UBC-Nepal Expedition

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Journal of Applied Physiology

DOI:
10.1152/japplphysiol.00583.2017

Published: 22/11/2017

Peer reviewed version

Submitted to: Journal of Applied Physiology

Title: UBC-Nepal Expedition: Acute alterations in sympathetic nervous activity do not influence brachial artery endothelial function at sea-level and high-altitude.

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Running head: Endothelial function during lower-body differential pressure

Keywords: Sympathetic nervous activity, lower-body negative pressure, lower-body positive pressure, endothelial function, high-altitude

Abstract

Evidence indicates that increases in sympathetic nervous activity (SNA), and acclimatization to high-altitude (HA), may reduce endothelial function as assessed by brachial artery flow-mediated dilatation (FMD); however, it is unclear whether such changes in FMD are due to direct vascular constraint, or consequential altered hemodynamics (e.g. shear stress) associated with increased SNA as a consequence of exposure to HA. We hypothesized that: 1) at rest, SNA would be elevated and FMD would be reduced at HA compared to sea-level (SL); and 2) at SL and HA, FMD would be reduced when SNA was acutely increased, and elevated when SNA was acutely decreased. Using a novel, randomized experimental design, brachial artery FMD was assessed at SL (344m) and HA (5050m) in 14 participants during mild lower-body negative pressure (LBNP; -10 mmHg) and lower-body positive pressure (LBPP; +10 mmHg). Blood pressure (finger photoplethysmography), heart rate (electrodcardiogram), oxygen saturation (pulse oximetry), and brachial artery blood flow and shear rate (Duplex ultrasound) were recorded during LBNP, control, and LBPP trials. Muscle SNA was recorded (via microneurography) in a subset of participants (n=5). Our findings were: 1) at rest, SNA was elevated ($P<0.01$), and absolute FMD was reduced ($P=0.024$), but relative FMD remained unaltered ($P=0.061$), at HA compared to SL, and 2) despite significantly altering SNA with LBNP (+60.3±25.5%) and LBPP (-37.2±12.7%) ($P<0.01$), FMD was unaltered at SL ($P=0.448$), and HA ($P=0.537$). These data indicate that acute and mild changes in SNA do not directly influence brachial artery FMD at SL or HA.

New and Noteworthy: The role of the sympathetic nervous system on endothelial function remains unclear. We used lower-body negative and positive pressure to manipulate sympathetic nervous activity at sea-level and high-altitude, and measured brachial endothelial function via flow-mediated dilation. We found that acutely altering sympathetic nervous activity had no effect on endothelial function.

Abbreviations:

CO, cardiac output

FMD, flow-mediated dilatation

HR, heart rate

LBNP, lower-body negative pressure

LBPP, lower-body positive pressure

MAP, mean arterial pressure

MSNA, muscle sympathetic nervous activity

SNA, sympathetic nervous activity

SpO_2, peripheral oxyhemoglobin saturation

SRAUC, shear rate area under the curve

SV, stroke volume

TPR, total peripheral resistance

Introduction

Brachial artery flow-mediated dilatation (FMD) is a non-invasive measurement of artery diameter changes in response to a transient increase in shear stress, and provides a clinical index of endothelial function [reviewed in: (48)]. Brachial FMD can be altered by several physiological factors such as: a) oxidative stress (20, 45); b) shear stress, and hemodynamics [e.g. cardiac output and blood pressure; (4, 12, 28, 32)]; c) inflammation (24, 27), and; d) sympathetic nervous system activity SNA) (1, 16, 26, 47, 50, 53). Given that increased SNA has been linked to cardiovascular disease and aging (8, 31, 37), from a clinical perspective, it is important to clearly understand the effects of SNA on vascular health in humans. In this context, the role of SNA on endothelial function has been examined by several investigations in young, healthy humans. These studies have revealed that FMD is impaired under conditions in which SNA is acutely elevated, such as lower-body negative pressure (LBNP) (26, 47), cold pressor test (16), mental stress (19), and immediately after cycling exercise (1, 4, 11, 29, 53). Additionally, exposure to hypobaric hypoxia (e.g. high-altitude) - which markedly elevates resting SNA (15, 23), has been demonstrated to reduce endothelial function in some (3, 35), but not all cases (5, 6, 52, 53). Differences in the degree and duration of altitude exposure, shear stress stimulus, and altitude ascent profile (passive vs. active) likely explain these variable findings on the influence of altitude on endothelial function.

It is clear that SNA is elevated during moderate or severe LBNP (43, 54), cold pressor test (16, 44), acute and chronic hypoxic exposure (13, 15, 23), and during lower-body cycling exercise (30). However, in addition to increasing SNA, each of these interventions have consequential changes in heart rate, stroke volume, blood pressure, and retrograde shear (i.e. altered hemodynamics) – these physiological factors can directly affect endothelial function (4, 11, 32, 33, 40, 41, 49). Currently, it remains unclear whether the current observed reductions in

114 brachial artery endothelial function are directly due to SNA related vascular constraint, or by
115 physiological consequences of SNA (e.g., increases in retrograde shear rate), which directly
116 impairs endothelial function (47).

117 We attempted to address this gap in the literature by investigating the role of SNA on
118 endothelial function independent of altered hemodynamics using an experimental design similar
119 to previous work (38), involving mild LBNP (-10 mmHg) and mild lower-body positive pressure
120 (LBPP; +10 mmHg), which alters both cardiopulmonary and arterial baroreflex activity (38). The
121 distinct advantage of employing a mild LBNP/LBPP model is that both modalities alter SNA
122 independent of changes in heart rate (18, 42), stroke volume (18), blood pressure (9, 18, 42), and
123 brachial artery vessel diameter (41). In supine position at rest, mild LBNP (-5 to -10 mmHg) has
124 demonstrated to significantly increase SNA by ~30-60% (9, 42, 43), while LBPP (+10 to +20
125 mmHg) decreases SNA by ~30% (18), in healthy individuals. Interestingly, the elevations in
126 SNA observed during mild LBNP (~30-60%) are comparable to those achieved with acute
127 hypoxia (F_IO_2 = 0.11) (13), which reduces brachial FMD via an α_1-adrenergic pathway (35). In
128 addition, due to SNA withdrawal, the novel approach of using LBPP may serve as a non-
129 pharmacological tool to elevate endothelial function, especially in the presence of hypobaric
130 hypoxia when resting SNA is markedly elevated (15, 23).

131 By employing a counter-balanced, randomized design, the primary purposes of the
132 current study were to investigate the role of the sympathetic nervous activity on endothelial
133 function at sea-level (344m), and during chronic exposure to hypobaric hypoxia (5050m) where
134 resting sympathetic nervous activity is chronically elevated (15, 23). By using a novel, purpose
135 built, light-weight, portable lower-body differential pressure chamber to alter sympathetic
136 nervous activity largely independent of hemodynamics, we hypothesized that: 1) at rest,

sympathetic nervous activity would be elevated, and endothelial function would be reduced at high-altitude compared to sea-level, 2) at sea-level and after acclimatization to high-altitude, endothelial function would be reduced during an acute increase in sympathetic nervous activity (induced by mild lower-body negative pressure), and elevated during an acute decrease in sympathetic nervous activity (induced by mild lower-body positive pressure), independent of changes in peripheral hemodynamics.

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 between September and November 2016. As such, participants took part in a number of studies conducted at the University of British Columbia (Kelowna, British Columbia; 344m) and during three weeks at the Ev-K2 CNR pyramid laboratory (Khumbu Valley, Nepal, 5050m). However, the *a priori*, primary research questions addressed in the current paper are novel and are exclusively dealt within this study alone.

Participants. Recruited participants (n=15; 1F) were normotensive (systolic blood pressure <140 and diastolic pressure <90 mmHg) at rest, and completed a medical history questionnaire. The participants were non-smokers, had no previous history of cardiovascular, cerebrovascular, or respiratory diseases. During the time of testing, one participant was taking oral contraceptives (i.e. birth control), and another was taking Mesalazine. At sea-level, two participants were excluded from data analyses for the following reasons: 1) testing was terminated on one participant due to being uncomfortable in the lower-body differential chamber, thus, testing was also not continued at high-altitude in this participant, and 2) a participant was omitted from data analysis at sea-level due to inadequate brachial artery imaging. However, this participant was included in our high-altitude data analysis (n=14). In summary, out of the 15 participants recruited for the current study, 13 and 14 participants were included in our data analysis at sea-level and high-altitude, respectively. In addition, cardiac output data was missing in one participant at high-altitude due to equipment malfunction. All participants arrived at the Ev-K2

CNR research facility within two days of each other, after following a similar ascent profile (7-8 day trek) as described in detail elsewhere (17, 35, 55). Upon ascent, all participants avoided taking oral acetazolamide (i.e. Diamox), a carbonic anhydrase inhibitor commonly used to prevent/treat high-altitude illness. Experimentation occurred between days 11 and 14 at high-altitude, and no participants had any symptoms of altitude illness during the time of testing, nor were any using aspirin, non-steroidal anti-inflammatory drugs, and phosphodiesterase-5 inhibitors.

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 four-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 (36, 39). 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.

Experimental protocol. After becoming comfortable within our custom lower-body differential pressure chamber (described below), participants were instructed to lie motionless in the supine position and breathe normally for 20-minutes to ensure that blood volume was comparably distributed prior to experimentation (21). At sea-level, muscle sympathetic nervous activity (MSNA) in the radial nerve was collected in a subset of participants (attempted: n=10; obtained: n=5) during the LBNP/LBPP protocol. Muscle SNA signals were obtained once the participant was instrumented while laying supine in our custom lower-body differential pressure chamber (described below). At sea-level and high-altitude, the protocol began with a five-minute eupneic breathing baseline period, after which, the pressure within the chamber was altered to one of the following: 1) -10 mmHg (LBNP trial), 2) remained unchanged at zero mmHg (control trial), or 3) +10 mmHg (LBPP trial). Once adequate pressure was achieved in the lower-body differential chamber, the participant was asked to remain quiet and relaxed, and after five-minutes a brachial artery FMD was performed on the participants left arm. Once the brachial artery FMD measurement was collected, the pressure of the lower-body differential pressure chamber was alleviated and the participant was given a five-minute recovery period. The protocol was then repeated for the remaining two randomized conditions (i.e. LBNP, control, or LBPP). Before each condition, a five-minute quiet resting baseline was endured (*Refer to figure 1 for a schematic of the protocol described above*).

Additionally, out of the five participants that we were able to obtain radial MSNA data at sea-level, MSNA signals were obtained in the peroneal nerve at rest at high-altitude in four of these participants in order to demonstrate the effects of altitude on resting MSNA. Previous work

has shown that there are no regional differences in MSNA between the radial and peroneal nerve (42).

Experimental Measurements.

Cardiovascular measurements. All continuously recorded cardiovascular measurements were acquired at 1000 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. 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. 90 degrees) from their body, an inflation/deflation cuff was placed on the participants left forearm, and their arm was fixed into position on a table at the level of the heart. 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 [J.C.T; performed brachial artery FMD in the following published investigations (51-53)], whom has a between day coefficient of

variation in FMD of 8.3 ± 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 mmHg for five-minutes. Recordings of diameter and velocity resumed 30-seconds prior to cuff deflation and continuously for three-minutes thereafter (48).

Lower-body differential pressure chamber. Mild LBNP and LBPP was elicited using a custom-built, light-weight, portable, lower-body differential pressure chamber (designed and built by the author M.M.T). The LBNP chamber was sealed at the level of the iliac crest of each participant using stretchable waist belts. Pressure within the chamber was generated using a 120V house-hold vacuum pump, and measured using a digital manometer (DigiMano 1000, 200–200IN, Netech Corporation, Farmingdale, NY, USA). The magnitude of negative pressure was manipulated using a 120-volt input/140-volt output variable transformer (Variac, Cleveland, OH, USA). Stable pressure of -10 mmHg LBNP or +10 mmHg LBPP were achieved within 10-15 seconds after turning on the vacuum pump.

Muscle sympathetic nerve activity. Recordings of MSNA were obtained by an experienced microneurographer (C.D.S and J.P.M). A tungsten microelectrode (50 mm long, 200 μm in diameter) was inserted percutaneously into the right radial nerve (at sea-level), and the right peroneal nerve (at high-altitude), using ultrasound guidance (12mHz linear array probe, GE Health Care, Canada) (10). A reference electrode was positioned subcutaneously 1–3 cm from the recording site. A suitable sympathetic nerve site was searched through manual manipulation of the tungsten microelectrode until a characteristic pulse-synchronous burst pattern was observed. Confirmation that the recorded signal represented MSNA was determined by the

absence of skin paresthesias and a signal that increased in response to voluntary apnea but not during arousal to a loud noise. Muscle sympathetic nervous activity was amplified 1,000× through a preamplifier and 100× by a variable-gain, isolated amplifier. The amplified, raw MSNA signal was band-pass filtered at a bandwidth of 700–2,000 Hz, sampled at 10,000 Hz and stored for offline analysis (LabChart V7.1, ADInstruments, Colorado Springs, CO, USA).

Data 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, while minimizing investigator bias (56). Antegrade, retrograde, and mean shear rates were calculated as four times the mean blood velocity, divided by vessel diameter and the oscillatory shear index as |retrograde shear rate| / (|antegrade shear rate| + |retrograde shear rate|). The FMD was calculated as the percent increase in vessel diameter from resting baseline diameter to peak diameter following cuff release, where baseline and peak diameters were automatically detected from the continuous data described above.

Muscle sympathetic nervous activity was analyzed using peak analysis software (LabChart V7.1, ADInstruments, Colorado Springs, CO, USA). Two minutes of MSNA data was averaged immediately prior to the end of each LBNP, control, and LBPP trial, and was expressed as the frequency of MSNA bursts per minute, and incidence per 100 heart beats.

Adjusted flow-mediated dilatation. The effects condition (i.e. LBNP, control, and LBPP) were analyzed within and between sea-level and high-altitude for brachial artery FMD. To determine if our FMD results were altered due to changes in baseline arterial diameter and/or shear rate area under the curve (SRAUC) in response to forearm cuff release, 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) (2).

Statistics

All statistical analyses were performed using SigmaStat V13 (Systat, Chicago, IL, USA), and were reported as mean ± SEM. Statistical significance was set at $P<0.05$. Paired t-tests were used to detect changes in cardiovascular variables between baseline and during the brachial artery FMD during LBNP, control, and LBPP at both sea-level and high-altitude (*see table 1*). One-way and two-repeated measures analysis of variance were used to detect any differences in brachial artery variables (*see table 2, figure 2, and figure 5*). One-way repeated measures analysis of variance was used to detect any differences in MSNA between LBNP, control, and LBPP trials at sea-level (*see figure 3*), and paired t-tests were used to assess any differences in MSNA between sea-level and high-altitude at rest (*see figure 4*). When significant F-ratios were detected, post-hoc comparisons were made using Bonferonni post hoc test for pair-wise comparisons.

Results

Participants

The participants included in the sea-level (n=13) and high-altitude (n=14) protocol data analysis had a mean ± SEM age of 27.2 ± 1.7 years, height of 179.5 ± 1.7 cm, and weight of 74.4 ± 2.5 kg. Participants had normal pulmonary health with an FVC of 5.5 ± 0.1 L (104.3 ± 2.4% of predicted), forced expiratory volume in one-second (FEV_1) of 4.3 ± 0.1 L (95.5 ± 3.3% of predicted), FEV_1/FVC of 78.3 ± 1.1, total lung capacity of 6.8 ± 0.2 L (98.5 ± 2.3% of predicted), and had a diffusing capacity of the lung for carbon monoxide of 33.1 ± 1.6 ml/min/mmHg (93.1 ± 3.9% 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.

Endothelial function between sea-level and high-altitude.

At high-altitude, absolute brachial artery FMD was reduced compared to sea-level by 0.10 ± 0.05 mm during the LBNP trial; 0.08 ± 0.05 mm during the control trial, and; 0.07 ± 0.04 mm during the LBPP trial (main effect: P=0.024; *see figure 2*). Additionally, there was no condition effect of LBNP, control, LBPP (P=0.243), nor interaction effect (P=0.835). Similarly, although relative brachial artery FMD was reduced at high-altitude compared to sea-level, this effect marginally missed our statistical significant criteria of P<0.05 (P=0.061). No differences were detected in relative brachial artery FMD for condition (P=0.343), nor interaction (P=0.856; *see figure 2*). In addition, when taking into account SRAUC and changes in baseline brachial artery diameter between sea-level and high-altitude, our results for brachial FMD were the same with a main

effect between sea-level and high-altitude (P=0.008), and no differences found for condition (P=0.250), nor interaction (P=0.693).

Muscle sympathetic nervous activity at sea-level and high-altitude.

Muscle sympathetic nervous activity was collected in a subset of participants (n=5) at sea-level (*see figure 3*). During the -10 mmHg LBNP trial, MSNA bursts per minute was elevated by 59.1 ± 25.2% compared to control (P=0.007), and by 140.1 ± 10.6% compared to the +10 mmHg LBPP trial (P=0.047). No differences were found between LBPP and the control trial with our one-way repeated measures analysis of variance; however, when comparing MSNA bursts per minute using a paired t-test, MSNA was significantly reduced by 39.2 ± 12.3% (P=0.03; *see figure 3*) during the LBPP trial. Similarly, MSNA burst incidence (per 100 heart beats) was elevated by 61.5 ± 25.9% compared to control (P=0.005), and by 131.9 ± 11.7% compared to the +10 mmHg LBPP trial (P=0.03). When comparing MSNA burst incidence using a paired t-test between LBPP and control trials, MSNA was significantly reduced by 35.1 ± 13.2% (P=0.04; *see figure 3*).

Out of the five participants that MSNA recordings were obtained at sea-level, we were able to obtain peroneal MSNA signals at rest in four of these participants at high-altitude (*see figure 4*). At high-altitude, MSNA bursts per minute was elevated compared to sea-level by 98.2 ± 39.5% (P=0.03), and although MSNA burst incidence was also higher at high-altitude compared to sea-level by 72.0 ± 35.2%, this elevation did not reach statistical significance (P=0.05; *see figure 4*).

Cardiovascular variables during LBNP and LBPP.

Sea-level: As expected, no change was present in HR, SV, CO, and TPR between baseline and FMD during LBNP (P=0.367, P=0.847, P=0.320, and P=0.614, respectively), control (P=0.854, P=0.155, P=0.472, and P=0.892, respectively), and LBPP (P=0.534, P=0.218, P=0.238, and P=0.785, respectively). Mean arterial pressure also remained unchanged from baseline during the LBNP and control trials (P=0.243 and P=0.257, respectively); however, it was elevated by 4.2 ± 1.2 mmHg during the LBPP trial (P=0.003; *see table 1*).

High-altitude: At high-altitude, HR, SV, CO, and TPR were the same between baseline and FMD during LBNP (P=0.703, P=0.677, P=0.992, and P=0.063, respectively), control (P=0.054, P=0.233, P=0.313, and P=0.453, respectively), and LBPP (P=0.201, P=0.355, P=867, and P=0.845, respectively). Mean arterial pressure was unchanged during the LBNP and control trial before and after brachial FMD (P=0.099 and P=0.171, respectively). In contrast, it was slightly elevated by 4.5 ± 0.9 mmHg during LBPP (P<0.001; *see table 1*).

Brachial artery responses during LBNP and LBPP.

Sea-level: Brachial artery diameter, blood velocity, blood flow, and vascular resistance were the same between LBNP, control, and LBPP trials (main effects: P=0.422, P=0.384, P=0.985, and P=0.867 respectively). Additionally, brachial mean, antegrade, and retrograde shear rates, and the oscillatory shear index were not different between LBNP, control, and LBPP (main effects: P=0.928, P=0.928, P=0.891, and P=0.919, respectively; *see table 2*).

High-altitude: No differences were detected in brachial artery diameter, blood velocity, blood flow, and vascular resistance was the same between LBNP, control, and LBPP trials (main

effects: P=0.993, P=0.224, P=0.405, and P=0.235, respectively). Additionally, brachial mean, antegrade, and retrograde shear rates, and the oscillatory shear index were not different between LBNP, control, and LBPP (main effects: P=0.304, P=0.563, P=0.119, and P=0.186, respectively; *see table 2*).

Endothelial function during LBNP and LBPP.

Sea-level: No difference was detected in SRAUC to peak diameter between LBNP, control, and LBPP trials (main effect: P=0.995). At sea-level, one participant was excluded from mean data analysis due to low-quality video files. Brachial artery FMD (n=13) did not change between LBNP, control, and LBPP (main effect: P=0.448).

High-altitude: There were no differences detected for SRAUC between LBNP, control, and LBPP trials (main effect: P=0.825) during the hypobaric hypoxia trial. Brachial artery FMD (n=14) did not change between LBNP, control, and LBPP trials (main effect: 0.537; *see figure 5*).

Discussion

Using a novel, and randomized experimental design, we examined the effect of acute alterations of SNA using mild LBNP and LBPP on brachial artery endothelial function at both sea-level (344m) and high-altitude (5050m). Our main findings were the following: 1) in support of previous studies, MSNA was elevated, and brachial artery endothelial function was reduced at high-altitude compared to sea-level after active ascent to 5050m, and 2) despite acutely increasing SNA with LBNP, and decreasing SNA with LBPP, we demonstrated that brachial artery endothelial function remained unchanged at sea-level and high-altitude. Our data indicates that mild and acute changes in SNA, at least in the absence of alterations in systemic hemodynamics, does not influence endothelial function.

Effect of high-altitude on endothelial function

The effects of high-altitude acclimatization on endothelial function, as assessed via brachial FMD, has been studied previously. These studies have reported contradictory results such as reduced FMD (3, 35), or no change in FMD upon acclimatization to high-altitude (5, 6, 52, 53). Despite the disparities between these studies, elevations in SNA is proposed to have a profound effect on brachial FMD (26, 53). After four-weeks of acclimatization to high-altitude (5260m), MSNA has been shown to be elevated by ~200% (23). The current study confirms these previous findings as we have demonstrated in four participants that MSNA was substantially elevated at rest after acclimatization to 5050m (*see figure 4*). This increase in SNA and total peripheral resistance is likely responsible for the substantial decrease in brachial artery blood flow observed at high-altitude (14).

An alternative explanation for the reported differences between these high-altitude FMD studies may lie within the mode of travel to high-altitude, and the severity of altitude exposure.

For example, the studies that have reported a decrease in brachial FMD took place after 5-10 days of trekking at high-altitude [4200m, (3); and 5050m, (35)]. In contrast, the studies that have reported no change in endothelial function arrived at a more moderate altitude passively by automobile [at 3800m (52, 53)], or cable car [at 3842m (5, 6)]. The high-altitude arm of the current study involved trekking ascent to 5050m over 7-8 days, and in support of our hypothesis and previous reports (3, 35), we found that brachial artery endothelial function was reduced at high-altitude compared to sea-level. This reduction may be due to long-term elevations in sympathetic nervous activity or marked elevations in oxidative stress, or both.

Altering sympathetic nervous activity non-invasively with lower-body negative and lower-body positive pressure.

There have been several investigations on the role of the SNA on endothelial function assessed by brachial FMD at sea-level (1, 16, 26, 47, 53); however, none of these studies have concurrently measured SNA using microneurography. Existing literature indicates that our mode of altering SNA (i.e. mild LBNP and LBPP) could provide a useful model to evaluate the role of SNA on endothelial function, assuming that this methodology significantly alters SNA independent of hemodynamics (9, 18, 38, 41-43). For the current project we developed a novel, light-weight, purpose built lower-body differential pressure chamber and measured its effectiveness of altering radial MSNA, which is representative of global MSNA (42), during our sea level trial (n=5). Our radial MSNA data indicates that SNA was elevated during LBNP and reduced during LBPP (*see figure 3*). Here, we established an effective methodological approach, to non-invasively increase and decrease SNA largely independent of systemic hemodynamics, however, the observed alterations in SNA failed to evoke a change in brachial artery resistance (*see table 2*) – a clear indicator of vascular constraint. Thus, since vascular constraint was not

434 significantly altered during acute and mild LBNP/LBPP, it is unclear if the experimental design
435 in its current form is effective when investigating the effects of SNA on endothelial function.
436 Future studies using a similar LBNP/LBPP experimental model should consider a longer
437 duration of stimulus (i.e. LBNP or LBPP), which may be more effective in altering peripheral
438 vascular resistance.
439
440 ***Effect of sympathetic nervous activity on endothelial function at sea-level.***
441 Although there have been several reports of SNA influencing endothelial function (1, 16, 26, 48,
442 53), it has been suggested that the method of altering SNA may yield different results (16). For
443 example, Dyson *et al*. (16) investigated the role of SNA (via epinephrine and norepinephrine
444 spillover) on brachial artery endothelial function, and discovered that the cold pressor test was
445 the only modality that reduced brachial artery FMD. Interestingly, Dyson *et al.* (16) found that
446 LBNP increased SNA, but it had no effect on brachial artery endothelial function, which
447 contrasts other studies that have found that LBNP reduces brachial artery endothelial function
448 (26, 47). The first report of LBNP significantly reducing brachial artery endothelial function was
449 by Hijmering *et al.* (26). Here, they discovered that the reduction in brachial artery endothelial
450 function was mediated through a α_1-adrenergic pathway as endothelial function was restored
451 during LBNP after administration phentolamine. This finding was supported by two recent
452 studies that used exercise as a method of increasing SNA (1, 53). Hijmering *et al*. (26) also
453 suggested that the observed reduction in brachial artery endothelial function could be directly
454 due to SNA, or indirectly via other mechanisms during LBNP such as altered hemodynamics
455 (e.g. increases in retrograde shear stress). Thijssen *et al*. (47) attempted to address this question
456 by using a local heating stimulus (to one arm) during LBNP in order to abolish the increase in
457 retrograde shear stress typically observed during moderate-to-severe magnitudes of LBNP (41,

47). Their findings revealed that brachial artery endothelial function was restored after the heat stimulus was applied and retrograde shear rate was reduced (47). However, altered hemodynamics (e.g. increased heart rate and reduced stroke volume) during LBNP were still present (47), and these physiological changes can directly affect endothelial function [reviewed in (22)].

The current study attempts to address this research question by manipulating SNA largely independent of changes in hemodynamics. This is the first study to investigate the role of SNA on endothelial function by increasing and decreasing SNA using LBNP and LBPP, respectively, findings confirmed (at sea-level) via microneurography. In contrast to our hypothesis, we did not observe any change in brachial artery endothelial function during LBNP - a finding that is consistent with at least one previous study (16), but opposes other reports (26, 47). It is possible that we did not increase SNA activity enough in order to influence endothelial function; however, the experimental design may be more important than the magnitude of SNA increase. For example, Dyson *et al.* (16) demonstrated that the only intervention that altered endothelial function during elevated SNA was not the intervention that evoked the largest SNA response. Interestingly, acute hypoxia (F_IO_2 = 0.11) has shown to reduce brachial artery endothelial function after 60-minutes (35), and this severity of hypoxia has been shown to increase SNA to approximately the same extent as our -10 mmHg LBNP stimulus (13). Additionally, it is possible that the current experimental design was too short in duration to evoke a change in vascular resistance and endothelial function. For example, a recent study demonstrated that 30-minutes of sustained moderate exercise reduced endothelial function via an α_1-adrenergic pathway; however, a ~10-minute maximal exercise test did not evoke the same results (53). Nevertheless,

our data indicates that acute and mild SNA activation and deactivation via LBNP and LBPP does not alter brachial artery endothelial function.

Effect of sympathetic nervous activity on endothelial function at high-altitude.

Lower-body negative pressure has been previously used to measure orthostatic tolerance in high-altitude Andean natives at high-altitude [4338m; (7)]; however, this is the first investigation to use LBNP above 5000m where MSNA is markedly elevated (*see figure 4*), in addition, this is the first study to use LBPP at high-altitude. Our research group has published the only other report investigating the role of SNA on endothelial function at high-altitude (53). Using moderate-intensity exercise to increase SNA, we found that brachial artery endothelial function is not reduced at high-altitude, indicating that after acclimatization to high-altitude neurovascular control may be altered (53). It is also unknown whether our previously reported findings were unique to exercise, and hence potentially, a different strategy to alter SNA may yield different results (16). Additionally, SNA stimulus (e.g. exercise) has been shown to be augmented with cycling exercise during hypoxia (30) – meaning that the alteration in SNA via LBNP and LBPP could be exacerbated at high-altitude, leading to a more pronounced effect on endothelial function. Therefore, we hypothesized that altering SNA using LBNP and LBPP would result in a decrease and increase in brachial artery endothelial function, respectively. To our surprise, similar to our sea-level data, we found that LBNP and LBPP did not change endothelial function at high-altitude. However, the lack of effect of LBNP and LBPP on endothelial function at high-altitude could be also be due to similar methodological reasoning outlined above: (a) our mode of altering SNA does not alter brachial endothelial function, and/or (b) the duration of SNA activation/deactivation was not long enough to elicit a change in endothelial function.

Methodological considerations

The degree of LBPP chosen for the current research project (i.e. +10 mmHg) was determined based on previous literature, which reported no changes of MAP (18, 38). However, during our LBPP trials at sea-level and high-altitude, LBPP elevated MAP elevated by ~4-5 mmHg, potentially due to LBPP associated transient fluid shifts. This result was likely not due to measurement drift from our continuous blood pressure monitor (i.e. finometer), since LBPP selectively increased MAP in both sea-level and high-altitude protocols, and the finometer was carefully calibrated before each trail. Changes in blood pressure could have a direct effect on brachial FMD (22); however, since mild LBNP and LBPP did not alter our other physiological variables (especially shear patterns), we feel that the small change in blood pressure is likely trivial. Although the recovery time between LBNP, control, and LBPP trials (5 minutes) was acute, previous data (18, 43), and our data indicates that participants research steady state following this short recovery period. Another consideration is that due to methodological constraints at high-altitude, we were unable to measure SNA via microneurography, therefore, the absolute effect of LBNP and LBPP on SNA at high-altitude is unknown. Additionally, it is important to consider that neurovascular transduction may be different at high-altitude compared to sea-level, but this is still under debate as there is evidence that neurovascular transduction is reduced (34), or increased (46), with exposure to hypoxia. We did, however, obtain MSNA recordings in the peroneal nerve at rest in a subset of participants (n=4) at both sea-level and high-altitude. We acknowledge that our MSNA data collected at sea-level and high-altitude were in the radial and peroneal nerves, respectively, but it has been previously demonstrated that MSNA does not differ between these two nerves during mild lower-body negative pressure and are both a reflection of global MSNA (42). Although our MSNA sample size was small, we still

detected statistical significance between LBNP and LBPP trials, which were recorded using a within subject design at sea-level.

Our experimental design warrants further comment. Our LBNP/LBPP methodological approach to bi-directionally alter SNA proved successful; however, the current study design failed to change brachial artery vascular resistance. We view our study design as a "double-edged sword", as it altered SNA largely independently of hemodynamics, yet it was not a potent enough stimulus to alter brachial artery resistance, making it unclear if our study design is appropriate to investigate the effects of SNA on peripheral vascular function. Lastly, menstrual cycle was not taken into consideration for our one female participant, and previous evidence indicates that brachial artery FMD changes throughout the menstrual cycle (25). However, our primary research objective was to look at the within-day comparison of brachial FMD between LBNP, control, and LBPP trials, therefore, the results of these data should not be affected by differences in menstrual cycle between sea-level and high-altitude. Importantly, changes in blood viscosity between sea-level and high-altitude was not taken into account when analyzing brachial artery FMD. However, a reduction in brachial artery FMD was still observed at high-altitude, even though hematocrit, thus shear stress, was likely higher during cuff release.

Conclusion

We used a novel experimental approach to investigate the relationship between sympathetic nervous activity and endothelial function by using mild lower-body negative pressure and lower-body positive pressure at both sea-level and high-altitude. We demonstrated for the first time using a novel, experimental design, that altering sympathetic nervous activity largely independent of hemodynamics (e.g. heart rate, stroke volume, shear stress) had no effect on

brachial artery endothelial function. These findings suggest that brachial artery endothelial function may not be directly mediated through sympathetic nervous activity associated vascular constraint. Together, our findings have implications for better understanding the consequential impact of sympathetic nervous activity on vascular function.

Acknowledgements: This study was carried out within the framework of the UBC International Research Expedition to Nepal, we thank the research stations staff for their friendly accommodation. The authors are grateful to the members of the UBC International Research expedition to the Ev-K2 CNR pyramid laboratory for their invaluable help with organization and implementation of this research study. M.M.T., and P.N.A., were responsible for conception and design of the current study. All authors contributed to the collection, assembly, analysis, and interpretation of the data, along with drafting the article or revising it critically for important intellectual content. All authors approved the final version of the manuscript and all persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Grants: This study was supported by the Natural Sciences and Engineering Research Council of Canada (PNA), the Canadian Foundation for Innovation and a Canada Research Chair (PNA). MMT was supported by a Natural Sciences and Engineering Research Council of Canada Doctoral CGS award.

Disclosures: The authors have no conflict of interest.

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List of Tables

Table 1: Cardiovascular variables at baseline, and during lower-body differential pressure at sea-level and high-altitude.

		Sea-level			High-altitude		
		LBNP	**Control**	**LBPP**	**LBNP**	**Control**	**LBPP**
HR (bpm)	*BL*	55.7 ± 3.0	54.9 ± 3.3	55.5 ± 3.2	61.4 ± 3.3	64.0 ± 4.1	61.9 ± 2.6
	FMD	54.9 ± 3.1	54.7 ± 2.8	55.1 ± 3.1	62.0 ± 3.4	59.6 ± 2.9	61.9 ± 3.2
SV (ml)	*BL*	99.8 ± 4.7	101.1 ± 3.8	99.9 ± 5.3	78.7 ± 3.4	79.1 ± 5.2	74.8 ± 4.3
	FMD	99.4 ± 4.7	104 ± 4.2	103.3 ± 5.5	77.8 ±4.4	82.4 ± 4.6	78.1 ± 3.9
CO ($l\ min^{-1}$)	*BL*	5.6 ± 0.5	5.6 ± 0.5	5.5 ± 0.6	4.7 ± 0.3	4.8 ± 0.4	4.5 ± 0.3
	FMD	5.5 ± 0.4	5.7 ± 0.5	5.7 ± 0.6	4.7 ± 0.3	4.8 ± 0.3	4.6 ± 0.3
MAP (mmHg)	*BL*	92.0 ± 1.9	91.1 ± 1.7	92.6 ± 2.2	99.3 ± 1.6	103.3 ± 2.2	102.5 ± 1.8
	FMD	93.8 ± 3.0	92.2 ± 1.8	96.8 ± 2.3*	101.7 ± 1.6	104.4 ± 1.6	107.0 ± 2.2*
TPR [$dyn\ s/cm^5$]	*BL*	1350.1 ± 106.7	1305.6 ± 94.5	1365.9 ± 96.3	1747.2 ± 125.5	1767.9 ± 135.6	1880.4 ± 132.6
	FMD	1370.0 ± 87.5	1309.6 ± 90.5	1352.0 ± 107.2	1822.3 ± 147.2	1752.0 ± 108.3	1863.1 ± 105.9
SpO_2 (%)	*BL*				83.3 ± 0.7	83.7 ± 0.9*	83.4 ± 0.9
	FMD				82.7 ± 0.7	81.6 ± 0.6	82.2 ± 0.8

Definition of Abbreviations: HR, heart rate; SV, stroke volume; CO, cardiac output; MAP, mean arterial pressure; TPR, total peripheral resistance; SpO_2, peripheral capillary oxygen saturation. *P<0.05, BL vs FMD.

Table 2: Brachial artery variables during the control and lower-body differential pressure trials at sea-level and high-altitude.

	Sea-level			High-altitude		
	LBNP	**Control**	**LBPP**	**LBNP**	**Control**	**LBPP**
BA diameter (mm)	4.6 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1
BA velocity (cm s^{-1})	14.0 ± 3.0	13.7 ± 2.7	14.4 ± 3.7	5.4 ± 1.0	6.3 ± 1.2	6.5 ± 1.2
BA flow (ml min^{-1})	147.0 ± 33.3	142.7 ± 30.6	146.0 ± 35.5	46.5 ± 9.5	53.1 ± 11.1	54.5 ± 11.4
BA resistance [mm Hg (ml min^{-1})$^{-1}$]	1.1 ± 0.3	1.0 ± 0.2	1.1 ± 0.2	3.0 ± 0.5	2.9 ± 0.5	2.7 ± 0.4
BA mean shear (s^{-1})	127.5 ± 27.4	136.1 ± 37.2	125.6 ± 33.4	50.0 ± 9.1	59.6 ± 10.9	62.4 ± 11.1
BA antegrade shear (s^{-1})	136.5 ± 26.0	145.6 ± 36.0	135.7 ± 32.0	70.7 ± 8.2	79.0 ± 9.6	77.1 ± 10.8
BA retrograde shear (s^{-1})	9.0 ± 2.2	9.5 ± 2.5	10.2 ± 3.6	20.6 ± 4.3	19.5 ± 4.0	14.8 ± 2.1
BA oscillatory shear (s^{-1})	0.09 ± 0.02	0.10 ±0.03	0.10 ± 0.03	0.23 ± 0.03	0.21 ± 0.03	0.18 ± 0.02

Definition of Abbreviations: BA, brachial artery.

Figure Legends

Figure 1: A schematic representation of the experimental protocol conducted at sea-level and high-altitude, and raw MSNA neurogram in two participants. After 20-minutes of supine rest, the protocol began with a five-minute eupneic breathing baseline period, after which, the pressure within the chamber was altered to one of the following: 1) -10 mmHg (LBNP trial), 2) remained unchanged at zero mmHg (control trial), or 3) +10 mmHg (LBPP trial). Once pressure was achieved, and maintained for five-minutes, a brachial artery FMD was performed on the participants left arm. Once the brachial artery FMD measurement was collected, the pressure of the lower-body differential pressure chamber was alleviated and the participant was given a five-minute recovery period. The protocol was then repeated for the remaining two randomized conditions (i.e. LBNP, control, or LBPP). Before each condition, a five-minute quiet resting baseline was endured.

Figure 2: A comparison of absolute and relative brachial artery flow-mediated dilation response between sea-level and high-altitude. These data highlight that absolute and relative brachial artery flow mediated dilation remained unchanged between sea-level and high-altitude within LBNP, control, and LBPP trials. Taking into account changes in baseline brachial artery diameter and SRAUC between sea-level and high-altitude, we found that relative endothelial function is still reduced at high-altitude compared to sea-level (P=0.008; *see results section*)

Figure 3: **Muscle sympathetic nervous activity during LBNP, control, and LBPP trials at sea-level.** Individual data of MSNA burst frequency (Panel A; bursts/minute), and burst incidence (Panel B; bursts per 100 heart beats) during LBNP (n=5), control (n=5), and LBPP (n=4) at sea-level. These findings illustrate a significant difference in MSNA between LBNP and LBPP. The gray line on the figure depicts the average between individuals during each trial.

Figure 4: **Muscle sympathetic nervous activity at rest between sea-level and high-altitude.** Individual data of MSNA burst frequency (Panel A; bursts/minute), and burst incidence (Panel B; bursts per 100 heart beats) between sea-level and high-altitude (n=4). These findings illustrate MSNA was significantly elevated in each individual at high-altitude, compared to sea-level. The gray line on the figure depicts the average between individuals during each trial.

Figure 5: Brachial artery shear rate and diameter response to forearm cuff release at sea-level and high-altitude. Panels A and B represent mean data ±SEM for shear rate response during brachial artery FMD during LBNP, control, and LBPP trials at sea-level (n=13) and high-altitude (n=14). Panels C and D represent mean data ±SEM for relative FMD during LBNP, control, and LBPP trials at sea-level (n=13) and high-altitude (n=14). These findings demonstrate that LBNP nor LBPP had no effect on brachial artery FMD, despite altering MSNA. The gray line on the figure depicts the average between individuals during each trial.

Figure 1

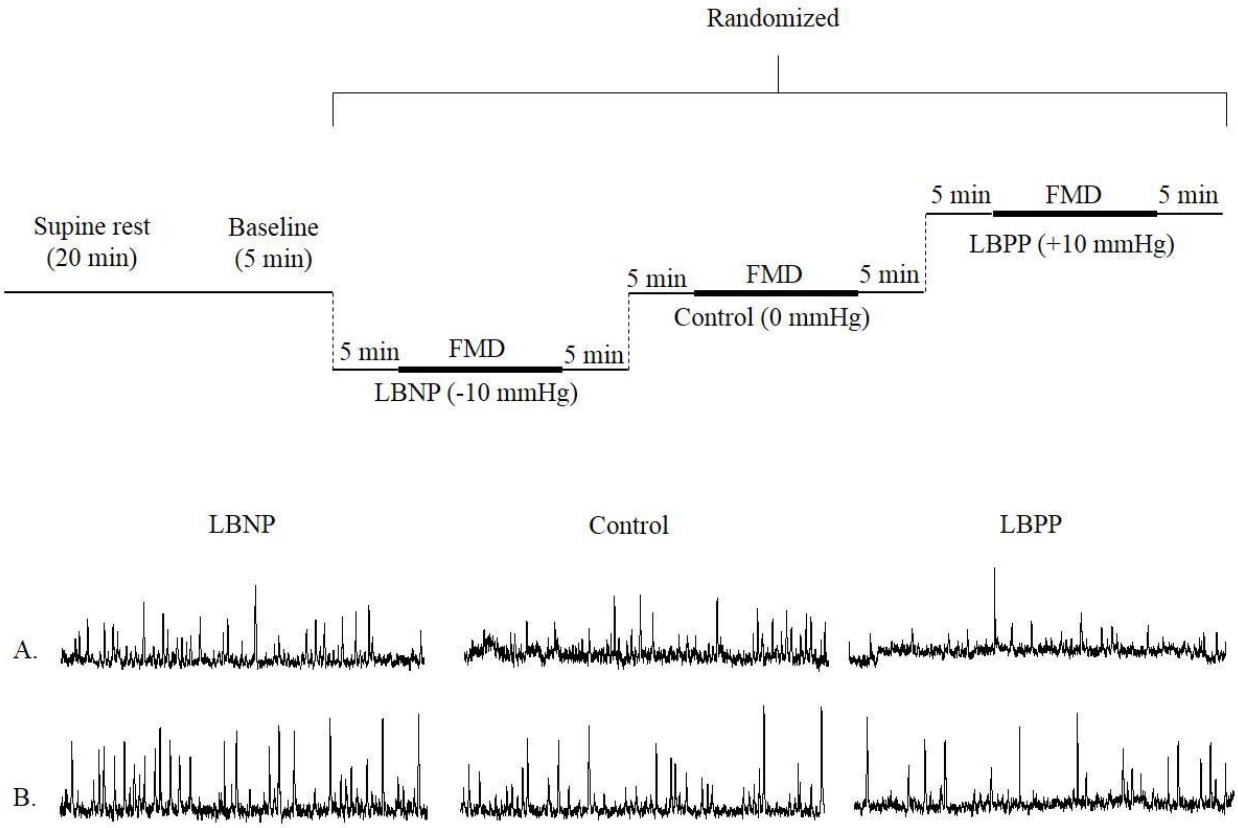

Figure 2

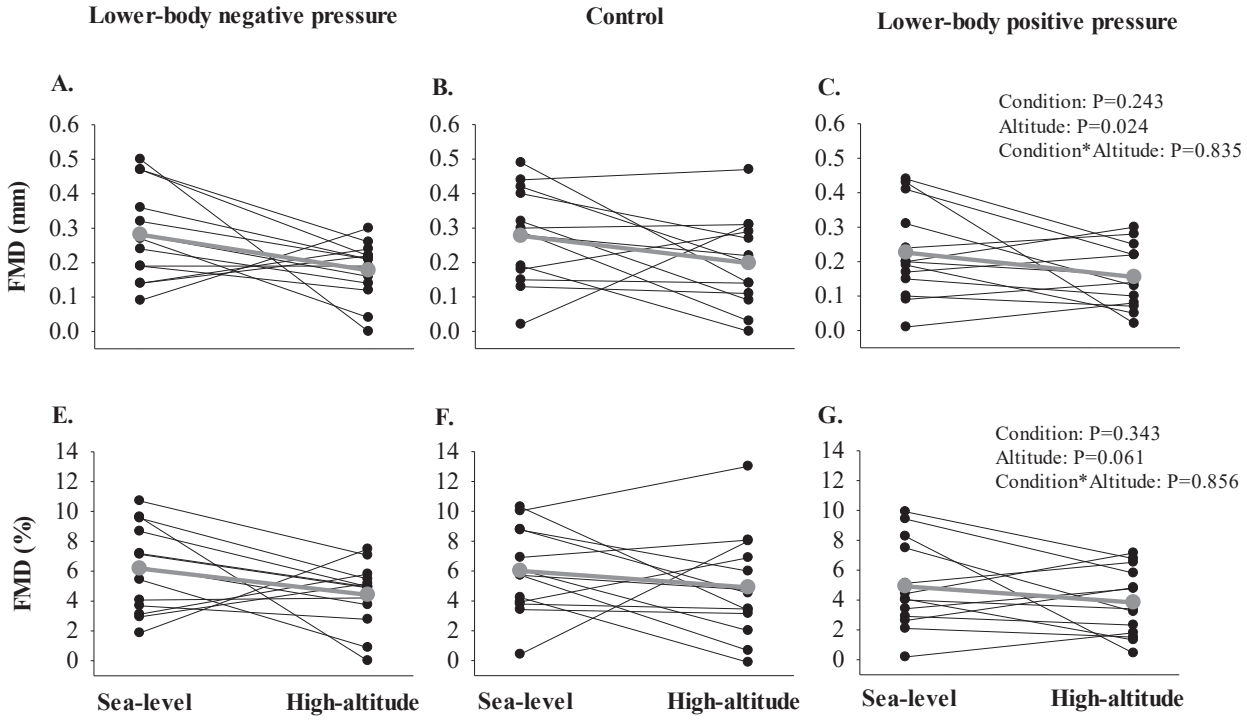

Figure 3

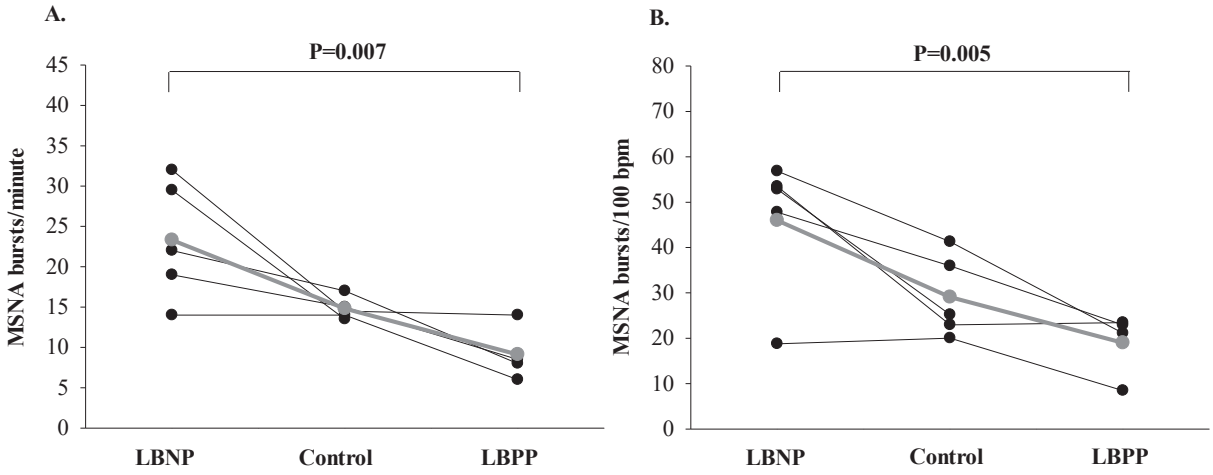

Figure 4

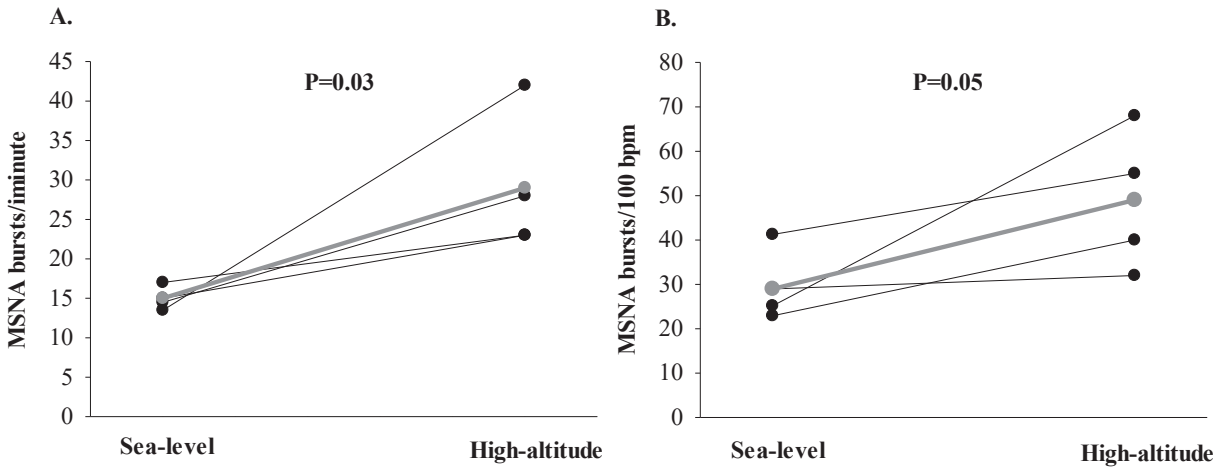

Figure 5