FMD with a standard deviation of 2%, we calculated that a sample size of 10 participants was required in order to achieve a statistical power >0.80 with a statistical difference of $P < 0.05$. Based on this power calculation, we recruited 11 participants for the sea-level and high-altitude arms of the study.

Maximal exercise. For the cardiovascular data obtained at sea-level and high-altitude, baseline measurements were averaged over one-minute immediately prior to exercise, peak maximal-exercise data were averaged over the last 30-seconds of the maximal exercise protocol, and post-maximal exercise data were averaged over one-minute, immediately prior to FMD cuff release after maximal exercise was terminated (i.e. approximately four-minutes post-exercise). Differences between peak Watt and maximal exercise time at sea-level and high-altitude were determined using a paired Student's t-test. For the cardiovascular data, a two-way repeated measures analysis of variance (2RM-ANOVA) was used to detect differences across time (baseline, maximal exercise, post-maximal exercise), and conditions (sea-level and high-altitude). For the brachial artery data measured during FMD, a 2RM-ANOVA was used to detect differences across time (baseline and post-maximal exercise), and conditions (sea-level and high-altitude).

Moderate-intensity exercise. For the cardiovascular data obtained at sea-level and high-altitude, baseline measurements were averaged over one-minute immediately prior to exercise. During moderate-intensity exercise, cardiovascular data were averaged over 30-seconds at every five-minute time-point (i.e. time = 5-, 10-, 15-, 20-, 25-, and 30-minutes). Post-exercise cardiovascular

data were averaged during the one-minute prior to FMD cuff release, immediately after, and 60-minutes after, moderate-intensity exercise. For the cardiovascular data, a 2RM-ANOVA was used to detect differences across time (baseline, exercise [six time-points], and post-exercise [two time-points]), and conditions (placebo and prazosin), at sea-level and high-altitude, separately. For the brachial artery FMD data, a 2RM-ANOVA was used to detect differences across time (baseline, post-exercise, 60-minutes post exercise), and conditions (placebo and prazosin), at sea-level and high-altitude, separately.

Adjusted flow-mediated dilatation. The effects of time and condition were analyzed within and between sea-level and high-altitude for FMD. To determine if our FMD results were different due to changes in baseline arterial diameter and/or shear rate area under the curve (SRAUC), we included these variables as covariates in a logarithmic-linked generalized linear model, where FMD was the dependent variable. This approach has been used to account for any changes in FMD that may be related to differences in baseline diameter or shear rate between conditions (i.e. time and condition) (Atkinson *et al.*, 2013).

Results

Participants and resting FMD data

The nine participants included in the sea-level and high-altitude protocol data analysis had a mean \pm SEM age of 26.9 ± 1.8 years, height of 176.3 ± 1.5 cm, and weight of 71.1 ± 2.5 kg. Participants had normal pulmonary health with an FVC of 5.5 ± 0.2 L ($109.0 \pm 3.2\%$ of predicted), forced expiratory volume in one-second (FEV_1) of 4.3 ± 0.2 L ($100.6 \pm 2.8\%$ of predicted), FEV_1/FVC of 78.7 ± 1.0 (no individuals reported under an $FEV_1/FVC < 75$), total lung capacity of 6.7 ± 0.3 L ($101.9 \pm 0.3\%$ of predicted), and had a diffusing capacity of the lung for carbon monoxide of 32.1 ± 5.7 ml/min/mmHg ($94.1 \pm 4.7\%$ of predicted). Recruited participants did not demonstrate any signs of small nor large airway obstruction characterized by an irregular expiratory flow tracing during the FVC maneuver. Additionally, participants were normotensive (systolic blood pressure = 130.6 ± 2.5 mmHg, diastolic blood pressure 68.7 ± 1.9 mmHg).

No differences were observed in FMD between sea-level and high-altitude during the placebo ($P=0.287$) and prazosin ($P=0.110$) trials at baseline (see figure 1).

Maximal exercise data

Figure 2 illustrates the cardiorespiratory data collected during the maximal exercise protocol at sea-level and high-altitude. At sea-level, peak Watt and maximal exercise protocol time (min) were greater than at high-altitude (275.4 ± 12.4 watts vs. 228.8 ± 11.2 watts, $P < 0.001$; 13.0 ± 0.5 min vs. 10.6 ± 0.5 min, $P < 0.001$). Cardiac output and stroke volume were higher at sea-level compared to high-altitude ($P=0.047$ and $P=0.032$, respectively). In contrast, heart rate at high-altitude was elevated compared to sea-level ($P=0.038$). No differences were observed between sea-level and high-altitude for mean arterial pressure or total peripheral resistance at baseline, peak exercise, and

post-exercise ($P=0.130$ and $P=0.055$, respectively). As expected, a main effect was observed for SpO_2 , as it was elevated at sea-level compared to high-altitude ($P<0.001$).

Table 1 illustrates brachial diameter, shear, and FMD data during baseline and post-maximal exercise at sea-level and high-altitude. Brachial artery diameter was greater at sea-level compared to high-altitude across all time-points ($P=0.030$). At both sea-level and high-altitude, brachial artery diameter was reduced post-maximal exercise compared to baseline by $4.8 \pm 2.5\%$ and $8.7 \pm 1.2\%$, respectively ($P=0.004$). Mean shear rate increased post-maximal exercise compared to baseline at sea-level by $211.2 \pm 26.5 \text{ l s}^{-1}$, and at high-altitude by $101.7 \pm 20.9 \text{ l s}^{-1}$ ($P<0.001$). Although a main effect was not present for mean shear rate between sea-level and high-altitude ($P=0.244$), an interaction effect was observed post-maximal exercise as mean shear was higher at sea-level by $36.1 \pm 19.0\%$ compared to high-altitude ($P=0.003$). Antegrade shear rate increased after post-maximal exercise compared to baseline at sea-level by $236.2 \pm 20.2 \text{ l s}^{-1}$, and at high-altitude by $163.8 \pm 20.3 \text{ l s}^{-1}$, respectively (both $P<0.001$); however, similar to mean shear rate, altitude had no effect ($P=0.833$). Retrograde shear rate increased (i.e. became more negative) between baseline and post-maximal exercise at sea-level by $-25.3 \pm 7.8 \text{ l s}^{-1}$ and at high-altitude by $67.4 \pm 11.7 \text{ l s}^{-1}$ ($P<0.001$). Retrograde shear was also greater (i.e. more negative) at high-altitude compared to sea-level ($P=0.031$).

No differences were found in absolute (mm) or relative changes (FMD) in brachial artery diameter between baseline and post-maximal exercise at sea-level or high-altitude ($P=0.453$ and $P=0.282$, respectively), or between sea-level and high-altitude ($P=0.380$ and $P=0.244$, respectively). Flow-mediated dilatation SRAUC was greater post-maximal exercise compared to baseline ($P=0.025$), but there was no difference between sea-level and high-altitude ($P=0.312$). When taking into account baseline diameter and SRAUC as covariates, the FMD results remained

the same as there was no effect of time ($P=0.614$), altitude ($P=0.291$), nor was there an interaction between these effects ($P=0.717$) (refer to Figure 3 for individual FMD data at baseline and post-maximal exercise).

Moderate intensity exercise at sea-level

Figure 4 (A-F) illustrates the cardiovascular data collected during moderate intensity exercise on placebo and prazosin at sea-level. No differences were detected for cardiac output between placebo and prazosin during baseline, exercise, and post-exercise ($P=0.444$). In contrast, prazosin increased heart rate ($P<0.001$), and decreased stroke volume ($P=0.026$), across all time-points compared to placebo. prazosin had no effect on mean arterial pressure ($P=0.701$), nor total peripheral resistance ($P=0.492$) during baseline, exercise, and post-exercise time-points. Additionally, there was no difference in SpO_2 between placebo and prazosin trials ($P=0.237$).

Figure 5 (A-C) highlights the brachial antegrade, retrograde, and mean shear rate data collected during baseline, exercise, and post-exercise on placebo and prazosin at sea-level. Mean and antegrade shear rate were not different between conditions ($P=0.567$ and $P=0.156$, respectively). Retrograde shear rate was lower (i.e. more negative) during the prazosin trial compared to placebo ($P=0.037$).

Figure 6 (A-D) illustrates brachial artery velocity, diameter, blood flow and conductance data collected during baseline, exercise, and post-exercise on placebo and prazosin at sea-level. No differences were found in mean blood flow ($P=0.285$), forearm vascular conductance ($P=0.294$), artery diameter ($P=0.623$), nor blood velocity ($P=0.400$) between the placebo and prazosin trials.

Figure 7 (A-B) displays FMD data collected at baseline, post-exercise, and 60-minutes post exercise on placebo and prazosin at sea-level. No differences in FMD were detected between placebo and prazosin ($P=0.916$), however, there was a time effect during the placebo trial, where FMD was reduced immediately post exercise compared to baseline ($P=0.043$), and 60-minutes post-exercise ($P<0.001$). Additionally, an interaction effect was present between placebo and prazosin immediately post-exercise, where FMD was greater during the prazosin trial by $2.9 \pm 1.3\%$ compared to the placebo trial ($P=0.039$). No differences were found in FMD between placebo and prazosin trials during baseline ($P=0.762$), nor 60-minutes post-exercise ($P=0.107$). When taking into account baseline diameter and SRAUC as covariates, the FMD results remained the same as there was still a main effect for time ($P=0.016$), no effect between conditions (i.e. placebo vs prazosin) ($P=0.450$), and an interaction effect ($P=0.026$).

Moderate intensity exercise at high-altitude

Figure 4 (G-L) outlines the cardiovascular data collected during baseline, moderate intensity exercise, and post-exercise on placebo and prazosin at high-altitude. No differences were detected for cardiac output between placebo and prazosin during baseline, exercise, and post-exercise ($P=0.825$). In contrast, heart rate was elevated ($P<0.001$), and stroke volume was decreased ($P=0.006$), while on prazosin compared to placebo. prazosin resulted in a lower mean arterial pressure ($P<0.001$), and total peripheral resistance ($P<0.001$) compared to the placebo trial. Interestingly, SpO_2 was elevated by $2.5 \pm 0.7\%$ ($P=0.005$) during the prazosin trial compared to placebo during the moderate-intensity exercise.

Figure 5 (D-F) illustrates brachial shear rate data collected during baseline, moderate-intensity exercise, and post-exercise on placebo and prazosin at high-altitude. Mean and antegrade

shear rates were elevated during the prazosin trial compared to the placebo trial across all time-points ($P=0.002$ and $P<0.001$, respectively). However, retrograde shear rate was not different between placebo and prazosin ($P=0.983$).

Figure 6 (E-H) highlights the brachial artery velocity, diameter, blood flow and conductance data collected during baseline, exercise, and post-exercise on placebo and prazosin at high-altitude. During the prazosin trial, mean blood flow, conductance, and blood velocity were elevated compared to the placebo trial ($P=0.004$, $P=0.008$, and $P=0.002$, respectively). No differences were found to brachial artery diameter between placebo and prazosin trials ($P=0.516$).

Figure 7 (C-D) illustrates FMD data collected at baseline, post-exercise, and 60-minutes post exercise on placebo and prazosin at high-altitude. No differences in FMD were detected between time-points (i.e. baseline, post-exercise, and post-60 exercise) ($P=0.474$), between placebo and prazosin ($P=0.099$). When taking into account baseline diameter and SRAUC as covariates, no differences were detected between time-points ($P=0.681$), nor were any interactions present ($P=0.474$), which was consistent with our original results. However, we found that there was a main effect for condition (i.e. prazosin vs placebo), where FMD was higher with prazosin compared to placebo ($P=0.032$).

Discussion

Using a double-blinded, counter-balanced, randomized and placebo-controlled design, this is the first study to examine the role of the SNS system on post-exercise peripheral vascular endothelial function at both sea-level (344m) and high-altitude (3800m). Our main findings were 1) at rest, brachial artery FMD remained unchanged between sea-level and high-altitude on both placebo and prazosin conditions, 2) FMD remained unchanged after maximal exercise at both sea-level and high-altitude, and 3) FMD decreased immediately after moderate-intensity exercise at sea-level, but not high-altitude. Prazosin abolished the observed post-exercise FMD decrease at sea-level, and resulted in an overall increase in FMD at high-altitude compared to placebo when SRAUC and baseline diameter were considered as covariates. These data demonstrate that hypobaric hypoxia counteracts the effect of moderate intensity exercise on FMD.

Endothelial function between sea-level and high-altitude at rest

Acclimatization to high-altitude results in several physiological changes, but the effect of high-altitude on endothelial function remains unclear. At sea-level, recent work has established that increases in SNS activity (Hijmering *et al.*, 2002, Dyson *et al.*, 2006, Atkinson *et al.*, 2015), and altered hemodynamics (e.g. increased retrograde shear) (Johnson *et al.*, 2012a, Dawson *et al.*, 2008, Birk *et al.*, 2013, Lamping and Dole, 1987, Millgard and Lind, 1998) can negatively affect vascular endothelial function as assessed by FMD. Since both of these physiological changes occur during high-altitude exposure, it is logical to hypothesize that endothelial function would be reduced at high-altitude, yet we found that there was no change. Comparisons of FMD between sea-level and high-altitude have been made previously, but studies have reported contradictory

results such as reduced FMD (Lewis *et al.*, 2014a, Bakker *et al.*, 2015), or no change in FMD upon acclimatization to high-altitude (Bruno *et al.*, 2016, Bruno *et al.*, 2015).

The major difference between these studies is the mode of transport to altitude – exercise versus cable car ascent. We sought to further investigate the effects of non-trekking arrival to high-altitude on endothelial function, and consistent with the results of Bruno *et al.* (2015), we found no change in endothelial function between sea-level and high-altitude at rest. This finding opposes our hypotheses that endothelial function would be impaired at high-altitude due to elevated resting SNS activity. Rather, our data indicate that exercise at high-altitude (i.e. trekking) may directly affect endothelial function, and we speculate the mechanism(s) for this may be due to increased vascular inflammation (Bruno *et al.*, 2016), oxidative stress (Quindry *et al.*, 2015), and reductions in nitric oxide bioavailability (Lewis *et al.*, 2014a).

Endothelial function before and after maximal exercise at sea-level and high-altitude

The degree of observed post-exercise reduction in FMD has been reported to be exercise intensity-dependent (for review see: Birk *et al.*, 2013); however, few studies have measured FMD after maximal exercise in healthy individuals in an inactive limb (i.e, free from exercise) (Thijssen *et al.*, 2006, Hwang *et al.*, 2012, McClean *et al.*, 2015). Moreover, no studies to date have reported FMD after maximal exercise at high-altitude. Based on previous reports, FMD following maximal exercise remains unchanged in the majority of studies (Thijssen *et al.*, 2006, Hwang *et al.*, 2012, McClean *et al.*, 2015) but this is not a universal finding (Hwang *et al.*, 2012). Studies methodologically similar to ours (Thijssen *et al.*, 2006, Hwang *et al.*, 2012, McClean *et al.*, 2015), we demonstrated and further confirm that at sea-level - and for the first time at high-altitude - FMD remained unchanged post-maximal exercise at both sea-level and high-altitude. Collectively, these

findings oppose the idea that post-exercise related decreases in FMD are inversely related to exercise intensity (Birk *et al.*, 2013). A likely explanation for this discrepancy is that the exercise duration of the maximal exercise test was too short in duration (i.e. exercise volume) to induce a reduction in FMD (Johnson *et al.*, 2012b, Dawson *et al.*, 2013). Interestingly, at high-altitude, FMD was elevated in a positive direction post-maximal exercise (6.3% to 8.5%); however, this observation was not statistically significant.

Endothelial function before and after moderate-intensity exercise at sea-level and high-altitude

Sea-level: In a different group of subjects, Atkinson *et al.* (2016) performed a similar investigation to the sea-level component of this study and demonstrated that FMD was transiently impaired immediately after 30-minutes of moderate-intensity exercise; these changes improved back to pre-exercise values 60-minutes post moderate-intensity exercise. Atkinson *et al.* (2016) attributed this reduction in FMD to exercise related increases in SNS activity. Congruent with Atkinson *et al.* (2016) and other studies with similar modern methodology to assess FMD (Goel *et al.*, 2007b, Dawson *et al.*, 2008, Jones *et al.*, 2010, Johnson *et al.*, 2012a, Birk *et al.*, 2013), we found similar results. We acknowledge, however, that such transient reductions in FMD following moderate-intensity exercise (at least at sea level) should not necessarily be interpreted that endothelial dysfunction; rather, that FMD that it is influenced by a myriad of factors that may well reflect physiological adaptation in other vascular beds, including resistance and conduct arteries.

High-altitude: Although there has been a recent investigation of post-exercise endothelial function after moderate-intensity exercise in acute normobaric hypoxia (Katayama *et al.*, 2016), our experiment was the first to investigate endothelial function after moderate-intensity exercise at

high-altitude (i.e. 3800m). In the study by Katayama *et al.* (2016), endothelial function was not different post 30-minutes of moderate-intensity (i.e. 60% VO_2 max) exercise between normoxia and hypoxia ($\text{F}_i\text{O}_2 = 0.12\text{-}0.13$) trials. However, longer exposure to hypoxia (i.e. 4-7 days at high-altitude) may well yield differential results due to cardiovascular adaptation (Lewis *et al.*, 2014a, Boos *et al.*, 2012, Rhodes *et al.*, 2011), and perhaps differential tonic SNS activity (Hansen and Sander, 2003). There is evidence suggesting increased SNS activity may be responsible for vascular dysfunction directly (Hijmering *et al.*, 2002), or indirectly by increasing retrograde shear rate (Thijssen *et al.*, 2014). Due to high-altitude related increases in SNS activity, and based on previous reports that endothelial function (via brachial FMD) is reduced upon arrival at high-altitude in some (Bakker *et al.*, 2015, Lewis *et al.*, 2014a), but not all studies (Bruno *et al.*, 2016), we anticipated a reduction in FMD immediately post moderate-intensity exercise at high-altitude. Our original hypothesis was not supported as no reduction in FMD was observed post moderate-intensity exercise, indicating that SNS related blood vessel regulation is different between sea-level and high-altitude. This latter notion suggests that post-exercise associated elevations in SNS activity may have a differential transduction to the peripheral vasculature compared to sea-level.

Effects of α_1 -adrenergic blockade on endothelial function at sea-level and high-altitude.

α_1 -adrenergic blockade, results in vasodilatation of the peripheral vasculature by reducing SNS transduction directly to smooth muscle cells. At sea-level, prazosin resulted in an increased heart rate and decreased stroke volume during exercise. However, the differential heart rate response between placebo and prazosin trials did not result in a different blood flow or mean and antegrade shear rate response to exercise. Similar to Atkinson *et al.* (2016), we reported that prazosin

administration abolished the reduction in endothelial function immediately post 30-minutes of moderate-intensity exercise.

At rest, SNS blockade has no effect on SpO₂ at sea-level (Liu *et al.*, 2007), nor high-altitude (Ainslie *et al.*, 2012). During exercise, SpO₂ remained the same at sea-level between conditions; however, at high-altitude, an unexpected observation was that participants on prazosin had a higher SpO₂ compared to the placebo trial. This was likely due to increased ventilation during exercise on the prazosin trial, perhaps due to baroreflex-chemoreflex interaction, supported by the increased heart rate, and reduced stroke volume and mean arterial pressure responses to exercise between placebo and prazosin trials. The ventilatory response to changes in arterial blood pressure is related to converging baroreceptor and chemoreceptor afferents at the nucleus tractus solitarius (Richter and Seller, 1975, Eckberg and Orshan, 1977). A reduction in arterial blood pressure potentiates the chemoreflex and results in an increase in ventilation, while an increase in arterial blood pressure dampens ventilation (Heistad *et al.*, 1974).

In contrast to our sea-level data, prazosin resulted in an increased mean and antegrade shear rate at high-altitude. These changes were likely facilitated by blockade of SNS vasoconstriction, which was likely enhanced at altitude. Increases in antegrade shear rate has been demonstrated to have a positive effect on endothelial function (Thijssen *et al.*, 2014). This enhanced antegrade shear response at altitude versus sea-level may explain the lack of post exercise FMD impairment, and could be in part responsible for the increase in endothelial function observed during the prazosin trial at altitude. The increase in FMD observed at high-altitude during the prazosin trial compared to the placebo trial suggests that there is indeed some SNS related vascular constraint. These data collected at high-altitude indicate that SNS activity is, in part, responsible for the FMD response.

Methodological considerations.

The intervention to reduce SNS activity was an α_1 specific adrenergic receptor blockade, and the dose of administration has been used to establish ~80% blockade in SNS activity, but this was not confirmed on an individual basis. An important methodological consideration for the current study is that prazosin- a specific α_1 -adrenergic receptor blockade was used; therefore, it is possible that during prazosin administration there is unopposed upregulation of α_2 -adrenergic and β -adrenergic receptor activity (Weisbrod *et al.*, 2001). These net effects likely result in competing vasoconstriction and vasodilation effects, respectively. Future studies investigating the role of the SNS on endothelial function should consider using both non-selective α - and β -adrenergic receptor blockades, along with a combined α - and β -adrenergic receptor blockade. Second, we can not exclude the possibility that the vascular response to changes in vasodilatory substances is also altered (Calbet *et al.*, 2014), and could hence influence our findings. Additionally, we achieved adequate power (>0.80) in nearly all of our measurements at sea-level and high-altitude. Although our small sample size was smaller than our calculated sample size ($n=9$ vs $n=10$), our sea-level data replicates previous findings by Atkinson *et al.* (2015), and we achieved a statistical effect. Moreover, additional novelty and statistical power of our study is within the nature of the double-blinded, counter-balanced, randomized and placebo-controlled design.

A final consideration is that we did not directly measure SNS activity directly via microneurography. Obviously, obtaining muscle SNS measurements in the leg (via peroneal nerve) would not be possible during exercise; however, past studies have reported in humans in the arm (via radial nerve) (Rea and Wallin, 1989). This approach would not have been feasible given our small sample size and the amount of measurements needed in each participant (i.e. twice

at both sea-level and high-altitude). Nevertheless, both at sea-level and high altitude, we have for the first time employed a powerful double-blinded, counter-balanced, randomized and placebo-controlled study to examine the role of SNS blockade both at rest and during exercise.

Conclusion.

Our findings illustrate that at sea-level, exercise related increases in SNS activity reduce FMD immediately following moderate-intensity exercise, but not at high-altitude. These findings indicate that differential governing mechanisms for endothelial function between sea-level and high-altitude. Together, our findings have implications for better understanding the chronic impacts of hypoxemia and exercise, and the interactions on sympathetic activity and vascular function.

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Table 1: Brachial artery diameter, shear, and flow-mediated dilatation data during baseline and post-maximal exercise at sea-level and high-altitude

	Sea-Level		High-Altitude	
	Baseline	Post-max	Baseline	Post-max
Diameter (mm)	4.4 ± 0.2	4.2 ± 0.2*	4.3 ± 0.2	3.9 ± 0.2*
	<i>Time: P=0.004, Altitude: P=0.030, Interaction: P=0.328</i>			
Mean Shear (1 s⁻¹)	86.4 ± 13.5	297.6 ± 26.2* [⊥]	111.8 ± 20.1	213.5 ± 27.7*
	<i>Time: P<0.001, Altitude: P=0.244, Interaction: P=0.003</i>			
Antegrade Shear (1 s⁻¹)	101.7 ± 13.7	337.9 ± 20.5* [⊥]	144.5 ± 17.3	308.3 ± 24.3* [⊥]
	<i>Time: P<0.001, Altitude: P=0.833, Interaction: P=0.012</i>			
Retrograde Shear (1 s⁻¹)	-15.2 ± 6.2	-40.5 ± 8.6*	-22.1 ± 7.1	-89.6 ± 14.9*
	<i>Time: P=0.001, Altitude: P=0.031, Interaction: P=0.009</i>			
Change in diameter (mm)	0.23 ± 0.05	0.25 ± 0.05	0.26 ± 0.04	0.32 ± 0.05
	<i>Time: P=0.453, Altitude: P=0.380, Interaction: P=0.581</i>			
FMD (%)	5.0 ± 1.1	6.2 ± 1.2	6.3 ± 1.3	8.5 ± 1.6
	<i>Time: P=0.282, Altitude: P=0.244, Interaction: P=0.532</i>			
FMD SRAUC (10³ s⁻¹)	23.3 ± 1.9	30.3 ± 2.8*	23.2 ± 2.7	22.6 ± 1.9*
	<i>Time: P=0.025, Altitude: P=0.312, Interaction: P=0.185</i>			

Definition of abbreviations: FMD, flow mediated dilatation; SRAUC, shear rate area under the curve. *P<0.05, pre-max data vs post-max data. [⊥]P<0.05, interaction effect baseline vs post-max

Table 2: Brachial artery diameter and flow-mediated dilatation data during baseline and post moderate-intensity exercise at sea-level and high-altitude

		Sea-Level			High-Altitude		
		Pre	Post	Post-60	Pre	Post	Post-60
Diameter (mm)	<i>Placebo</i>	4.5 ± 0.2	4.8 ± 0.2*	4.4 ± 0.2*	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.2
	<i>Prazosin</i>	4.5 ± 0.2	4.6 ± 0.2	4.6 ± 0.2	4.2 ± 0.2	4.3 ± 0.1	4.2 ± 0.2
		<i>Time: P=0.015, Condition: P=0.783, Interaction: P=0.008</i>			<i>Time: P=0.496, Condition: P=0.398, Interaction: P=0.501</i>		
Change in diameter (mm)	<i>Placebo</i>	0.21 ± 0.03	0.12 ± 0.02	0.30 ± 0.05†	0.26 ± 0.03	0.22 ± 0.04	0.25 ± 0.03
	<i>Prazosin</i>	0.20 ± 0.04	0.22 ± 0.05	0.22 ± 0.03†	0.29 ± 0.03	0.31 ± 0.05	0.35 ± 0.05
		<i>Time: P=0.022, Condition: P=0.954, Interaction: P=0.074</i>			<i>Time: P=0.502, Condition: P=0.093, Interaction: P=0.501</i>		
FMD (%)	<i>Placebo</i>	4.9 ± 0.7	2.1 ± 0.8*	7.0 ± 1.2†	6.3 ± 0.8	5.3 ± 1.0	5.8 ± 0.6
	<i>Prazosin</i>	4.5 ± 1.0	4.9 ± 1.3⊥	4.0 ± 0.9	6.8 ± 0.8	7.3 ± 1.2	8.5 ± 1.2
		<i>Time: P=0.013, Condition: P=0.916, Interaction: P=0.033</i>			<i>Time: P=0.474, Condition: P=0.099, Interaction: P=0.455</i>		
FMD SRAUC (10 ³ s ⁻¹)	<i>Placebo</i>	19.8 ± 2.4	37.9 ± 4.5*†	18.3 ± 2.7	26.9 ± 2.8	40.2 ± 5.3*†	25.5 ± 1.7
	<i>Prazosin</i>	27.4 ± 2.1	39.8 ± 4.7*†	23.7 ± 2.5	30.5 ± 2.5	50.2 ± 3.0*†	37.6 ± 3.2
		<i>Time: P<0.001, Condition: P=0.060, Interaction: P=0.448</i>			<i>Time: P<0.001, Condition: P=0.005, Interaction: P=0.287</i>		

Definition of abbreviations: FMD, flow mediated dilatation; SRAUC, shear rate area under the curve. *P<0.05, vs pre-exercise data. †P<0.05, Post-60 vs Post. ⊥P<0.05, Interaction within Post FMD. **Bolded Condition: P=0.099**, when accounted for baseline diameter and shear rate, P=0.032.

Figure Legends

Figure 1. FMD data collected during baseline on placebo and prazosin at sea-level and high-altitude. White bars represent sea-level data \pm SEM, and black bars represent high-altitude data \pm SEM in 9 participants. *Definitions of abbreviations:* FMD, flow-mediated dilatation. These findings illustrate that there were no differences in resting FMD between sea-level and high-altitude after passive ascent to 3800m.

Figure 2. Cardiovascular data during baseline, maximal exercise, and post-maximal exercise at sea-level and high-altitude. Open circles (o) represent sea-level data \pm SEM, and closed circles (●) high-altitude data \pm SEM in 9 participants. * $P < 0.05$, for interaction effects. Statistics for main effects and interactions are displayed on the top right of each figure panel. *Definitions of abbreviations:* BL, baseline; Max-Ex, maximal exercise; Post-Max, post-maximal exercise; SpO₂, percent oxygen saturation of hemoglobin; SV, stroke volume, HR, heart rate; CO, cardiac output; MAP, mean arterial pressure; TPR, total peripheral resistance. Collectively, these findings reveal that SV, HR, CO, and SpO₂ were all elevated at sea-level compared to high-altitude during a maximal exercise test.

Figure 3. Individual FMD data collected at baseline and post-maximal exercise at sea-level and high-altitude. Mean data (n=9) is represented by the gray line plot. *Definitions of abbreviations:* BL, baseline; Post-Max, post-maximal exercise; FMD, flow-mediated dilatation. These data reveal that we found no change in FMD post maximal exercise at sea-level and high-altitude.

Figure 4. Cardiovascular data during baseline, moderate-intensity exercise, and post-exercise on placebo and prazosin at sea-level and high-altitude. Open circles (o) represent placebo data \pm SEM, and closed circles (●) represent prazosin data \pm SEM in 9 participants. *P<0.05, for interaction effects. Statistics for main effects and interactions are displayed on the top right of each figure panel. *Definitions of abbreviations:* BL, baseline; Post, immediately post-exercise; Post 60, 60-minutes post-exercise; SpO₂, percent oxygen saturation of hemoglobin; SV, stroke volume, HR, heart rate; CO, cardiac output; MAP, mean arterial pressure; TPR, total peripheral resistance. These findings demonstrate that at sea-level, SV was elevated, while HR was reduced on placebo compared to prazosin during 30-minutes of moderate-intensity exercise. At high-altitude, SPO₂ and HR were lower, while MAP and HR were higher on placebo compared to prazosin during 30-minutes of moderate-intensity exercise.

Figure 5. Brachial artery shear rate data during baseline, moderate-intensity exercise, and post-exercise on placebo and prazosin at sea-level and high-altitude. Open circles (o) represent placebo data \pm SEM, and closed circles (●) represent prazosin data \pm SEM in 9 participants. *P<0.05, for interaction effects. Statistics for main effects and interactions are displayed on the top right of each figure panel. *Definitions of abbreviations:* BL, baseline; Post, immediately post-exercise; Post 60, 60-minutes post-exercise. These data reveal that retrograde shear rate was greater after prazosin administration at sea-level, while at high-altitude, mean and antegrade shear rate were greater after prazosin administration compared to placebo during 30-minutes of moderate-intensity exercise.

Figure 6. Brachial artery velocity, diameter, blood flow and conductance data collected during baseline, moderate-intensity exercise, and post-exercise on placebo and prazosin at sea-level and high-altitude. Open circles (o) represent placebo data \pm SEM, and closed circles (●) represent prazosin data \pm SEM in 9 participants. * $P < 0.05$, for interaction effects. Statistics for main effects and interactions are displayed on the top right of each figure panel. *Definitions of abbreviations:* BL, baseline; Post, immediately post-exercise; Post 60, 60-minutes post-exercise. These findings illustrate that there were no differences found in brachial artery velocity, diameter, blood flow and conductance at sea-level between placebo and prazosin trials, however, at high-altitude, we found brachial artery velocity, blood flow and conductance were reduced while participants were on placebo compared to prazosin during the 30-minute moderate-intensity exercise test.

Figure 7. Individual FMD data collected during baseline and post-exercise on placebo and prazosin at sea-level and high-altitude. Mean data (n=9) is represented by the gray line plot. * $P < 0.05$, represents time effect, where FMD Post-EX was lower compared to baseline, and Post-60 (for more details, see results section). *Definitions of abbreviations:* Post, immediately post-exercise; Post 60, 60-minutes post-exercise; FMD, flow-mediated dilatation. Collectively, these findings reveal that at sea-level, FMD was reduced immediately after 30-minutes of moderate-intensity exercise while on placebo, and the reduction in FMD was abolished after administration of prazosin. At high-altitude, there was no observed reduction in FMD immediately after 30-minutes of moderate-intensity exercise after administration of placebo or prazosin. Additionally, after taking into account for changes in brachial baseline diameter and shear rate between placebo

and prazosin trials, FMD was elevated after administration of prazosin compared to placebo at high-altitude.