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Title: ONE SESSION OF REMOTE ISCHEMIC PRECONDITIONING DOES NOT IMPROVE VASCULAR FUNCTION IN ACUTE NORMOBARIC AND CHRONIC HYPOBARIC HYPOXIA

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Running Title: Remote Ischemic Preconditioning and Vascular Function

Abstract: ABSTRACT Application of repeated short duration bouts of ischemia to the limbs, termed remote ischemic preconditioning (RIPC), is a novel technique that may have protective effects on vascular function during hypoxic exposures. In separate parallel-design studies, at sea-level (SL; n=16), and after 8-12 days at high-altitude (HA; n=12; White Mountain, 3800m), participants underwent either a sham protocol or one session of 4x5 minutes of dual-thigh cuff occlusion with 5-minutes recovery. Brachial artery flow-mediated dilation (FMD; ultrasound), pulmonary artery systolic pressure (PASP; echocardiography), and internal carotid artery flow (ICA; ultrasound) were measured at SL in normoxia and isocapnic hypoxia [end-tidal P_{O_2} (P_{ETO_2})

maintained to 50mmHg], and during normal breathing at HA. The hypoxic ventilatory response (HVR) was measured at each location. All measures at SL and HA were obtained at baseline (BL), 1 hour, 24 hours, and 48 hours post-RIPC or sham. At SL, RIPC produced no changes in FMD, PASP, ICA flow, end-tidal gases or HVR in normoxia or hypoxia. At HA, although HVR increased 24 hours post RIPC compared to BL (2.05{plus minus}1.4 vs. 3.21{plus minus}1.2 L•min-1•%SaO2-1, $p<0.01$), there were no significant differences in FMD, PASP, ICA flow, resting end-tidal gases. Accordingly, a single session of RIPC is insufficient to evoke changes in peripheral, pulmonary, and cerebral vascular function in healthy adults. Although chemosensitivity may increase following RIPC at HA, this did not confer any vascular changes. The utility of a single RIPC session seems unremarkable during acute and chronic hypoxia.

New Findings: What is the central question of this study? It is suggested that remote ischemic preconditioning (RIPC) may offer protection against ischemia-reperfusion injuries, yet the utility of RIPC in high-altitude settings remains unclear. What are the main findings and its importance? We found that RIPC offers no vascular protection relative to pulmonary artery pressure or peripheral endothelial function during acute, normobaric hypoxia, and at high-altitude in young, healthy adults. However, peripheral chemosensitivity was heightened 24 hours following RIPC at high altitude.

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ONE SESSION OF REMOTE ISCHEMIC PRECONDITIONING DOES NOT IMPROVE VASCULAR FUNCTION IN ACUTE NORMOBARIC AND CHRONIC HYPOBARIC HYPOXIA

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ABSTRACT

Application of repeated short duration bouts of ischemia to the limbs, termed remote ischemic preconditioning (RIPC), is a novel technique that may have protective effects on vascular function during hypoxic exposures. In separate parallel-design studies, at sea-level (SL; n=16), and after 8-12 days at high-altitude (HA; n=12; White Mountain, 3800m), participants underwent either a sham protocol or one session of 4x5 minutes of dual-thigh cuff occlusion with 5-minutes recovery. Brachial artery flow-mediated dilation (FMD; ultrasound), pulmonary artery systolic pressure (PASP; echocardiography), and internal carotid artery flow (ICA; ultrasound) were measured at SL in normoxia and isocapnic hypoxia [end-tidal PO_2 ($PETO_2$) maintained to 50mmHg], and during normal breathing at HA. The hypoxic ventilatory response (HVR) was measured at each location. All measures at SL and HA were obtained at baseline (BL), 1 hour, 24 hours, and 48 hours post-RIPC or sham. At SL, RIPC produced no changes in FMD, PASP, ICA flow, end-tidal gases or HVR in normoxia or hypoxia. At HA, although HVR increased 24 hours post RIPC compared to BL (2.05±1.4 vs. 3.21±1.2 L·min-1·%SaO_2-1, $p<0.01$), there were no significant differences in FMD, PASP, ICA flow, resting end-tidal gases. Accordingly, a single session of RIPC is insufficient to evoke changes in peripheral, pulmonary, and cerebral vascular function in healthy adults. Although chemosensitivity may increase following RIPC at HA, this did not confer any vascular changes. The utility of a single RIPC session seems unremarkable during acute and chronic hypoxia.

Key Words: Remote ischemic preconditioning; High-altitude; Hypoxia; Pulmonary artery pressure; Vascular function; Cerebral blood flow; Chemosensitivity

INTRODUCTION

Remote ischemic preconditioning (RIPC) is a non-invasive procedure that has substantial effects on protecting various organs in the body against ischemia-related injuries. In humans and animal models alike, cyclic 5-10 minute periods of occlusion and reperfusion of blood flow through a limb, with total treatment times ranging from 40-60 minutes produces significant protection against ischemia in the heart (Murry *et al.*, 1986), lungs (Kinoshita, 2015), kidneys (Wever *et al.*, 2011), liver (Yan *et al.*, 2015) and brain (Koch *et al.*, 2011). The exact mechanisms responsible for these effects remain unclear, but are likely a result of activation of various anti-inflammatory and anti-oxidative pathways [for review see: (Koch *et al.*, 2014)].

Recent findings suggest that RIPC may also play a role in protection from hypoxic and altitude-related injuries (Berger *et al.*, 2015*b*). For example, in athletes, one 4x5 minute RIPC treatment in the lower limb attenuates the rise in pulmonary artery systolic pressure (PASP) normally seen after 90 minutes of normobaric hypoxia (Foster *et al.*, 2011), and 5 days of consecutive treatment has shown similar pulmonary vascular protective effects when conducted prior to travel to high altitude (Foster *et al.*, 2014). Together, these findings suggest that preconditioning may offer a degree of protection against the onset of high altitude pulmonary edema. In addition, RIPC has also been linked to reduced oxidative stress and lower symptoms of acute mountain sickness after acute exposure to normobaric hypoxia (Berger *et al.*, 2015*a*); however, these findings were only observed transiently (0-12hours). After a brief period of no observable protection, a second delayed window of protection appears after ~18-20 hours and lasts for 1-2 days (Miguel, 2004; Koch *et al.*, 2014). While clinical outcomes as a result of RIPC seem promising (Thielmann *et al.*, 2013), only a very limited number of studies have investigated

RIPC and potential further protection against hypoxia (Foster *et al.*, 2011, 2014; Berger *et al.*, 2015*a*).

High-altitude represents an experimental model that allows for the study of hypoxic adaptation in healthy humans. In addition to marked changes in PASP and cerebral blood flow (Stembridge *et al.*, 2014; Willie *et al.*, 2014), ascent to high altitude is typically associated with a decline in vascular function, as demonstrated by impaired endothelial-dependent flow-mediated dilation (FMD) (Lewis *et al.*, 2014; Bakker *et al.*, 2015) and endothelial-independent dilation (Lewis *et al.*, 2014). Such impairment in vascular function may further compromise the body's ability to tolerate the various stresses associated with hypoxia. Increased sympathetic nerve activity (SNA) and disturbed blood flow may contribute to the hypoxia-associated reduction in FMD (Lewis *et al.*, 2014; Tremblay *et al.*, 2016). Additionally, the impairment may be attributed to an increase in oxidative stress, which may interfere with the intracellular signalling processes required for smooth muscle relaxation (Munzel *et al.*, 2004). Remote ischemic preconditioning reduces oxidative stress after ischemic injuries (Chen *et al.*, 2015), and these same activated pathways may also attenuate the decline in vascular function at high altitude. Furthermore, RIPC has been demonstrated to preserve FMD immediately after strenuous exercise (Bailey *et al.*, 2012), a period that is normally associated with elevations in SNA and a temporary impairment in vascular function (Atkinson *et al.*, 2015; Tymko *et al.*, 2016*b*).

Therefore, the primary aim of this study was to explore the potential protective benefits of one single session of RIPC (4 x 5 min) on integrative vascular function during both acute and chronic exposure to hypoxia. The previously observed reduction in PASP and improved haemoglobin

saturation at altitude in response to RIPC (Foster *et al.*, 2014) could potentially be explained by a larger hypoxic ventilatory response (HVR), although this possibility has not yet been investigated. Therefore, a secondary objective of this study was to examine if RIPC evokes any changes in peripheral chemosensitivity to hypoxia. Finally, based on reports that RIPC offers two distinct protective windows (Koch *et al.*, 2014) - from 0-12 hours, and again from 18-72 hours - measurements were repeated immediately (1 hour), 24 hours and 48 hours after the initial RIPC treatment. We hypothesized that RIPC of the lower limbs would reduce the pulmonary artery pressure increase normally observed in hypoxia (Swenson, 2013), as well as attenuate the hypoxic impairment in FMD (Lewis *et al.*, 2014). We also reasoned that the RIPC intervention would increase the HVR, thereby explaining the previously reported elevations in peripheral oxygen saturation (Foster *et al.*, 2014). Given that >80 million of people live above 2500 meters and many more travel to altitude per year, with 10-85% of these getting some form of altitude illness (Hackett *et al.*, 1976; Maggiorini *et al.*, 1990), determining the impact of RIPC may provide a simple and inexpensive strategy to alleviate high altitude related illnesses.

MATERIALS AND METHODS

This experiment was conducted in two separate parts, with one protocol taking place near sea-level (Kelowna, Canada; 344 m), and the second protocol starting after 8-10 days at high altitude (Barcroft Station, White Mountain, California; 3800 m). This study was a part of a series of experiments that took place over the course of a two-week research expedition to Barcroft Station, starting with rapid ascent to high altitude (3800 m, <6-hour drive) on day one. There was no overlap between participation in this study and participation in other investigations relative to carry over effects of drugs and/or exercise, and the questions addressed in this paper are dealt with exclusively within this study alone. During the entirety of their stay at Barcroft Station,

participants had access to regular meals and fluids *ad lib.* All participants abstained from vigorous exercise, caffeine and alcohol for 12 hours prior to testing, and were asked to consume a light snack two hours before coming to the laboratory.

Participants:

All participants were free of overt cardiovascular, respiratory and cerebrovascular disease, were non-diabetic, and not taking any prescription medications (other than oral contraceptives, n=2) at the time of their participation, as determined by a screening questionnaire. Each subject provided written informed consent prior to arrival at the lab for familiarization. Participants were different for the SL and HA components of the study, with select characteristics described below. This study was approved by the University of British Columbia Clinical Research Ethics board and conformed to standards set by the Declaration of Helsinki, except for registration in a database, and the Canadian Government Tri-Council Policy Statement for Integrity in Research.

Experimental Design:

Part 1: Sea Level (Acute hypoxia)

Participants came into the lab on three consecutive days, where the protocol was performed on the first day (Baseline) and then repeated again at three time points: 1 hour (Day 1), 24 hours (Day 2), and 48 hours (Day 3). The testing protocol is depicted in Figure 1. Immediately after BL, subjects were randomly allocated into either dual-thigh RIPC or a Sham treatment, both of which are described below.

For each session (BL, 1 hour, 24 hours, 48 hours), subjects lay supine while being instrumented for the tests (described further in "Experimental Measurements"). After 20 minutes of supine rest normoxic echocardiographic images were obtained. Next, a baseline FMD test of the brachial artery was performed, followed by 5 minutes of rest, where baseline VE (ventilation), SpO_2% (peripheral oxygen saturation), BP (blood pressure) and MCAv (middle cerebral artery blood velocity) were collected. Subjects breathed on an end-tidal air forcing system (described below) where the end-tidal partial pressures of O_2 and CO_2 ($P_{ET}O_2$ and $P_{ET}CO_2$, respectively) were clamped to match previously measured room air values. Once steady state was achieved, the ultrasonographer began scanning the internal carotid artery (ICA) and obtained at least one minute of satisfactory recordings before the isocapnic hypoxia stage. The sonographer held the image of the ICA in place while $P_{ET}O_2$ was subsequently and rapidly dropped to 50 mmHg, while $P_{ET}CO_2$ was maintained at room-air values. From the establishment of steady state, ICA images were then obtained continuously over the first 10 minutes of hypoxia. Starting at minute 15, a second brachial artery FMD was performed, followed by echocardiographic image acquisition (for PASP) at minute 30. Therefore, room air and hypoxia measures were collected in every visit. The level of hypoxia ($P_{ET}O_2$ = 50 mmHg) was selected in order to provide an approximated, comparable hypoxic stimulus to that experienced during the subsequent HA component of the study at Barcroft station (Severinghaus *et al.*, 1966).

Part 2. High Altitude (Chronic hypoxia)

A similar time profile was used to the sea-level study. Subjects were tested on three consecutive days, between the 8^{th} and 12^{th} day of continuous residence at the Barcroft Station (3800m), where the protocol was performed on the first day (BL), and then repeated at the three time

points: 1 hour (Day 1), 24 hours (Day 2), and 48 hours (Day 3). The experimental protocol is depicted in Figure 2. Immediately after BL, subjects were randomly allocated to either dual thigh- RIPC or a sham treatment which acted as time-based control. Consequent to our limited participant pool at HA, eight participants were selected to receive RIPC in order to more clearly identify any physiological changes after treatment, and the remaining four served as a time-control subset. Cardiorespiratory changes throughout acclimatization have been well-documented, and after the first week, day-to-day changes in HR, VE, and SPO_2 are minimal (Swenson & Bärtsch, 2014).

While resting in the supine position, subjects were instrumented for testing. After 20 minutes of rest, echocardiographic images (for PASP) were acquired, followed by a FMD test of the brachial artery. Baseline ventilatory measurements were then taken followed by end-tidal forcing, clamping $P_{ET}O_2$ and $P_{ET}CO_2$ to BL values. ICA velocity and diameter were measured for at least 1 minute, followed by a subsequent isocapnic drop in $P_{ET}O_2$ to 45 mmHg. This level of hypoxia was selected in order to provide a significant hypoxic stimulus beyond what was already being experienced by the participants at Barcroft station. Once steady state was achieved, ICA measures were taken for 10 minutes, along with measures of VE, SpO_2%, BP and MCAv.

Remote Ischemic Preconditioning:

Participants were seated in a chair with blood pressure cuffs placed around both legs at mid-thigh level. At sea level, an automated rapid inflation system was used to quickly inflate the cuffs to 225 mmHg, whereas at high altitude a manual hand pump was used to increase the cuff pressure. The cuffs remained inflated for 5 minutes, followed by a 5 minute period of deflation allowing

for reperfusion of blood flow to the limb. This process was repeated 4 times, for a total treatment time of 40 minutes (Foster *et al.*, 2014). In the time control and sham conditions, the cuffs were only inflated to <20mmHg. We chose this method of dual-thigh occlusion in order to simulate an easily-reproducible treatment that could be applied on short notice in remote locations, with minimal time and equipment. In practice, it could be used in situations where rapid ascent to altitude is required with short notice, or as a treatment to mitigate risk in those already in a hypoxic setting.

Experimental Measurements:

Cardiorespiratory

In both studies at sea level and high altitude, cardiorespiratory variables were sampled continuously throughout the protocol at 1KHz via an analogue-to-digital converter (Powerlab, 16/30; ADInstruments, Colorado Springs, CO). A 3-lead electrocardiogram (ADI bioamp ML132) was used to measure heart rate (HR), and beat-to-beat blood pressure was recorded by finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands). The Finometer reconstructed brachial waveform was used for calculation of mean arterial pressure (MAP) after back-calibrating to the average of three automated brachial blood pressure cuff measurements made at rest (Tango+; Suntech, Morrisville, NC). Respiratory flow and minute ventilation (V_E) was measured by a pneumotachograph (HR800L, HansRudolph, Shawnee, KS) connected in series to a bacteriological filter, and a calibrated gas analyzer (ML206, ADInstruments) was used to record the partial pressure of both end-tidal CO_2 and end-tidal O_2, sampled at the level of the mouth. Arterial O_2 saturation was measured continually using pulse oximetry (ADInstruments). All measures, unless otherwise stated, are reported as

averages over 1-minute bins. In the acute hypoxia protocol, hypoxic cardio-respiratory measurements are taken between minutes 25-30 of isocapnic hypoxia. In the chronic hypoxia protocol, hypoxic measurements are taken between minutes 9-10 of isocapnic hypoxia. These time-frames were chosen as the most suitable representations of steady-state for each test.

End-tidal forcing

A dynamic end-tidal forcing system was used to control $P_{ET}CO_2$ and $P_{ET}O_2$ during the normoxic and isocapnic hypoxic periods of the protocols. This system has previously been described in detail (Tymko *et al.*, 2015, 2016*a*), and is able to effectively control end-tidal gases independent of ventilation at low and high altitudes. $P_{ET}CO_2$ was kept constant at resting room air values throughout the two protocols, while $P_{ET}O_2$ was rapidly dropped during the room-air to hypoxia transition until steady-state was achieved; this was determined as at least three consecutive breaths within 1 mmHg of the desired target.

Peripheral chemosensitivity

Following the onset of hypoxia, ventilatory and end-tidal data were averaged into 15-second bins. The HVR was calculated using the peak 15-second bin of ventilation following the transition from normoxia to isocapnic hypoxia on the end-tidal forcing system (described above). This peak 15s bin was compared to a 30s bin of data collected immediately prior to the hypoxic drop, and the HVR was thus calculated using the formula: $HVR = \Delta VE / \Delta ScO_2\%$, with ScO_2 (calculated oxygen saturation) calculated from the end-tidal O_2 trace using the equation described by Severinghaus, (Severinghaus, 1979). We used this equation to calculate saturation over pulse oximetry in order to more accurately reflect the timing of changes in blood oxygenation.

Flow-mediated dilation

Reactive hyperemia flow-mediated dilation was performed according to internationally-recognized guidelines (Thijssen *et al.*, 2011). Participants were lying supine with their left arm extended in a fixed position ~80 degrees perpendicular from their body. All measurements were taken after at least 20 minutes of supine rest in a quiet, dark room. 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 images were acquired by the same experienced ultrasonographer (JCT), 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 220-250 mmHg for five-minutes. Recordings of diameter and velocity continued 30-seconds prior to cuff deflation and continuously for three-minutes thereafter. Video recordings were anonymized and stored for later offline analysis using specialized edge-detection software (Woodman *et al.*, 2001; Thijssen *et al.*, 2011). Flow-mediated dilation was calculated as the peak increase in diameter following cuff deflation. The FMD stimulus was calculated as the shear rate area under the curve (SRAUC) from the onset of reactive hyperemia to FMD (Pyke & Tschakovsky, 2007).

Measurement of extra- and intra-cranial blood flow

A 10MHz multi-frequency linear array duplex ultrasound (Terason T3200, Teratech, Burlington, MA) was used to measure blood velocity and diameter of the internal carotid artery (ICA). These recordings were acquired using simultaneous B-mode imaging (diameter) and pulse-wave mode (velocity). In order to eliminate recordings of turbulent and retrograde flow, measurements were

taken at least 1.5cm distal to the common carotid bifurcation. To avoid any artificial changes in arterial wall brightness/thickness, there was no alteration in B-mode gain upon acquisition of the first ultrasound image. Data were anonymized and later analysed offline using the same edge-detection software as described above, and values are reported as an average over a minimum of 12 consecutive cardiac cycles. Flow-reactivity was calculated using a 30-second bin encompassing the peak response to hypoxia.

Blood velocity through the left middle vertebral artery (MCAv) was recorded using a 2MHz transcranial Doppler ultrasound (Spencer Technologies, Seattle, WA). A specialized headband (model M600 bilateral head frame, Spencer Technologies) was used to secure the probe in place. Insonation was achieved through the trans-temporal window using previously described location and standardization techniques (Willie *et al.*, 2011), and data are reported as the average across selected 30-second bins during each stage.

Echocardiography

All echocardiographic images were obtained by the same experienced sonographer on a commercially available ultrasound machine (Vivid Q (Sea-Level) / E9 (High Altitude), GE, Fairfield, CT). M5-S 1.5-4.6 MHz and 4V 1.5-40 MHz transducers were used to collect echocardiographic images, which were saved for offline analysis (Echopac v.113, GE, Fairfield, CT). Subjects lay in the supine left lateral decubitus position. The modified Bernoulli equation was used to calculate pulmonary artery systolic pressure, where $PASP = 4V^2 + 5mmHg$, where V equals the peak tricuspid regurgitation velocity and 5 mmHg was added for right atrial pressure where the inferior vena cava collapsed under inspiration (Lawrence G Rudski MD *et al.*, 2010). Measures of PASP are reported using the average of at least three cardiac cycles.

Statistical Analysis:

Based upon previous studies investigating FMD and PASP during hypoxia, sample sizes of 9-12 (Foster *et al.*, 2014; Jones *et al.*, 2014*b*; Lewis *et al.*, 2014) were able to show significant changes. We therefore attempted to have >10 participants in each group; however, the time allocated and subject availability for this study only allowed us to have 8 participants in each group at SL, and a small subset of four time-control participants in the HA trial. For example, during isocapnic hypoxia at SL, if we were to assume a PASP of 30 mmHg, and wished to detect a 10% reduction following RIPC, with a standard deviation of the differences of 2.5 mmHg, a power of 0.8 and alpha of 0.05, we would need 10 participants. Results from this study must therefore be treated as preliminary, rather than confirmatory and further studies employing larger sample sizes are needed.

In Study 1 (Acute hypoxia), a two-way repeated measures ANOVA was used for the cardiovascular measures in each treatment group (Factors: Time & O_2). In addition, a two-way repeated measures ANOVA (Factors: Time & Treatment) was used to identify differences in the HVR and ICA reactivity, as well as the change from normoxia to hypoxia for FMD and PASP. In study 2 (Chronic hypoxia), independent one-way ANOVAs were used on both the RIPC group and the time-control group (Factor: Time). Upon detection of significant main effects, pairwise comparisons were made using Dunnett's t-tests. While it has recently become convention to allometrically scale FMD changes (Atkinson & Batterham, 2013) and scale for SRAUC as a covariates (Atkinson & Batterham, 2015) baseline diameter and SRAUC did not differ between groups in this study, and were therefore not corrected for. Furthermore, normality

of all main outcome variables (FMD, PASP, qICA, VE, and HVR) was confirmed using a Shapiro-Wilk test. All data were analysed using SPSS (version 24, IBM, Surrey, UK). Results are reported as mean ± standard deviation unless otherwise indicated. Statistical significance was defined as $P<0.05$.

RESULTS:

Part 1. Sea level (acute hypoxia)

Cardiovascular responses

Table 2 presents cardiovascular and respiratory variables during baseline and during isocapnic hypoxia ($P_{ET}O_2$ clamped to 50 mmHg). There were no significant differences in the magnitudes of responses to hypoxia in HR (RIPC: P=0.91; Sham: P=0.91), MAP (RIPC: P=0.51, Sham: P=0.37), or SpO_2 (RIPC: P=0.84, Sham: P=0.98) at any time point in either the RIPC or Sham condition.

Pulmonary artery pressures

Pulmonary pressures during normoxia and hypoxia are presented in Table 2**.** One subject was excluded from each group due to a lack of suitable echocardiographic images for measuring tricuspid regurgitation; thus, statistical analyses was based on seven participants in each group. While hypoxia consistently increased PASP during each trial ($P<0.01$ for both RIPC and Sham), the magnitude of this rise did not differ between trials (Figure 3**,** P=0.60).

Flow-mediated dilation

Brachial artery flow-mediated dilation (%) responses during normoxia and hypoxia are shown in Table 2. There were no differences in baseline diameter (RIPC: P=0.67, Sham: P=0.83) or SRAUC (RIPC: P=0.40, Sham: P=0.99), and subsequently no changes in FMD response at any time in either condition. The FMD (% dilation) is presented in Table 2**.**

ICA flow and intra-cranial velocity

Isocapnic hypoxia resulted in a comparable increase in blood flow through the ICA (P<0.01 for both RIPC & Sham) and blood velocity in the MCA (P<0.01 for both RIPC & Sham) in both conditions; although neither response differed between RIPC and Sham at any point through the protocol (Table 2 for MCA & Figure 3 for ICA).

Ventilation

Ventilation was elevated during hypoxia (P<0.01 for both RIPC and Sham); however there were no differences in ventilation across any of the testing times (RIPC: P=0.58, Sham: P=0.89, see Table 2). The HVR (Figure 3) was also unaffected by either RIPC or sham treatment (P=0.45).

Part 2. Chronic hypoxia (high altitude)

Cardiovascular responses

Table 3 presents selected resting cardiorespiratory variables after 8-12 days at high altitude, while breathing room air, as well as during isocapnic hypoxia. There were no significant changes within each condition (normoxia vs. hypoxia) in HR, MAP, or SPO_2 in either the RIPC or the Sham group (P>0.05 for all).

Pulmonary artery pressures

Reliable echocardiographic images were obtained from 7 participants from the RIPC group and 4 from the time control group. PASP (Figure 4) was unchanged from baseline after treatment in either group (RIPC, P = 0.64 vs. time-control, P=0.88).

Flow-mediated dilation

FMD of the brachial artery did not differ from baseline at any point after RIPC (P=0.89) or after time-control (P=0.41) (Figure 4). There were no significant differences in baseline diameter or SRAUC in either group (P>0.05 for all).

ICA flow and intra-cranial velocity

Blood flow through the ICA and velocity in the MCA both increased upon the transition from room air to isocapnic hypoxia (P<0.05 for both, see Table 3), although the magnitude of this increase did not change after either treatment (RIPC, P=0.51 and time-control, P=0.39).

Ventilation

Resting room air ventilation was unaffected by time in either treatment group; however, the hypoxic ventilatory response was higher than baseline after 24 hours treatment with RIPC. For example, HVR at baseline was 2.05±1.4 L·min/% $SaO_{2,}$ compared to 1 hour (2.44±1.3, p=0.80), 24 hours (3.21±1.2, p=0.04), and 48 hours (2.79±1.5, p=0.21) post RIPC (Figure 5). This increase was driven by an elevation in VE peak (P=0.02) as well VE average (P=0.03) during hypoxia, 24 hours after RIPC treatment (Table 3).

DISCUSSION

The aim of this study was to explore the potential protective effects of a single session of 4x5 minutes of RIPC on the peripheral, cerebral, and pulmonary vasculature during exposures to acute and chronic hypoxia. By using tightly controlled hypoxic episodes within the laboratory, as well prolonged high altitude exposure in the field, we were able to test early and late protective windows of RIPC in acute and chronic hypoxic models. We hypothesized that our method of RIPC would induce prophylactic vascular benefits during exposure to acute hypoxia, as well as therapeutic benefits during chronic hypoxia. Contrary to our hypotheses, we did not observe any discernable benefits in the form of reduced PASP, improved peripheral vascular function (i.e., FMD), or alterations in cerebral perfusion or cerebrovascular reactivity to hypoxia. We do, however, report an increase in the isocapnic HVR in the late protective window (24 hours) after RIPC treatment at high altitude (3800 m). Although hypoxic chemosensitivity may increase following RIPC at HA, the absence of any accompanying benefits on resting oxygenation or the vasculature suggests that the utility of a single RIPC session seems unremarkable during acute and chronic hypoxia.

Experimental Paradigms of RIPC

Previous research investigating the utility of RIPC has been largely focused on clinical populations with the goal of improving outcomes after stroke (Dave *et al.*, 2006; Ren *et al.*, 2008)**,** myocardial infarction (Gho *et al.*, 1996; Costa *et al.*, 2013; Man *et al.*, 2017), and a number of ischemia related injuries [reviewed in: (Tapuria *et al.*, 2008)]. RIPC appears to reduce biomarkers of tissue and neuronal damage after numerous surgical interventions [reviewed in: (Candilio *et al.*, 2012)]; however, two recent large scale clinical trials (with concurrent propofol use) have failed to show an improvement in clinical outcomes when using RIPC prior to coronary artery bypass grafting (Hausenloy *et al.*, 2015; Meybohm *et al.*, 2015).

Despite extensive research into clinical utility, very little research has investigated whether RIPC elicits vascular benefits to those exposed to hypoxia, and if a minimum stimulus is required to elicit any of the potential benefits. For example, RIPC can be structured a number of different ways, with ischemic episodes ranging from 4-10 minutes, repeated 3-6 times within a treatment, and the volume of total treatments ranging from one or two days, to weeks/months of consecutive daily treatment (Eisen *et al.*, 2004; Meng *et al.*, 2012). We sought to pursue a simple, conservative, and potentially practical form of treatment, using 4 consecutive 5-minute periods of dual-thigh cuff occlusion, interspersed with 5 minutes of reperfusion, for one single forty-minute session. This approach was chosen as it is logistically simple, and would be easy to perform as a field tool in normal trekking circumstances. We took repeated measurements at 3 separate points after the treatment to evaluate differences in previously established early (1 hour-16 hours) and late (18 hours – 2-3 days) protective windows (Koch *et al.*, 2014). While the protective effects of RIPC have commonly been attributed to the upregulation of anti-oxidative and anti-inflammatory pathways, the exact pathways being utilized seem to vary based on the target organ, and the mechanisms of activation are still unclear. Further, how these pathways translate into physiological changes is relatively unknown.

RIPC and peripheral vascular function

In humans, long-term RIPC (daily, 1 month) has been associated with improvements in resting endothelial function (Kimura *et al.*, 2007). This is likely due to RIPC-triggered signalling for the upregulation of the NO system (Gattullo *et al.*, 1999; Kimura *et al.*, 2007; Arroyo-Martínez *et al.*, 2016) as well as the release of vasoactive molecules such as adenosine and bradykinin (Lim & Hausenloy, 2012)**,** which may trigger increases in reactivity of the endothelial lining. Furthermore, recent research suggests that RIPC treatment may reduce sympathetic tone

(Lambert *et al.*, 2016), which would likely alter reactivity of the peripheral arteries to changes in shear stress. Improvements in resting FMD in healthy subjects have also been reported after 7 consecutive days of RIPC treatments (Jones *et al.*, 2014*a*), and it has been demonstrated that one session of RIPC offers protection against ischemia-reperfusion injury-induced (Liu *et al.*, 2015), and exercise-induced (Bailey *et al.*, 2012) impairments in FMD. However, in this latter study, and consistent with our findings, it was reported that resting FMD immediately after one treatment was unchanged. Our findings suggest that baseline and hypoxic FMD responses are unaffected by single-session RIPC treatment, although we also report no significant reduction in FMD upon the acute transition from normoxia to hypoxia. Two probable causes may contribute to this finding: the duration or level of hypoxia was too small to elicit a reduction in FMD (measured after 15 minutes in acute hypoxia); or, the magnitude of reduction in FMD was too small to identify with our sample size. There is emerging evidence that impairment in FMD is not a uniform response to hypoxia and may depend on severity, duration, concomitant exercise (Lewis *et al.*, 2014; Tremblay *et al.*, 2016; Tymko *et al.*, 2016*b*)**,** or the presence of cardiovascular risk factors (Frøbert *et al.*, 2008). In the present study, failure to alter FMD with RIPC could subsequently be explained by the lack of a meaningful vascular impairment in our model of hypoxia.

RIPC and pulmonary vascular tone

It has been previously reported that a single session of RIPC prior to breathing a hypoxic gas mixture for 90 minutes blunts the rise in PASP (Foster *et al.*, 2011). Furthermore, after pre-treatment with 5 consecutive days of RIPC prior to rapid ascent to high altitude (followed by an

exercise challenge), the rise in PASP was also mitigated (Foster *et al.*, 2014). Mechanisms for this attenuation of hypoxic pulmonary vasoconstriction remain speculative and are poorly understood. During the chronic hypoxia (HA) component of our study, the rise in PASP could be at least partially attributed to a degree of pulmonary vascular remodelling, a complex process with proliferative and inflammatory components (Stenmark *et al.*, 2006) that may be affected by RIPC, however the magnitude and time course of remodelling with hypoxia is still unclear. Results from our study suggest there is no change in the amplitude of the PASP response to hypoxia after RIPC in either acute or chronic hypoxia; however, our measures were taken only 30 minutes into the acute hypoxic challenge at point where PASP is still rising (Dorrington *et al.*, 1997). Furthermore we used a single session versus a 5-day repeated protocol, which may lead to dose dependent effects. Different, overlapping mechanisms may be responsible for the initial rapid increase, and subsequent slow intensification of pulmonary artery pressures (Vejlstrup *et al.*, 1997) in response to hypoxia, and delaying our measurements to 90-120 minutes may have yielded different results. However, at altitude, where subjects were hypoxic for 8-12 days, RIPC failed to elicit any changes in PASP indicating that time of measurement likely does not explain our lack of effect at sea level. At high altitude, all participants exhibited a moderate elevation in PASP that is comparable with other high altitude studies (Antezana *et al.*, 1998). Based on reports of reduced measures of NO-bioavailability and increased production of endothelin-1 (Sartori *et al.*, 1999; Bailey *et al.*, 2010), we speculate that RIPC may differentially affect those with exaggerated HPV responses who are susceptible to high altitude pulmonary edema, although this hypothesis has yet to be tested.

RIPC and peripheral chemosensitivity

A novel finding of this study is that RIPC augments the HVR during chronic hypoxia (Figure 4). We did not attempt to investigate any mechanisms underlying changes caused by RIPC, although this finding broadly supports previous observations by Foster and colleagues (Foster *et al.*, 2014) that RIPC improves oxygen saturation upon ascent to high altitude. Sensitivity of the carotid body relies on the balance of numerous systemic factors, many of which vary significantly on a daily basis, leading to a large daily intra-individual variation in the hypoxic ventilatory response. Mechanisms underpinning the observed increase in chemosensitivity are purely speculative, but might involve increased release of neurotransmitters such as 5-hydroxytryptamine, or a hypoxia-inducible factor-1 aided improvement in oxygen sensing (Nurse, 2010; Prabhakar & Semenza, 2016).

Methodological considerations

A large amount of uncertainty still exists over the nature and magnitude of the ischemic stimulus required to optimally elicit the desired protective benefits of RIPC. An emerging pattern seems to suggest that there may be a dose-response relationship, i.e. thigh occlusion is more effective than arm occlusion; dual thigh treatment is more effective than single thigh; and more ischemic repeats and more consecutive days of treatment have an additive effect compared to single sessions (Koch *et al.*, 2014). Inevitably, caution must be exercised, since the goal of ischemic preconditioning is to deliver small, non-lethal bouts of ischemia to an area, rather than creating an actual ischemic injury in the distal tissue. If we had utilized a longer duration of ischemia, more repeats, or added consecutive days of treatment we may have observed different results; however, the aim of this study was to explore the efficacy of one simple session of RIPC, with the goal of offering a simple prophylactic treatment to those travelling to high altitude on short

notice, or a quick therapeutic and practical aid for those who may be experiencing signs of mountain sickness.

With a treatment such as RIPC, it is difficult to deliver a true "sham" protocol, as it is impossible to blind a subject to the obvious physical sensations associated with cuffs being inflated to 225 mmHg over the thighs. For this reason, naïve subjects were verbally informed that the aim of the study was to explore differences between arterial (200mmHg) and venous (20mmHg) occlusion during their respective trials.

We also acknowledge that the lack of an equally-weighted control group in the high altitude arm; however, with limited time and a small participant pool afforded to the study, we were only able to use four subjects to act as a time control. The most significant changes in acclimatization status occur within the first few days of arrival to high altitude (Swenson & Bärtsch, 2014), and we observed no changes in ventilation or any other physiological parameters in our time control group, suggesting that any acclimatization occurring between days 8-12 over the course of the study had little effect on our findings.

Given the small sample size and lack of a repeated-measures control, these data must be interpreted judiciously and considered more exploratory than confirmatory. For example, power calculations indicate that a much larger sample size (n>250) would have been needed to observe significant changes (>1%) in FMD with a power of 0.8. However, this is not feasible in high altitude research and supports the negligible influence of RIPC on FMD in hypoxia.

Conclusions

Our preliminary results indicate that one session of 4x5 minute dual-thigh RIPC treatment seems to have no benefit in terms of pulmonary vascular protection or preserving peripheral endothelial

function during acute and chronic hypoxia. Continued exploration of potential protective benefits that may be derived from utilizing different RIPC treatment strategies may be useful. An improved HVR 24 hour after preconditioning during chronic hypoxia suggests that RIPC can influence the hypoxic chemosensitivity of the carotid body. The HVR has been positively related to exercise performance at high (<6100 m) altitudes (Schoene *et al.*, 1984) however, since resting SpO_2 was unchanged, and an enhanced peripheral chemosensitivity may augment periodic breathing at altitude [reviewed in: (Ainslie *et al.*, 2013)]- whether these changes are of advantage seems unlikely.

ADDITIONAL INFORMATION

Competing Interests

The authors have no conflict of interest.

Author contributions

All authors contributed to data collection, critically assessed the manuscript for scientific content and approved the final manuscript. Study Conception: MGR, RLH, PJS, JNA, PNA; Data Analysis: MGR; Data interpretation: MGR, RLH, JCT, MS; Drafting manuscript: MGR, RLH, PNA.

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FIGURES:

Figure 1. Schematic of the experimental protocol for Part 1 of the study representing acute hypoxia, performed near sea level (Kelowna, 344m). PASP = pulmonary artery systolic pressure measurement; FMD = flow-mediated dilation measurement, Q_{ICA}=blood flow through the internal carotid artery; HVR = period of assessment of the hypoxic ventilatory response.

Figure 2. Schematic of the experimental protocol for Part 2 of the study, during hypoxia. Performed at Barcroft Station after 8-12 days at high altitude (White Mountain, 3800m). PASP = time of pulmonary artery systolic pressure measurement; FMD = time of flow-mediated dilation measurement, Q_{ICA}= time of measurement of blood flow through the internal carotid artery; HVR = period of assessment of the hypoxic ventilatory response.

Figure 3: Acute Hypoxia. Selected vascular and respiratory responses to hypoxia at baseline, and 1 hour, 24 hours, and 48 hours after dual-thigh RIPC. **A)** Absolute increase in pulmonary artery systolic pressure (PASP) from normoxia to 30 minutes of hypoxia. **B)** Change in brachial artery flow-mediated dilation (FMD) response from normoxia to hypoxia (i.e. normoxia % - hypoxia %) **C)** Peak flow-reactivity of the internal carotid artery (ICA) upon the transition from normoxia to hypoxia. **D)** The hypoxic ventilatory response (HVR). Black bars represent means for RIPC, grey bars represent means for Sham. End-tidal partial pressure of O_2 was clamped at 50 mmHg under isocapnic conditions in the hypoxia phase. Testing was to model acute hypoxia, performed at low-altitude (344m). Individual data points are shown, bars represent group means. A 2x4 ANOVA (factors: time, treatment) was used to identify significant differences, $P>0.05$ for all interactions.

Figure 4. Chronic hypoxia. Selected vascular and respiratory parameters at baseline, and 1 hour, 24 hours, and 48 hours after dual-thigh RIPC or time control, after 8-12 days at high altitude (3800m). **A)** Pulmonary artery systolic pressure (PASP), **B)** Flow-mediated dilation (FMD) of the brachial artery, **C)** Peak hypoxic flow-reactivity of the internal carotid artery (ICA), **D)** The hypoxic ventilatory response (HVR). Black bars represent means for RIPC, grey bars represent means for Time control. For C) & D), end-tidal partial pressure of O_2 was clamped to 45 mmHg under isocapnic conditions in the hypoxia phase. Individual data points are shown, bars represent group means. ANOVA was used to determine the effect of time for each condition. *P-value represents difference from baseline.

Table 1. Study subject characteristics

	Acute Hypoxia		**Chronic Hypoxia**	
Condition	RIPC	Sham	RIPC	Time Control
N (females)	8 (3)	8 (3)	8 (3)	4 (0)
Age (years)	24.1±3.6	24.7±3.7	23.9±3.4	27.5±2.9
Weight (kg)	69.1±6.2	71.3±9.6	70.4±9.9	74.75±10.2
Height (cm)	173.3±6.2	175.7±4.6	177.1±5.4	175.0±2.2

There were no differences in subject demographics (age, height, weight) between treatment conditions or between acute and chronic hypoxia branches of the study ($P>0.05$).

Table 2. Acute Hypoxia

		RIPC				Sham			
		BL	1 hour	24 hours	48 hours	BL	1 hour	24 hours	48 hours
FMD (%)	*normoxia*	7.74±3.0	7.90±2.7	6.78±2.3	7.16±2.2	7.90±2.7	8.15±2.8	8.05±2.9	6.92±2.4
	hypoxia	7.24±2.9	7.34±2.3	6.24±2.8	6.77±3.4	7.45±3.0	7.47±2.3	7.36±2.8	6.56±2.8
		Time: P = 0.652, O_2: P=0.235, Interaction P=0.856				Time: P = 0.623, O_2: P=0.187, Interaction P=0.995			
PASP (mmHg)	*normoxia*	22.3±3.6	21.9±3.2	21.7±4.9	21.1±2.5	22.53±2.1	23.00±2. 2	23.4±2.46	22.7±2.71
	hypoxia	25.2±3.9	25.3±3.4	24.5±5.0	24.1±4.9	26.1±2.1	25.8±3.0	28.9±3.2	26.4±2.6
		Time: P = 0.999, O_2: P=0.009, Interaction P=0.993				Time: P = 0.652, O_2: P<0.001, Interaction P=0.991			
ICA flow (mL/min)	normoxia	242.7±30.2	232.5±38.3	237.8±42.2	235.9±32.8	230.6±31.0	231.8±23.3	220.8±32.8	213.3±82.8
	hypoxia	286.1±42.1	282.6±41.9	277.4±67.8	276.2±54.2	294.4±6	293.7±46	275.8±41	249.9±98
		Time: P = 0.325, O_2: P=0.003, Interaction P=0.561				Time: P = 0.767, O_2: P<0.001, Interaction P=0.880			
MCAv (cm/s)	*normoxia*	56.6±3.6	54.7±3.1	55.9±5.9	56.8±6.5	58.4±14.7	61.2±12.9	57.2±9.8	57.5±10.3
	hypoxia	65.3±7.5	65.2±5.9	63.3±4.4	62.4±9.5	67.3±16.0	68.0±15.7	67.1±10.1	62.7±12.1
		Time: P = 0.877, O_2: P<0.001, Interaction P=0.924				Time: P = 0.430, O_2: P<0.001, Interaction P=0.343			
HR (bpm)	*normoxia*	66.0±18.6	66.2±16.0	64.1±12.5	63.8±11.8	63.9±12.7	68.1±13.3	64.1±12.7	64.0±14.0
	hypoxia	71.5±11.8	71.8±14.4	71.5±12.3	70.4±10.4	75.7±18.4	77.9±16.4	72.9±14.5	76.1±9.7
		Time: P = 0.949, O_2: P<0.001, Interaction P=0.909				Time: P = 0.899, O_2: P<0.001, Interaction: P=0.906			
MAP (mmHg)	*normoxia*	89.9±8.4	92.2±13.7	93.4±17.9	87.2±7.3	85.1±8.3	86.4±7.0	84.8±7.0	82.5±10.8
	hypoxia	89.9±14.4	93.0±13.4	88.0±13.7	86.3±9.7	88.3±7.2	86.4±10.9	85.1±10.6	89.2±7.3
		Time: P = 0.342, O_2: P=0.312, Interaction P=0.505				Time: P = 0.945, O_2: P=0.421, Interaction: P=0.790			
VE (l/min)	*normoxia*	11.5±2.6	11.9±1.8	11.3±1.8	11.4±1.3	11.4±2.6	12.6±2.4	11.4±1.1	11.1±2.5
	hypoxia	20.7±4.9	20.9±5.5	20.0±3.7	21.0±6.6	19.6±5.9	18.4±4.4	19.1±4.7	19.3±4.6
		Time: P = 0.577, O_2: P<0.001, Interaction P=0.514				Time: P = 0.889, O_2: P<0.001, Interaction: P=.631			
$PETCO_2$ (mmHg)	*normoxia*	40.7±3.0	39.3±3.3	40.7±2.7	41.0±2.8	40.3±1.6	40.5±2.6	40.5±0.8	41.2±2.3
	hypoxia	41.7±2.3	40.6±3.0	40.2±2.6	41.3±2.3	41.6±2.9	41.0±2.5	41.5±3.0	41.7±2.4
		Time: P = 0.798, O_2: P=0.342, Interaction P=0.956				Time: P = 0.821, O_2: P=0.344, Interaction: P=0.999			
$PETO_2$ (mmHg)	*normoxia*	95.9±4.3	96.5±3.3	95.5±5.2	94.9±7.1	99.3±3.9	100.1±5.2	99.4±3.4	98.7±4.8

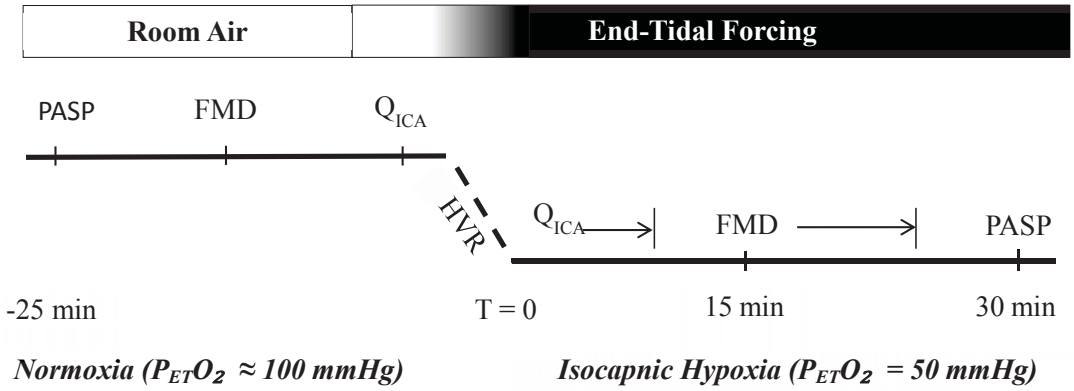
	hypoxia	49.8±1.6	50.0±1.2	50.3±1.9	50.4±1.7	49.8±0.9	49.9±1.3	50.1±1.5	49.4±0.8
		Time: P = 0.879, O_2: P<0.001, Interaction P=0.853				Time: P = 0.967, O_2: P<0.001, Interaction: P=0.999			
SPO_2 (%)	*normoxia*	98.4±1.0	97.9±1.2	97.9±1.0	97.3±1.4	98.1±1.2	98.2±0.9	97.5±1.4	97.7±1.3
	hypoxia	82.6±3.6	83.0±4.1	83.0±4.2	83.1±3.6	80.5±3.7	79.7±4.3	80.0±3.8	81.0±3.4
		Time: P = 0.999, O_2: P<0.001, Interaction P=0.843				Time: P = 0.899, O_2: P<0.001, Interaction: P=0.976			

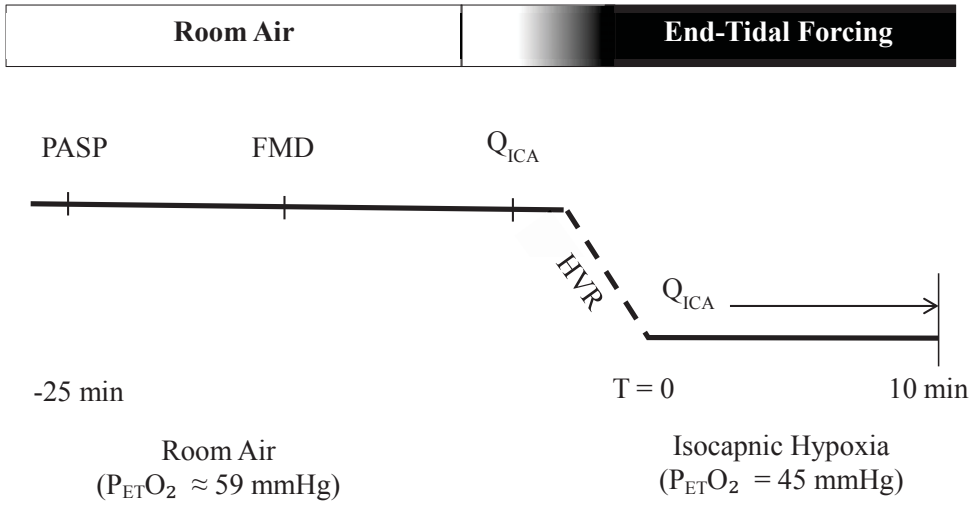
Acute hypoxia. Selected cardiovascular and respiratory parameters at baseline, and 1 hour, 24 hours, and 48 hours after dual-thigh RIPC, during room-air breathing and acute hypoxia. End-tidal partial pressure of O_2 was clamped to 50 mmHg under isocapnic conditions in the hypoxia phase. Testing was performed at low-altitude (344m). Data represents group means ± SD. A 2-way ANOVA was used to evaluate statistical differences in each group.

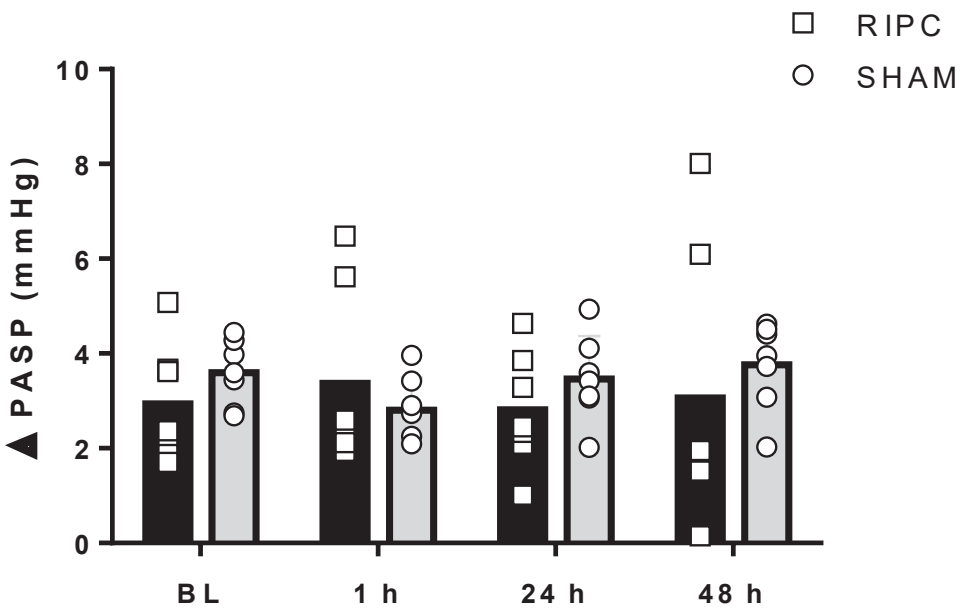
Table 3. Chronic Hypoxia

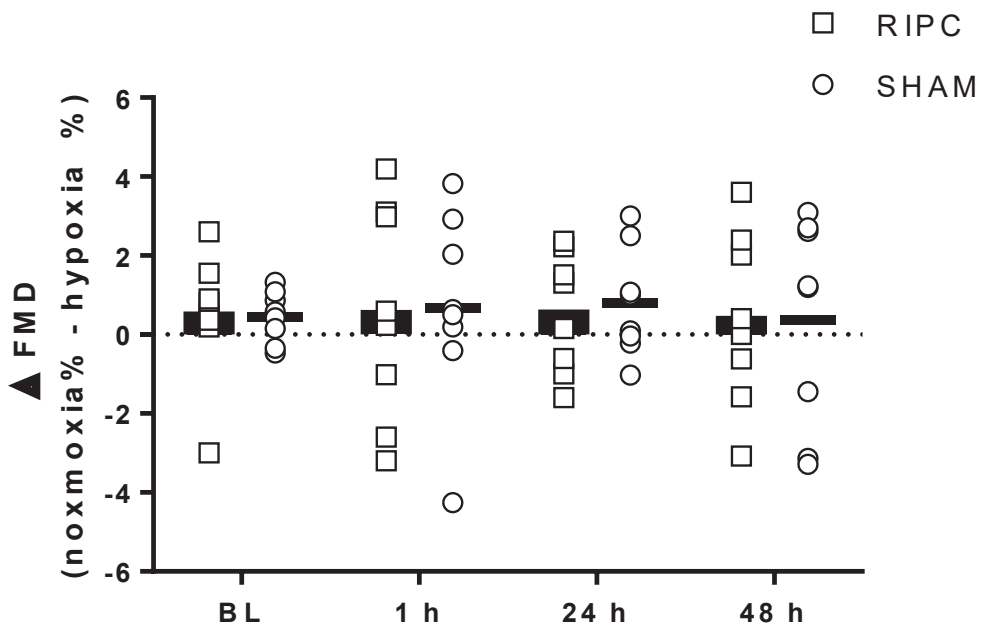
	RIPC					Time control				
	Baseline	1 hour	24 hours	48 hours	P-value	Baseline	1 hour	24 hours	48 hours	P-value
Room Air (3800m)										
HR (bpm)	67.4±11.3	68.2±14.0	67.0±13.1	66.4±10.3	0.53	59.3±18.8	58.0±16.0	60.1±16.3	59.9±12.9	0.35
MAP (mmHg)	95.2±4.6	90.5±10.3	93.8±8.2	93.6±6.2	0.63	92.5±6.5	98.7±6.9	89.7±12.6	94.2±10.8	0.77
SPO_2 (%)	90.2±2.1	89.6±1.4	89.2±1.1	89.3±1.9	0.59	87.5±4.1	90.3±3.0	88.8±2.0	89.5±0.6	0.6
VE (l/min)	10.8±3.9	13.8±2.7	14.1±1.4	13.6±1.4	0.22	12.1±1.4	12.8±0.9	13.0±0.5	13.1±1.5	0.55
$PETO_2$ (mmHg)	59.5±2.8	59.0±1.9	58.7±2.7	58.4±1.5	0.32	53.6±2.9	55.9±4.4	55.0±3.0	55.7±0.6	0.31
$PETCO_2$ (mmHg)	28.1±1.8	27.9±1.6	28.4±1.9	28.4±1.9	0.93	30.8±1.9	30.0±2.2	30.9±1.9	30.6±0.6	0.52
MCAv (cm/s)	52.7±11.6	53.7±10.7	51.4±8.8	54.1±10.2	0.64	61.7±4.0	62.5±4.2	62.8±6.8	58.4±7.9	0.41
ICA flow (mL/s)	227.0±46.7	246.8±34.2	248.1±25.2	238.9±25.6	0.31	262.0±27.0	290.3±31.7	262.0±47.0	521.0±28.4	0.62
Isocapnic Hypoxia ($PETO_2$ = 45mmHg)										
HR (bpm)	73.1±11.7	74.4±13.6	72.8±14.9	72.9±13.2	0.31	61.5±17.7	62.2±17.8	66.4±19.2	67.9±17.9	0.47
MAP (mmHg)	102.3±8.2	99.5±10.4	102.5±7.8	99.3±9.3	0.6	96.7±1.4	96.2±12.6	89.7±4.0	97.8±15.3	0.32
SPO_2 (%)	78.0±2.0	78.6±2.9	78.2±3.2	79.0±2.2	0.54	79.5±3.2	80.2±0.5	80.0±1.75	78.9±1.9	0.85
MCAv (cm/s)	61.5±12.0	59.6±13.5	58.8±11.2	61.3±10.9	0.71	66.1±3.3	66.7±7.1	65.5±10.8	67.0±5.6	0.64
ICA flow (mL/min)	296.5±26.0	308.2±37.4	291.1±23.3	297.0±38.2	0.73	296.6±29.1	302.3±52.0	350.2±25.5	334.4±38.6	0.88
VE peak (L/min)	29.2±16.7	37.2±15.0	42.6±11.1*	40.7±15.2	**0.02**	32.7±15.4	30.9±10.6	36.4±18.2	37.6±11.3	0.51
VE average (L/min)	18.2±9.0	23.8±6.1	28.9±8.3*	25.7±11.7	**0.04**	25.5±11.0	24.0±12.8	25.8±7.6	30.6±6.4	0.48

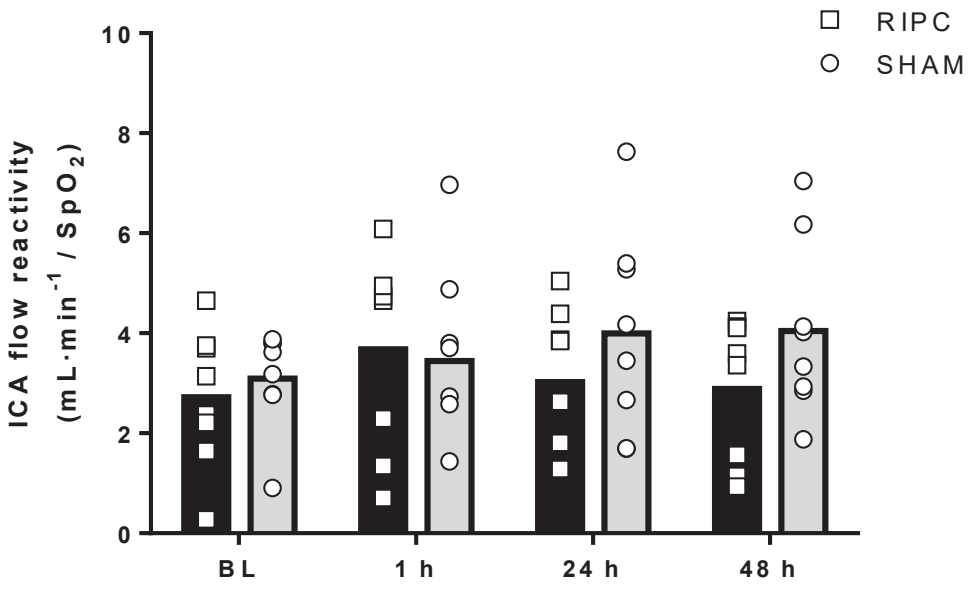
Selected vascular and respiratory parameters at baseline, and 1 hour, 24 hours, and 48 hours after dual-thigh RIPC or time control, after 8-12 days at high altitude (3800m). End-tidal partial pressure of O_2 was clamped to 45 mmHg under isocapnic conditions in the hypoxia phase. P-value represents between-groups significance. * represents difference from baseline ($P<0.05$).

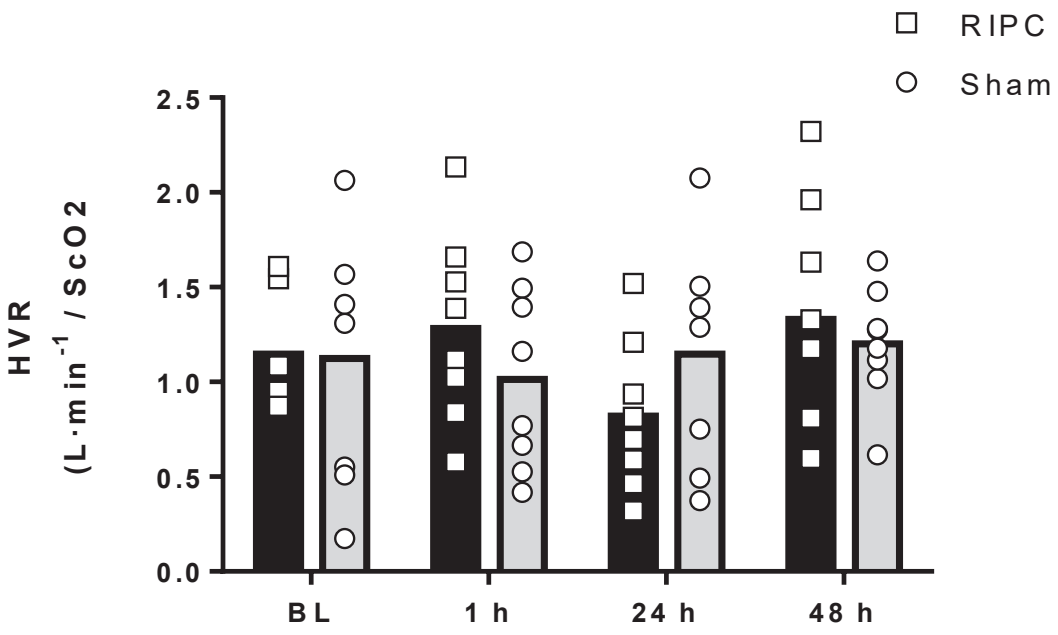

Room Air
End-Tidal Forcing
PASP
FMD
Q_{ICA}
HVR
Q_{ICA}
FMD
PASP
-25 min
T = 0
15 min
30 min
Normoxia ($P_{ET}O_2$ ≈ 100 mmHg)
Isocapnic Hypoxia ($P_{ET}O_2$ = 50 mmHg)

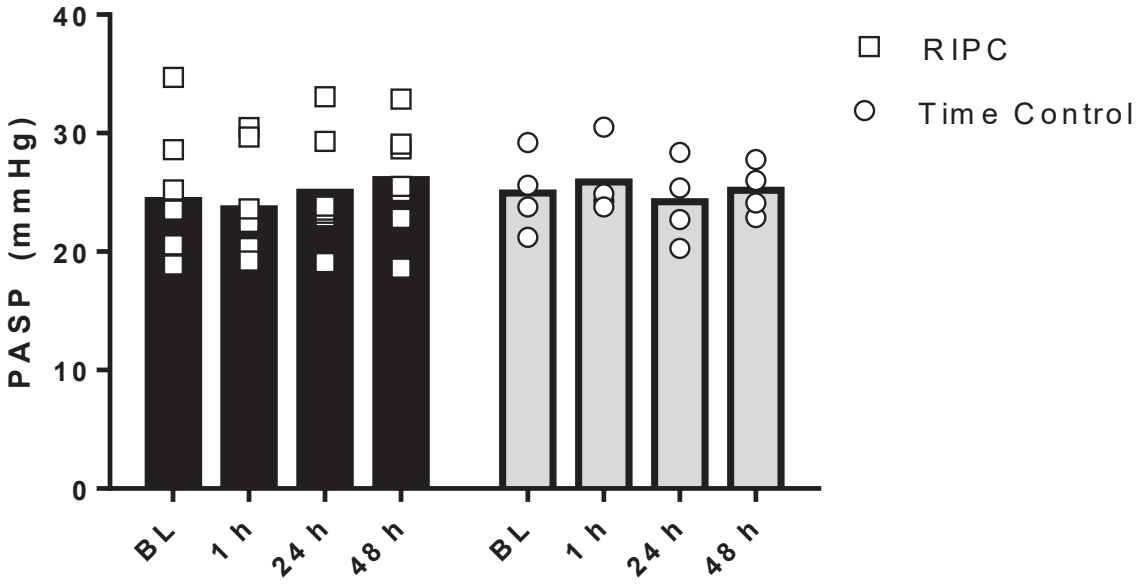

Room Air
End-Tidal Forcing
PASP
FMD
Q_{ICA}
HVR
Q_{ICA}
-25 min
T = 0
10 min
Room Air
($P_{ET}O_2$ ≈ 59 mmHg)
Isocapnic Hypoxia
($P_{ET}O_2$ = 45 mmHg)

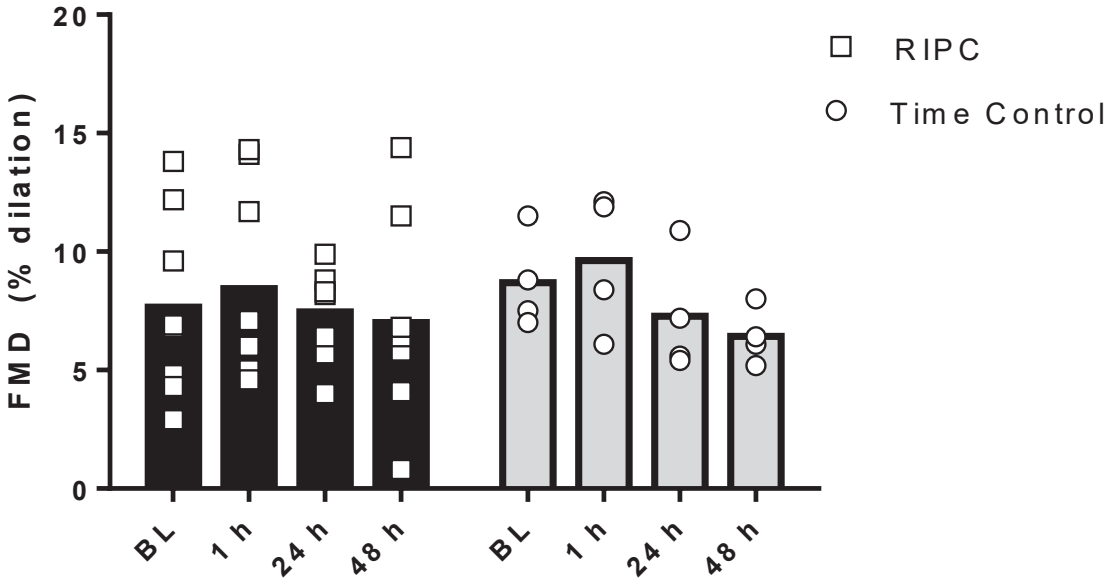

RIPC
SHAM
▲PASP (mmHg)
10
8
6
4
2
0
BL
1 h
24 h
48 h

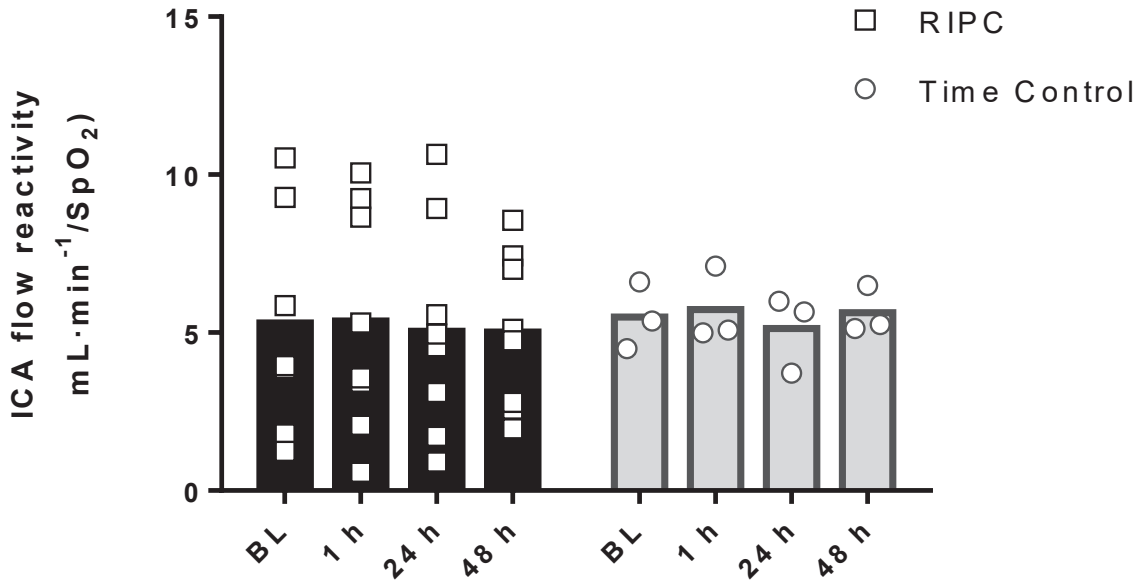

RIPC
SHAM
▲ FMD
(noxmoxia% - hypoxia %)
6
4
2
0
-2
-4
-6
BL
1 h
24 h
48 h

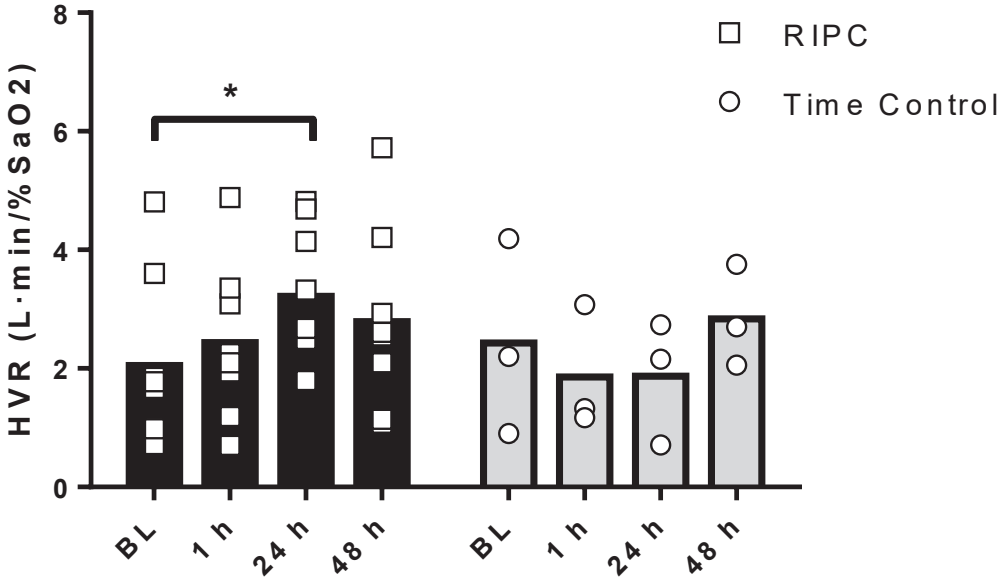

RIPC
SHAM
ICA flow reactivity
(mL·min-1 / SpO2)
10
8
6
4
2
0
BL
1 h
24 h
48 h


RIPC
Sham
HVR
(L·min-1 / ScO2
2.5
2.0
1.5
1.0
0.5
0.0
BL
1 h
24 h
48 h


40
30
20
10
0
PASP (mmHg)
BL
1 h
24 h
48 h
BL
1 h
24 h
48 h
RIPC
Time Control


20
15
10
5
0
FMD (% dilation)
BL
1 h
24 h
48 h
BL
1 h
24 h
48 h
RIPC
Time Control


RIPC
Time Control
ICA flow reactivity
mL·min-1/SpO2)
15
10
5
0
BL
1 h
24 h
48 h
BL
1 h
24 h
48 h


HVR (L·min/%SaO2)
8
6
4
2
0
*
RIPC
Time Control
BL
1 h
24 h
48 h
BL
1 h
24 h
48 h