**Conduit artery structure and function in lowlanders and native highlanders: relationships with oxidative stress and role of sympathoexcitation**

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**Key point summary**

- Information describing alterations in vascular function during either acute or prolonged normobaric or hypobaric hypoxia is sparse and often confounded by pathology and methodological limitations.

- We show that high altitude exposure in lowlanders is associated with impairments in both endothelial and smooth muscle function, and with increased central arterial stiffness. Furthermore, in all of these respects, lowlanders' vasculature becomes comparable to that of natives born and raised at altitude.

- Changes in endothelial function occur very rapidly in normobaric hypoxia, and partly under the influence of sympathetic nerve activity.

- Thus, a lifetime of high altitude exposure neither attenuates nor intensifies the impairments in vascular function observed with short-term exposure in lowlanders; such impairment and altered structure likely translate into an elevated cardiovascular risk.

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**Abstract**

Research detailing the normal vascular adaptions to high altitude is minimal and often confounded by pathology (e.g., chronic mountain sickness) and methodological issues. We examined vascular function and structure in: 1) Healthy lowlanders during acute hypoxia and prolonged (~2 weeks) exposure to high altitude, and 2) High-altitude natives at 5050 m (highlanders). In 12 healthy lowlanders (aged 32±7 y) and 12 highlanders (Sherpa; 33±14 y) we assessed brachial endothelium-dependent flow mediated dilation (FMD), endothelium-independent dilation (via glyceryl trinitrate; GTN), common carotid intima thickness (CIMT) and diameter (ultrasound), and arterial stiffness via pulse wave velocity (PWV; applanation tonometry). Cephalic venous biomarkers of free radical-mediated lipid peroxidation (lipid hydroperoxides, LOOH), nitrite ($NO_2^-$) and lipid soluble antioxidants were also obtained at rest. In lowlanders, measurements were performed at sea level (334 m) and between days 3-4 (acute high altitude) and 12-14 (chronic high altitude) following arrival to 5050 m. Highlander were assessed once at 5050 m. Compared with sea level, acute high altitude reduced lowlanders' FMD (7.9 ± 0.4 vs. 6.8 ± 0.4%; P=0.004) and GTN-dilation (16.6 ± 0.9 vs. 14.5 ± 0.8%; P=0.006), and raised central-PWV (6.0 ± 0.2 vs. 6.6 ± 0.3 m/s; P=0.001). These changes persisted at days 12-14, and after allometrically scaling FMD to adjust for altered baseline diameter. Compared to lowlanders at sea level and high altitude, highlanders had a lower carotid wall: lumen ratio (~19%, P≤0.04), attributable to a narrower CIMT and wider lumen. Although both LOOH and $NO_2^-$ increased with high altitude in lowlanders, only LOOH correlated with the reduction in GTN-induced dilation evident during acute (*n*=11, r=-0.53) and chronic (*n*=7, r=-0.69; P≤0.01) exposure to 5050 m. In a follow-up, placebo-controlled experiment (n=11 healthy lowlanders) conducted in a normobaric hypoxic chamber ($FIO_2$=0.11; 6 h), a sustained reduction in FMD was evident within 1 hr of hypoxic exposure when compared to normoxic baseline (5.7 ±1.6 vs. 8.0 ±1.3%; P<0.01); this decline in FMD was largely reversed following $\alpha_1$-adrenoreceptor blockade. In conclusion, high-altitude exposure in lowlanders caused persistent impairment in vascular function, which was mediated partially via oxidative stress and sympathoexcitation. Although a lifetime of high altitude exposure neither intensifies nor attenuates the impairments seen with short-term exposure, chronic high altitude exposure appears to be associated with arterial remodeling.

**Abbreviations list**

BP, blood pressure; CCA, carotid artery; CIMT, carotid intima media thickness; CV, coefficient variation, ECG, electrocardiogram; FMD, flow-mediated dilatation, GTN, gyceryl trinitrate, HR, heart rate; LOOH, lipid peroxidation; MAP, mean arterial blood pressure; NO, Nitric oxide; $NO_2^-$, Nitrite; PWV, pulse-wave velocity; $SaO_2$, Arterial oxygen saturation; SR, shear rate.

## Introduction

Ambient hypoxia associated with high altitude is a potent activator of the sympathetic nervous system (Saito *et al.*, 1988; Marshall, 1994; Duplain *et al.*, 1999; Xie *et al.*, 2001; Hansen & Sander, 2003), which causes vascular dysfunction (Hijmering *et al.*, 2002). Indeed, intermittent or sustained hypoxia in pathologies such as sleep apnoea, have been associated with vascular dysfunction (Lurie, 2011; Phillips *et al.*, 2013). However, information describing alterations in vascular function during either acute or prolonged exposure to high altitude is sparse and often confounded by pathology (i.e. acute / chronic mountain stickiness, metabolic syndrome) and methodological issues. Previously, acute exposure to high altitude (3,450 m and 4,770 m) has been reported to impair endothelial function (Rhodes *et al.*, 2011). However, these conclusions were based on measurements derived from finger photoplethysmography and derived estimates of changes in arterial stiffness and tone, rather than the more direct and widely adopted non-invasive measures of pulse-wave velocity (PWV; a measure of arterial stiffness (Laurent *et al.*, 2006)) and endothelium flow-mediated dilatation (FMD; as a measure of vascular function (Corretti *et al.*, 2002; Green, 2005)).

Frick et al. (2006) assessed endothelial-dependent FMD following acute (1 day) and prolonged (3 week) exposure to a moderate altitude (1,700 m) in individuals with the metabolic syndrome. Endothelial-dependent FMD was unchanged during acute exposure but impaired following the prolonged exposure (despite participation in an exercise intervention over this period). However, methodological issues existed which cloud interpretation of those data, including: lack of a control group; uncertain non standardisation of cuff placement during the FMD assessment, and lack of continuous monitoring of artery diameter and blood flow during reactive hyperaemia (Thijssen *et al.*, 2011). Specifically, the lack of continuous monitoring of artery diameter and blood flow can result in measurement error, and the true peak response may have been missed (Black *et al.*, 2008). Furthermore, 1700 m is a relatively weak hypoxic stimulus relative to altitudes that are readily accessed or lived in by many, thus the potential negative effect of high-altitude exposure on vascular function and structure in lowlanders, especially in a non-diseased population has not been adequately established.

Impairments in brachial vascular function and structure have been recently documented in native Andean highlanders, with chronic mountain sickness (Rimoldi *et al.*, 2012; Bailey *et al.*, 2013).

Despite elevated levels of oxidative stress, endothelial-dependent FMD in the highlanders free from chronic mountain sickness was found to be comparable to healthy lowlanders at sea level (Rimoldi *et al.*, 2012). Yet, healthy lowlanders exposed to acute normobaric hypoxia exhibited vascular dysfunction, based on indirect biomarkers of oxidative stress (Bailey *et al.*, 2013). Whilst the research findings of Rimoldi et al. (2012) and Bailey et al. (2013) are relevant to chronic mountain sickness, vascular function in healthy lowlanders travelling from sea level to high altitude has not been comprehensively examined, nor compared with permanent high-altitude residents. The latter is of particular interest given that native highlanders have been reported to show earlier cardiovascular degeneration changes with ageing, in particular arterial wall stiffening (Otsuka *et al.*, 2005).

We therefore aimed to 1) Examine potential alterations in vascular function and structure in healthy lowlanders following initial ascent and ~2 weeks of acclimatisation to high altitude (5050 m), and 2) Examine the potential effect of chronic exposure to hypoxia on vascular function and structure in native Sherpa's (highlanders) born and permanently residing at high altitude. We hypothesised that: 1) Lowlanders' endothelial-dependent FMD and arterial stiffness would be impaired following acute exposure to high altitude, and further impairments would be evident following a more chronic stay at high altitude; 2) Measures of vascular function and structure in highlanders would be comparable to lowlanders at sea level; however, following ~2 weeks of high-altitude exposure, measures of vascular function and structure would be impaired in lowlanders compared to highlanders. To provide some mechanistic insight into potential alterations in vascular function, hypoxic-mediated alterations in systemic oxidative stress, nitrite ($NO_2^-$) and antioxidants were also quantified from plasma samples. It was anticipated that increased oxidative stress and concomitant reductions in $NO_2^-$ at high altitude would be related to impairments in vascular function.

In addition, based on the findings from this study (study 1) we conducted a follow-up study (study 2) to gain further insight on the mechanisms and time-course changes in the FMD response during acute hypoxic exposure. An increase in sympathetic outflow has previously been shown to attenuate FMD by ~57% (relative), yet this attenuation was completely abolished by alpha (α)-adrenergic blockade (Hijmering *et al.*, 2002). We reasoned that the FMD impairment seen with high altitude might also have been mediated via elevations in sympathetic nerve

activity via an α-adrenergic mechanism; therefore, blocking the hypoxic-induced elevations in sympathetic nerve activity may potentially restore FMD. To address this possibility, we conducted a placebo-controlled experiment (study 2; n=11) in a normobaric hypoxic chamber ($FIO_2$=~0.11; ~6 h) following selective $\alpha_1$ -sympathetic receptor blockade (1 mg/20 kg body mass; prazosin). We hypothesised that: 1) FMD would be progressively impaired in normobaric hypoxia, and 2) $\alpha_1$-adrenergic blockade would partially normalize the hypoxic-induced reductions in FMD.

## Methods

### Ethical approval

Study 1 and 2 were approved by the Human Ethics Committee of the University of British Columbia and the Nepal Health Medical Research Council, and conformed to the standards set by the *Declaration of Helsinki*. Verbal and or written consent was obtained by all participants. Sea-level experiments took place at the University of British Columbia, Okanagan (344 m; barometric pressure 755 ± 7 mm Hg) while high-altitude experiments were conducted at the Ev-K2-CNR Pyramid Laboratory in the Khumbu Valley in Nepal (5050 m; barometric pressure 413 ± 1 mm Hg).

### Study 1 - high altitude

### Participants

*Lowlanders*: Twelve healthy normotensive volunteers (10 males; 2 females) with a mean ± SD age of 32 ± 7 yrs, body mass 76 ± 13 kg, height 176 ± 7 cm and body mass index 25 ± 4 $kg/m^2$, were recruited for this experiment. Participants were non-smokers, had no previous history of cardiovascular, cerebrovascular or respiratory diseases, and were not taking any cardiovascular medications. All participants were born and lived close to sea level (<1000 m) and none had been to high altitude for >2 years.

*Highlanders*: Following informed consent, twelve male natives Sherpa's (33 ± 14 yrs; body mass 69 ± 16 kg, height 170 ± 8 cm, body mass index 24 ± 5 kg/m$^2$) were screened through Nepalese translation by a medical practitioner, and had no known previous history of cardiovascular, cerebrovascular or respiratory diseases, nor were they taking any cardiovascular medications. All participants were born and permanently residing at high altitude between 3440 m – 6119 m (mean ± SD: 4372 ± 673 m).

**Design and ascent protocol**

Following screening and familiarisation with the experimental procedures, lowland participants underwent the experimental session at sea level, upon initial arrival at high altitude (between days 3 and 4; acute-high altitude) and following 12-14 days of acclimatisation (chronic-high altitude; see figure 1), while the highlander group completed one session at high altitude (5050 m) only (testing procedures were identical except for brachial artery vasodilator capacity test, see below). Lowland participants spent 6 days at Kathmandu (1400 m) before flying to Lukla (2860 m). Participants then trekked to the Ev-K2-CNR Pyramid Laboratory over a 9-11-day period, which included rest days at Namche Bazar (3450 m), Pangboche (3985 m) and Pheriche (4371 m). Arrival at 5050 m for the lowland group was staggered to facilitate measures within the desired time course across the two weeks, consequentially, participants arrived at 5050 m after spending 1 (*n*=6), 2 (*n*=4) or 3 (n=2) rest days at 4371 m. Participants were given low-dose acetazolamide (125 mg bd) for the first 7 days of ascent to minimise acute mountain sickness prophylaxis, as recommended (Basnyat *et al.*, 2006). Importantly, treatment was discontinued on day 8 to allow for sufficient clearance time (i.e., >48 h) (Ritschel *et al.*, 1998). Experimental testing began after 12-h abstinence from alcohol, caffeine, and strenuous exercise, and a 4-h fast. This study was part of a larger research expedition and consequently participants took part in a number of studies conducted during 3 weeks at the Pyramid Laboratory. The recovery time between the various testing sessions was managed to prevent any potential for confounding results (e.g., >48 hours between all drug and/or exercise intervention studies).

**Measurements**

All measures were made following a minimum of 15 min of supine rest. Arterial oxygen saturation ($SaO_2$) was monitored via pulse oximetry (Pulse Oximeter MD300K1; Vacumed,

Canada). Continuous beat-to-beat measures of arterial blood pressure (BP; finger photoplethysmography; Finapres Medical Systems, Biomedical Instruments, The Netherlands) and heart rate (HR, 3-lead ECG; ML132, ADInstruments, Colorado Springs CO, USA) were recorded. Manual sphygmomanometer BP recordings were obtained during supine rest to confirm the accuracy of the finger photoplethysmography measurements. All data were sampled continuously using an analogue-digital converter (PowerLab/4S ML750; ADInstruments) interfaced with a computer and displayed in real time during testing. Data were stored for subsequent off-line analysis using the commercially available software (Chart v7, ADInstruments). All tests were completed in the following order:

***Arterial stiffness***: Adhering to the international guidelines (Laurent *et al.*, 2006), hand held-tonometry (SPT-301 Millar Instruments, Houston, Texas) was employed to assess central (carotid-femoral PWV) and peripheral (carotid-radial PWV) arterial stiffness. Twenty reproducible carotid-femoral artery waveforms and 20 separate carotid-radial artery waveforms were recorded simultaneously using mechanotransducers, which were applied directly to the skin and over the area of greatest pulsation. The distance from the $4^{th}$ intercostal space in the midline of the sternum to the individual carotid, femoral and radial artery pulse sites were measured along the surface of the body using a measuring tape. This technique was used as it has been shown to have the best agreement with aortic PWV measured invasively using cardiac catheterization (Weber *et al.*, 2009). The foot to foot method was used to determine pulse transit time, using a bandpass filter (5-30 Hz) to identify the foot or "notch" of the carotid-femoral and -radial waveform, and the difference in time from R interval to systolic upstroke at each location. Pulse distance was determined by subtracting the distance from carotid measurement to the sternal notch from the distance from the sternal notch to the femoral and radial pulse site measurement. Pulse-wave velocity was then determined by dividing distance by pulse transit time. The day-to-day intra-observer coefficient variation (CV) for central and peripheral PWV (n=6) was 4.8% and 4.7%, respectively (Lewis et al., unpublished observations).

Local artery stiffness in the common carotid artery (CCA) was also assessed. Carotid ultrasound images and carotid BP were collected simultaneously over 20-30 cardiac cycles. Left CCA BP waveforms, a representative of carotid arterial pressure, were obtained using the hand-held

tonometer positioned over the greatest pulsation. Right CCA ultrasound images were obtained ~1-2 cm proximal to the bifurcation of the external and internal carotid arteries using a 10-MHz multifrequency linear array probe, attached to a B-mode high-resolution ultrasound machine [Terason 3000TM, Teratech, Burlington, MA, USA]. Maximal and minimal lumen diameters were calculated using edge-detection software (described below). Ten carotid and brachial waveforms along with 10 complete cardiac cycles of carotid diameter change were averaged, and carotid arterial compliance, distensibility and ß-stiffness index were calculated (Tanaka *et al.*, 2000) (Figure 2).

***Carotid Intima-Medial Thickness (CIMT)***: The right carotid artery was imaged in the anterolateral, posterolateral and mediolateral planes, 1-2 cm proximal to the carotid bulb (Stein *et al.*, 2008), using a high-resolution ultrasound machine (Vivid-q, GE, Fairfield, CT, USA) attached to an 8L-RS MHz high frequency linear array transducer. Participants were measured in the supine position with a slight hyperextension of the neck at a 45° angle. The CIMT at end-diastole (1 frame prior to the R-interval) of 10 successive beats were recorded at each of the three angles and averaged. All analysis was completed offline using commercial edge-detection software (EchoPAC PC, GE Healthcare; Figure 2). To correct for differences in diameter, we also calculated the wall-to-lumen ratio.

***Brachial artery vascular function***: A 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Terason 3000$^{TM}$, Teratech) was used to image the brachial artery in the right arm during the three tests employed to examine brachial vascular function:

*Endothelium-dependent FMD*: FMD was assessed according to international guidelines (Black *et al.*, 2008). With the occluding cuff placed distal to the ultrasound probe, 1 min of diameter and flow recordings preceded forearm cuff inflation (>200 mm Hg) for 5 min. Diameter and flow recordings resumed 30 s prior to cuff deflation and continued for 3 min thereafter. In the current study the day-to-day intra-observer CV for FMD was 3.6% (n=6; Lewis et al., unpublished observations).

*Brachial artery vasodilator capacity**:*** Following a 10-min resting period, the occluding cuff was positioned above the imaged part of the brachial artery, i.e., proximally on the upper arm.

Following 1 min of diameter and velocity recordings, the cuff was inflated (>200 mm Hg) for 5 min. During the middle 3 min of cuff occlusion, ischaemic handgrip exercise was performed. Diameter and flow recordings resumed 30 s prior to cuff deflation and continued for 3 min thereafter. This protocol results in a near-maximal dilatation of the brachial artery in humans and provides a valid index of peak vasodilator capacity (Tinken *et al.*, 2008). In the current study the day-to-day intra-observer CV was 6.7% (n=6; Lewis et al., unpublished observations).

*Endothelium-independent FMD (GTN)*: Following 10 min of rest, brachial diameter and velocity were recorded for 1 min. Participants then received a sublingual dose of glyceryl trinitrate (GTN; 400 µg spray). Diameter and flow recordings were taken continuously for a 10-min period thereafter**.**

**Metabolic measurements:** In lowlanders only, venous blood was obtained without stasis from a forearm antecubital vein after at least 20 min of supine rest, at sea level (*n*=11) as well as one day (*n*=11) and one week (*n*=7) after arrival at high altitude. Blood was collected into EDTA and serum vaccutainers. EDTA-plasma was drawn after centrifugation (3000 rpm at 4 °C) for 10 min and serum was drawn after leaving vaccutainers at room temperature for 60 min prior to centrifugation. Plasma and serum were placed in a -80 °C freezer at sea level, or -40 °C freezer at high altitude, for up to 10 days where they remained frozen during transport on dry-ice to the UK prior to batch analysis.

*Biomarkers of oxidative stress and NO bioavailability*:
*Antioxidants:* Plasma $\alpha$/$\gamma$-tocopherol, $\alpha$/$\beta$-carotene, retinol, lycopene, zexanthin, $\beta$-cryptoxanthin and lutein were determined using an HPLC method (Catignani & Bieri, 1983; Thurnham *et al.*, 1988). The intra and inter-assay CV were both <5%.

*Lipid hydroperoxides (LOOH):* Serum LOOH was determined using the ferrous iron/xylenol orange assay (Wolff, 1994) with modification. The intra/inter-assay CV were both <5%.

*Nitrite ($NO_2^-$):* Plasma $NO_2^-$ was measured via ozone-based chemiluminescence (OBC Model 280i, NOA®, Sievers, Boulder, CO, USA) following reduction by potassium iodide in acetic acid

according to established methods (Pelletier *et al.*, 2006). The intra and inter-assay CV were both <5%.

**Study 2 - Normobaric hypoxia and $\alpha_1$-adrenoreceptor blockade**

Eleven healthy normotensive volunteers (7 males: 4 females) with a mean (± SD) age of 24 ± 3 years, body mass 75 ± 10 kg, height 174 ± 6 cm and body mass index 25 ± 3 kg/m$^2$, participated in this randomized placebo-controlled experiment. Experimental sessions commenced between 08:00 and 09:00 hrs and measurements of FMD, $SaO_2$ and BP (as described above) were made in normoxia and during normobaric hypoxia ($FIO_2$=0.11) at 60 min, 210 min and 330 min, and then 60 min after returning to normobaric normoxia (Figure 1). This level of hypoxia was chosen as it equivalent to an altitude of 5,000 m. Participants ingested the $\alpha_1$-adrenoreceptor blocker, prazosin (1 mg/20 kg body mass) or an identical placebo capsule 90 min before the last assessment in hypoxia. Prazosin has been shown to induce systemic peripheral arterial-dilation and venodilation via the removal of sympathetic nerve activity (Awan et al., 1977; Jauernig et al., 1978). This clinically-acceptable dose of prazosin has previously been shown to have a functional block of ~80% and peak activity 90- to 180-min post-ingestion (Ogoh et al., 2008; Jones et al., 2011; Lewis et al., 2012, 2013). Each experiment was separated by ≥ 7 days, and a small standardised snack and 250 mL of water were provided following each FMD assessment.

**Data Analysis**

*Calculations:* Baseline measures of BP and HR were averaged over 1 min following 15-min supine rest. Custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias, was utilised for the analysis of CCA and brachial diameter and brachial blood flow velocity [(Woodman *et al.*, 2001; Black *et al.*, 2008) see Figure 2]. This software provides continuous and simultaneous diameter, velocity and shear rate (SR; 4 x velocity / diameter) measurements, as well as post hoc calculation of FMD, vasodilator capacity and GTN responses. This semi-automated software provides higher reproducibility of diameter measurements and reduces both observer error and bias with a reported intra-observer CV for FMD% of 6.7% (Woodman *et al.*, 2001). Data are presented as absolute (millimetres) and relative (percentage) rises from the preceding baseline diameter and are calculated based on

standardised algorithms applied by the software (Black *et al.*, 2008). In accordance with procedural recommendations (Pyke & Tschakovsky, 2005, 2007; Atkinson *et al.*, 2009), we also measured the post-deflation area under the shear rate curve in order to best interpret any changes in FMD. Recent evidence has highlighted that the general use of FMD% is associated with statistical bias as it may fail to consider the difference in baseline artery diameter following an intervention or between groups (Atkinson & Batterham, 2013; Atkinson *et al.*, 2013). Therefore, using recent guidelines that provide a statistical quantification of FMD, which is independent of baseline artery size (Atkinson & Batterham, 2013; Atkinson *et al.*, 2013); we adopted an allometric scaling approach to adjust for variability in baseline diameter. These results are presented as "corrected" FMD %. This approach is reported to improve the specificity and interpretation of the FMD protocol (Atkinson & Batterham, 2013; Atkinson *et al.*, 2013) and is used herein as a complementary measure of vascular function.

*Statistical analysis:* All data were analysed using SPSS (version 21, IBM, Surrey, UK) and expressed as mean ± SD. Statistical significance was defined as $P \leq 0.05$ and distribution normality confirmed using repeated Shapiro-Wilk *W* tests. *Study 1:* Trial differences within lowlanders between sea-level, initial-high altitude and prolonged-high altitude were analysed using a one-way repeated measures ANOVA. Pearson's correlation analysis was used to examine the relationship between measures. Difference between highlanders and lowlanders at were explored using independent t-tests. *Study 2*: The time course changes of hypoxia in the placebo trial were examined using one-way repeated measures ANOVA, to establish the effect of the hypoxic stimulus on FMD. To examine the interaction between the time course change and experimental condition (placebo vs. $\alpha_1$-blockade) a two-way repeated measures ANOVA was used; to further exposure any significant interaction effect, paired t-tests were used to exposure the effect of the $\alpha_1$-blockade on the vascular changes with hypoxia.

## Results

### Study 1

*Lowlanders at high altitude*: Arterial oxygen saturation was markedly lower and HR was significantly higher following acute-high altitude (-18 ± 2% and +21 ± 4 beats·min$^{-1}$,

respectively; P<0.001) and chronic prolonged-high altitude exposure (-16 ± 3% and +18 ± 4 beats·min$^{-1}$; P<0.001; Table 1) compared with sea level. Arterial blood pressure, CIMT, CCA pulse pressure, CCA distensibilty, CCA wall: lumen ratio, and β-stiffness were unaltered with high-altitude exposure (Table 1; Figure 3).

Compared to sea level, central-PWV was greater following acute-high altitude (9%; +0.6 ± 0.06 m·s$^{-1}$; P=0.04) and even more so following chronic-high altitude exposure (13%; +0.8 ± -0.1 m·s$^{-1}$; P=0.006; Figure 4). No difference in peripheral-PWV was evident with high-altitude exposure (*n*=11; Figure 4). Compared to sea level, carotid compliance was higher following initial arrival to high altitude (by 0.05 ± -0.01 mm$^2$·mm Hg; P=0.006); however, this difference was not evident following ~2 weeks of high-altitude exposure (Table 1). Both systolic and diastolic diameter of the CCA increased with acute exposure to high altitude, and remained so following chronic exposure (~0.5 mm; P≤0.007; Figure 3).

Compared with sea level, brachial FMD and GTN were reduced by 1.1 ± 0.1% (relative change ~14%) and 2.4 ± 1.6% (relative ~14%), respectively (P≤0.02; Figure 5) following acute altitude exposure. Following ~2 weeks at high altitude, both FMD and GTN remained reduced when compared with sea level, by 0.6 ± 0.6% (relative 8%; P=0.01) and 1.8 ± 2.1% (relative 11%;P=0.06), respectively (Figure 5). Compared with sea level, vasodilator capacity (*n*=11) displayed a trend towards being reduced (P=0.07) by 1.8 ± 2.4 (relative ~14%) and 1.0 ± 2.4 (relative ~8%) following initial arrival to altitude and following ~2 weeks, respectively. Compared with initial arrival to 5050 m, FMD shear rate area under the curve was lower following ~2 weeks at altitude (by 81,568 ± 3,856 AUC , P=0.03; Table 1), while no other differences were evident across the time spent at 5050 m (Table 1). Compared to sea level, allometrically "corrected" FMD was also reduced by 1.2 ± 0% (relative ~15%) and 0.6 ± 0% (relative ~8%) following acute and chronic high altitude exposures, respectively (P≤0.03; Table 1).

*Markers of oxidative stress and NO bioavailability*: Serum LOOH and plasma $NO_2^-$ increased in lowlanders following acute high-altitude exposure, by 17% (P=0.01) and 72% (P=0.06), respectively (see Table 2). A significant negative correlation between LOOH and GTN-induced dilation was evident for initial arrival (*n*=11, r=-0.53; P=0.01) and chronic exposure (*n*=7, r=-

0.69; P≤0.001); according to the coefficient of determination, elevations in LOOH statistically accounted for 28% and 48% of the decrease in GTN with acute- and chronic-high altitude exposure, respectively. Although significance was not reached, a trend for a positive correlation between LOOH and central arterial stiffness was also evident following initial arrival to 5050 m (r=+0.39; P=0.07). No other correlations were evident. Plasma retinol following initial arrive to 5050m, showed a 9% increase from sea level; the concentration of the other measured lipid soluble antioxidants were significantly unaltered with high altitude exposure (Table 2).

*Comparison between lowlanders and native highlanders*: Compared with lowlanders at sea level, highlanders had lower mean arterial blood pressure (-10 ± 2 mm Hg; P=0.03), $SaO_2$ (-16 ± 1%; P<0.001), CCA pulse pressure (-8 ± 4 mm Hg; P=0.04), and a higher HR (+21 ± 1 beats·min$^{-1}$; P<0.001) and central-PWV (17%; +1.0 ± 0.7 m·s$^{-1}$; P=0.05; Figure 4); however, once lowlanders were exposed to high altitude these between-group differences were not present (Table 1, Figure 4). Compared with lowlanders at sea level, highlanders systolic and diastolic CCA diameters were larger (11%; +0.7 ± 0.1 mm and +0.8 ± 0.2 mm, respectively; P≤0.005; Figure 3), and both FMD and GTN dilation displayed a trend of being reduced by 1.6 ± 3.0% (relative ~20%; P<0.10) and 3.5 ± 4.7% (relative ~ 21%; P=0.07; Figure 5). However, again these between-group differences were absent when lowlanders were exposed to high altitude. In contrast, highlander's wall: lumen ratio was ~19% lower when compared to lowlanders at both sea level and at high altitude (P≤0.04; Figure 3).

**Study 2 - Normobaric hypoxia and $\alpha_1$-adrenoreceptor blockade**

*Time course alterations to hypoxia within the placebo trial:* Compared to normoxic baseline (0 min) $SaO_2$ was reduced and HR was elevated during hypoxic exposure (Table 3). Due to technical issues (i.e. loss of one recording), FMD time course related data within the placebo trial were obtained in 10 out of the 11 participants. Compared to normoxic baseline, FMD was reduced following 60 min, 210, and 330 min of hypoxia by 1.6 ± 0.2 % (relative 28%), 2.5 ± 0.1 % and 2.8 ± 0.2 % (relative 36%), respectively (P≤0.003; Figure 6). Although no significant difference was evident between normoxic conditions (baseline and 60-min post-hypoxia

recovery), FMD was 1.3 ± 0.2 % (relative 16%) higher in normoxic recovery from hypoxia exposure.

*The effect of the $\alpha_1$-adrenoreceptor blockade:* Two-way repeated measures ANOVA (*n*=9) revealed a significant interaction between time and condition for HR and FMD. Pair t-test analysis revealed that compared to the placebo trial, HR and FMD were 5 ± 2 beats·min$^{-1}$ (P=0.03; Table 3) and 1.8 ± 1.4 % (relative 35%; P=0.002; Figure 6) higher following $\alpha_1$-adrenoreceptor blockade after 330 min of hypoxic exposure.

**Discussion**

This is the first study to comprehensively examine the time course and potential mechanisms of alterations in vascular function and structure in healthy lowland individuals during normobaric hypoxia, upon ascent to and following a partial acclimatization to high altitude, and to provide relevant comparisons with high-altitude natives. Acute exposure to 5050 m in lowlanders was associated with an impairment in both endothelial-dependent (FMD) and endothelial-independent (GTN) dilatation, and an increase in central-PWV and CCA diameter. These changes were neither exacerbated nor resolved with chronic-high altitude exposure. Compared to lowlanders at sea level, highlanders had a lower FMD and GTN dilation response, a higher central-PWV and a larger CCA diameter. These between-group differences were removed when lowlanders were exposed to 5050 m, with only one exception (carotid wall: lumen ratio). In lowlanders, we found that alterations in elevations in oxidative stress were partially and selectively related to the reductions in GTN-induced vasodilation at 5050 m. In a follow-up placebo-controlled experiment conducted in a normobaric hypoxic chamber, we found that sustained reductions (-28-36%) in FMD occur within 60 min and could be partially reversed following $\alpha_1$-adrenoreceptor blockade. We conclude that high altitude exposure in lowlanders was associated with persistent impairment in vascular function, and was potentially mediated via oxidative stress and sympathoexcitation. Although a lifetime of high-altitude exposure does not intensify the observed vascular function impairments seen with acute exposure, chronic high-altitude exposure appears to be associated with altered arterial structure; whether this is an adaptive or maladaptive response remains to be established.

*Influence of high altitude on vascular structure and function in lowlanders*: Flow mediated dilation, an index of cardiovascular risk(Mullen *et al.*, 2001; Green *et al.*, 2005; Green *et al.*, 2012; Maruhashi *et al.*, 2013), was reduced by 14% in healthy lowlanders upon initial exposure to 5050 m, and did not change following ~2 weeks at this elevation. These findings contrast with those reported by Frick et al. (2006), where FMD following 3 weeks at 1700 m was reduced by ~49%. However, given the methodological issues discussed above, comparison with this study is difficult. Given the comparable brachial baseline diameter and FMD shear stress between sea level and arrival to high altitude, the initial reduction in FMD with high altitude does not appear to be explained by alterations in diameter or the shear stress stimulus on the vessel. Moreover, our findings persisted following allometric scaling to control for variability in baseline diameter and therefore, improved specificity and interpretation of the FMD protocol (Atkinson & Batterham, 2013; Atkinson *et al.*, 2013).

We also quantified the peak vasodilatory capacity of the brachial artery, and found this too was reduced by 14% at high altitude. This measure is a valid index and surrogate measure for arterial structural remodelling (Naylor et al. 2005). The artery dilation associated with the assessment of vasodilator capacity in the current study is less dependent on NO than the FMD assessment. For example, it has previously been shown that when cuff occlusion is proximal to the site of vascular imaging (as used in the current study for testing vasodilator capacity), NO accounts for only 40% of the dilation response (Doshi *et al.*, 2001; Green *et al.*, 2012); the degree of shear stress and release of multiple vasoactive substances contribute to the additional artery dilation (Green *et al.*, 2012). It would seem that a balance of NO and other vasoactive substances, in addition to neurogenic factors, underpin the vascular changes observed at high altitude, and are considered below.

*Potential mechanisms of action for initial reduction in FMD:* A positive relationship between $SaO_2$ and FMD (r=0.62) has previously been reported, and 100% oxygen inhalation has been shown to improve FMD in hypoxemic ($SaO_2$ <90%), but not in normoxemic ($SaO_2$ <90%) control participants, suggesting that vascular dysfunction to high altitude, is partly influenced by hypoxemia (Rimoldi *et al.*, 2012). In the current study, a similar weak-to-moderate relationship was evident between FMD and $SaO_2$ (r=0.33, P=0.05), and calculation of the coefficient of

determination indicates that the change in $SaO_2$ potentially explained around 11% of the change in FMD; confirming the multifactorial nature of vascular dysfunction and that other mechanism(s) are involved in the impairment of FMD at high altitude.

In contrast to our hypothesis, and previous findings (Frick *et al.*, 2006), GTN-induced dilation in lowlanders was also reduced at both time points at high altitude. It is accepted that GTN-induced dilation provides an index of the maximum obtainable vasodilator response, and represents vascular smooth muscle function (Corretti *et al.*, 2002; Maruhashi *et al.*, 2013). This particular assessment is used as a control test for FMD measures, as the assessment of FMD is based on the premise that endothelium-independent dilation is not altered, and any alterations seen in FMD measures is resultant of endothelial dysfunction, and not vascular smooth muscle dysfunction(Maruhashi *et al.*, 2013). Given that GTN-dilation and FMD were each reduced by ~14% with acute exposure to 5050 m, these findings indicate that alterations in vascular smooth muscle function and/or structure contributed to the decline in FMD at high altitude. The potential mechanism(s) by which ambient hypoxia alters vascular smooth muscle function/structure is unknown. Endothelial dysfunction associated with acute hypoxia at sea level, has been associated with a reduced NO-bioavailability in both humans (Cosby *et al.*, 2003; Maher *et al.*, 2008) and rodents (Reboul *et al.*, 2005; Hernandez-Guerra *et al.*, 2013); however, in agreement with others (Janocha *et al.*, 2011; Beall *et al.*, 2012) we found that vascular NO bioavailability (as indexed by plasma $NO_2^-$) increased with high altitude. Thus, it seems that vascular dysfunction clearly cannot be explained by reduced NO bioavailability *per se*, but is more likely related to additional factors including the direct vasculo-toxic/constrictive effects of elevations in LOOH, as confirmed by the observed correlation. Consistent with these findings, there is evidence to indicate that hypoxic upregulation of superoxide production (Dweik, 2005) potentially hinders the intravascular signalling processes in smooth muscle cells required for relaxation (e.g., soluble guanylyl cyclase and cGMP-dependent kinase; (Munzel *et al.*, 2003; Maruhashi *et al.*, 2013). The elevations in NO at high altitude may well be an adaptive response to maintain circulatory homeostasis through both vasodilation and metabolic suppression [reviewed in: (Umbrello *et al.*, 2013)].

It is known that hypoxic-induced sympathoexcitation (Saito *et al.*, 1988; Hansen & Sander, 2003) may potentially modulate functional, and/or mechanical properties of large arteries via an increase in vasomotor tone (Hijmering *et al.*, 2002; Dyson *et al.*, 2006; Fok *et al.*, 2012). To explore this possibility, we conducted a follow-up study in normobaric hypoxia to examine whether a similar reduction in FMD would occur in response to acute normobaric hypoxia, and the contributory role of increased sympathetic nerve activity via the $\alpha_1$-adrenoreceptor. Broadly consistent with our hypothesis, we found that FMD was markedly reduced in normobaric hypoxia (~28-35%). Moreover, following administration of the $\alpha_1$-blockade, these reductions in FMD were largely reversed back to normoxic baseline. Thus, our findings clearly support the notion that changes in FMD occur early within exposure to hypoxia and are under the influence of elevations in sympathetic nerve activity. We acknowledge that differences between normobaric and hypobaric hypoxia are possible, and the potential mechanism regulating the vascular response to shear stress may also differ.

*Arterial stiffness*: Lowlanders experienced an 11% increase in central-PWV with high altitude exposure, with no change in peripheral-PWV. Carotid to femoral PWV is considered the non-invasive gold standard measure of arterial stiffness (Laurent *et al.*, 2006), and is influenced by the tone of arterial smooth muscle irrespective of the signalling pathway in which it is modulated (Fok *et al.*, 2012). Vascular smooth muscle tone is affected by both endothelial cell signalling and the sympathetic nervous system (Wilkinson & McEniery, 2004; Bruno *et al.*, 2012). As highlighted above, the FMD and GTN findings in the current study support impairment in endothelial function and increase in smooth muscle tone, both of which alone or in combination may explain our observed increase in central-PWV.

It is important to note that the anatomy of arteries and the extent to which smooth muscle tone influences stiffness is not homogenous across the vascular tree, e.g., muscular arteries (radial) unlike elastic arteries (carotid) show little stiffening with ageing (Avolio *et al.*, 1985; Stewart *et al.*, 2003). Additionally, structural changes can be present without obvious functional changes and vice versa (Naghavi, 2009). The results of the current study support this concept. In support of arterial differences along the vascular tree with ambient hypoxia, our lowlander group

experienced a ~7% increase in CCA diameter at 5050 m, whereas brachial diameter was unchanged. Although the regional mechanisms are unknown, the differential effects of hypoxia on vessel dilation would seem of physiological benefit in that dilation of the CCA would act to redistribute blood flow towards the cerebral vessels to help maintain a normal oxygen flux to the brain in the face of arterial hypoxemia (Ainslie & Ogoh, 2010).

*Comparisons between lowlanders and highlanders:* When compared to lowlanders at sea level, we provide evidence of impaired vascular function in highlanders, as indexed by ~20% reduction in FMD/ GTN and elevation in central-PWV of ~17%. Interestingly, these observed changes in vascular function and central-PWV in natives were remarkably comparable to lowlanders at 5050 m. These comparable alterations in vascular function indicate that these changes might not be dependent on the time spent at high altitude. Compared to lowlanders at both sea level and high altitude, highlanders had a significantly lower (~19%) carotid wall: lumen ratio. This difference likely reflects the consistently lower CIMT and larger lumen diameter in the highlanders. It is unclear if this difference is reflective of a beneficial adaptation high altitude, or the specific mechanism(s) governing this response; however, a smaller wall thickness and larger lumen diameter have been reported in individuals following chronic exercise exposure (Rowley *et al.*, 2012). It has been suggested that exercise training induces an increase in shear stress and transmural pressure (Tuttle *et al.*, 2001; Laughlin *et al.*, 2008; Green *et al.*, 2011); these collective changes can increase the circumferential strain placed on the blood vessel and stimulate structural arterial remodelling and an anti-atherogenic effect, resulting specifically in luminal expansion (Laughlin *et al.*, 2008; Rowley *et al.*, 2012). Although we do not provide evidence to support the above concept with high altitude exposure, the increase in carotid diameter in lowlanders with high altitude exposure may indicate that arterial remodelling is an adaptive response to high altitude.

**Perspectives**

The high-altitude induced changes in FMD/GTN dilation and central-PWV in lowlanders and the comparison with highlanders has potential clinical relevance. This is especially evidenced in native highlanders who have been reported to show earlier cardiovascular degeneration changes with aging, in particular arterial wall stiffening (Otsuka *et al.*, 2005). It has been estimated that a

1% (absolute) decrease in FMD is associated with a 9% increase in cardiovascular risk (Green *et al.*, 2012); and a 1 $m \cdot s^{-1}$ increase in aortic stiffness (central-PWV) accounts for a 15% increase in cardiovascular and all-case mortality (Vlachopoulos *et al.*, 2010). The absolute mean differences in FMD and central-PWV between highlanders at high altitude and lowlanders at sea level were 1.6% and 1.0 $m \cdot s^{-1}$, respectively; and the absolute change in lowlanders upon exposure to high altitude from sea level was 0.9% and 0.7 $m \cdot s^{-1}$. These findings indicate that: 1) natives to high altitude are at greater risk of advanced vascular aging, due to impairment in endothelial and smooth muscle function; thus, lifelong adaption to high altitude does not appear to provide any functional cardioprotective benefits; and 2) the vascular alterations/adaptions in vascular endothelial and smooth muscle function with high-altitude exposure are significant enough to induce an increase in cardiovascular risk in healthy lowlanders. These findings may be particularly relevant to 'at-risk' populations who ascend to high altitude (e.g. patients with sleep apnoea, heart failure, lung disease, etc.). The potential risk and level of altitude required to induce adverse changes in vascular function remains to be established.

**Methodological considerations**

Due to the nature of high-altitude research, our sample size was relatively small. Nevertheless, clear significant differences were evident, indicating that additional numbers would be unlikely to alter our findings. An important consideration in the assessment of FMD in cross-sectional and longitudinal studies is that of consistency in the brachial baseline diameter and shear-stress stimulus and related normalisation (Atkinson *et al.*, 2009; Green *et al.*, 2012). Following ~2 weeks at 5050 m, FMD was unaltered compared to initial arrival to high altitude. It is important to highlight that compared to initial exposure at 5050 m, the SR area under the curve stimulus influencing the FMD response was reduced by ~40% following the prolonged stay at 5050 m. Despite this marked reduction in shear stress, the FMD reduction was markedly consistent across time at high altitude. Whether or not these changes in SR area under the curve reflect a subtle functional improvement in vascular function is unclear. In addition, the changes in FMD persisted following allometric scaling to control for variability in baseline diameter.

Although logistically difficult in field conditions or prolonged laboratory studies, complimentary measures of sympathetic nerve activity either via noradrenaline concentrations or muscle sympathetic nerve activity would have added to mechanistic interpretation to our findings. It is

also known that physical fitness influences the brachial baseline diameter and hence FMD response (Green *et al.*, 2013). Although there were no significant differences in brachial baseline diameter between conditions (e.g., time at altitude) or groups (e.g., lowlanders vs. highlanders), complementary measures of cardiorespiratory fitness may have been useful.

It has been reported that a linear relationship exists between central-PWV in elderly individuals with cardiovascular disease and HR when >70 beats·min$^{-1}$ (Lantelme et al., 2002). For example, a 6 beats·min$^{-1}$ increase in HR above 70 beats·min$^{-1}$ corresponds to a <0.3 m·s$^{-1}$ increase in central-PWV (Stewart *et al.*, 2003). How relevant these findings are to an otherwise young, non-cardiovascular-diseased population is currently unknown, and the translation to the current study is questionable given that the lowest central-PWV reported by Lantelme et al. (2002) at a HR of ~60 beats·min$^{-1}$ was double that present in the current study in healthy lowlanders free of overt cardiovascular disease even when exposed to the vascular stress associated with high altitude. As anticipated, the peak HR in the current study occurred following acute exposure to 5050m (77 beats·min$^{-1}$) and was reduced over 2-3 weeks to 73 beats·min$^{-1}$. Because the mean difference in central-PWV between sea level and acute-high altitude was 0.6 m·s$^{-1}$ (which persisted over time despite reductions in HR), it is possible that HR may have contributed up to half of the increase seen in central-PWV with acute-altitude exposure; however, given that the increase in central-PWV persisted with chronic exposure, when HR had declined, it seem other mechanisms influenced the changes in central-PWV with high altitude. Additionally, we would have expected small changes in blood viscosity and haemoconcentration over 2 weeks at altitude (Lucas *et al.*, 2011), however, such changes would appear to have a negligible impact on vascular function measures including FMD, PWV and carotid compliance (Parkhurst *et al.*, 2012); therefore, we feel that changes in blood viscosity are unlikely to explain our findings within or between groups.

Although we provided some insight into the role of oxidative stress and NO as potential mechanisms of vascular dysfunction, we were unable to collect these blood markers in the high altitude natives. As such, we do not know if similar changes and relationships may also be evident in this group. It is also important to acknowledge that evidence exists to indicate that highland groups with different evolutionary histories, e.g. Andean and Tibetan populations, differ from one another genetically, resulting in different adaptation responses to hypoxia (Beall,

2007; Bailey *et al.*, 2013). Therefore, as Tibetan highlanders were selected in the current study, caution is needed when comparing or interpreting the results of the current study with Andean populations. Further research is needed to explore vascular function in different highland groups.

## Conclusion

Our findings are the first to show that vascular function alterations in healthy lowlanders during high altitude exposure are potentially attributed to impairment in both endothelial and vascular smooth muscle function, associated with an increase in central arterial stiffness. The mechanisms underpinning the changes in vascular function with hypoxia seem to be related at least partly to elevations in oxidative stress and sympathetic nerve activity. It appears that the effects of ambient hypoxia on the vascular tree are not uniform, and that a lifetime of high altitude exposure neither exacerbates nor protects against the vascular function impairments seen following arrival to high altitude. In contrast, highland Sherpa had a consistently lower carotid wall: lumen ratio than did lowlanders at sea level and high altitude; these findings potentially indicate that arterial remodelling is an adaptive response to chronic high altitude. The extent to which these changes may potentially translate into an elevated cardiovascular risk remained to be determined.

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**Conflict of Interest/Disclosure**

None

**Authors Contribution**

Nia C.S Lewis: Conception and design of the experiment, data collection, analysis and interpretation of data and drafting the article or revising it critically for important intellectual content. Also approved the final version of the manuscript; Damian M. Bailey: Data analysis and interpretation, and approved the final version of the manuscript. Gregory R. duManoir: Data collection, analysis and drafting the article or revising it critically for important intellectual content, and approved the final version of the manuscript. Laura Messinger: Conception and design of the experiment, data collection, drafting the article or revising it critically for important intellectual content, and approved the final version of the manuscript. Samuel J.E. Lucas: Data collection, drafting the article or revising it critically for important intellectual content, and approved the final version of the manuscript. James D. Cotter: Data collection, drafting the article or revising it critically for important intellectual content, and approved the final version of the manuscript; Joseph Donnelly: Data collection, and approved the final version of the manuscript. Jane Emery: Data analysis. Ian Young: Data analysis. Mike Stembridge: Assisted with data collection and analysis. Keith R. Burgess: Data collection, and approved the final version of the manuscript. Aparna S. Basnet: Drafting the article or revising it critically for important intellectual content. Also approved the final version of the manuscript; Philip N. Ainslie: Conception and design of the experiment, interpretation of data, drafting the article or revising it critically for important intellectual content and approved the final version of the manuscript.

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**Tables**

**Table 1:** The effect of high altitude (5050 m) on cardiovascular variables in lowlanders and comparison with highlanders.

| Group | **Lowlanders** | | | **Highlanders** |
|---|---|---|---|---|
| Location/altitude duration | Sea level | Acute-HA | Chronic-HA | Lifelong-HA |
| SBP (mm Hg) | 118 ± 7 | 119 ± 9 | 115 ± 9 | 106 ± 16 |
| DBP (mm Hg) | 77 ± 7 | 73 ± 9 | 72 ± 9 | 72 ± 10 |
| MAP (mm Hg) | 94 ± 10 | 86 ± 8 | 87 ± 7 | 83 ± 11 ‡ |
| HR (beats·min$^{-1}$) | 56 ± 10 | 77 ± 15 ***** | 73 ± 15 ***** | 77 ± 11 ‡ |
| $SaO_2$ (%) | 99 ± 1 | 81 ± 3 ***** | 83 ± 4 ***** | 83 ± 2 ‡ |
| Carotid compliance cm$^2$/mmHg | 0.17 ± 0.04 | 0.22 ± 0.03 ***** | 0.19 ± 0.04 | 0.22 ± 0.08 |
| Carotid distensibilty mmHg$^{-1}$ | 0.006 ± 0.001 | 0.006 ± 0.002 | 0.005 ± 0.001 | 0.006 ± 0.003 |
| β stiffness | 4.5 ± 1.2 | 4.2 ± 1.2 | 4.5 ± 0.7 | 4.8 ± 2.0 |
| Carotid pulse pressure (mm Hg) | 37 ± 7 | 32 ± 6 | 34 ± 6 | 30 ± 11 ‡ |
| ***FMD*** | | | | |
| Baseline diameter (mm) | 4.2 ± 0.5 | 4.2 ± 0.5 | 4.1 ± 0.5 | 4.6 ± 0.5 |
| Peak diameter (mm) | 4.4 ± 0.5 | 4.5 ± 0.6 | 4.4 ± 0.5 | 4.9 ± 0.5 |
| Time to peak (s) | 60 ± 24 | 62 ± 20 | 59 ± 16 | 68 ± 21 |
| $SR_{AUC}$ (AUC) | 20327 ± 5401 | 22961 ± 9163 | 14802 ± 5306 † | 24490 ± 7230 |

| | | | | |
|---|---|---|---|---|
| Allometrically corrected FMD (%) | 7.9 ± 1.4 | 6.7 ± 1.4 | 7.3 ± 1.4 | 6.7 ± 2.1 |
| ***GTN*** | | | | |
| Baseline diameter (mm) | 4.2 ± 0.5 | 4.4 ± 0.6 | 4.2 ± 0.5 | 4.6 ± 0.7 |
| Peak diameter (mm) | 4.9 ± 0.5 | 4.9 ± 0.6 | 4.9 ± 0.5 | 5.3 ± 0.7 |
| Time to peak (s) | 365 ± 56 | 372 ± 104 | 338 ± 70 | 368 ± 46 |
| FMD:GTN ratio | 0.49 ± 0.12 | 0.49 ± 0.15 | 0.50 ± 0.14 | 0.49 ± 0.13 |
| ***VD*** | | | | |
| Baseline diameter (mm) | 4.1 ± 0.4 | 4.0 ± 0.4 | 4.0 ± 0.5 | |
| Peak diameter (mm) | 4.6 ± 0.4 | 4.4 ± 0.4 | 4.5 ± 0.5 | |
| Time to peak (s) | 97 ± 32 | 90 ± 31 | 105 ± 36 | |
| $SR_{AUC}$ (AUC) | 61089 ± 45870 | 63078 ± 25056 | 51850 ± 21826 | |

Values expressed as mean ± SD. HA, high altitude; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate; $SaO_2$, arterial oxygen saturation; CIMT, carotid intima media thickness; FMD, flow mediated dilation; GTN, glyceryl trinitrate, VD, vasodilator capacity; shear rate area under the curve ($SR_{AUC}$). Different probe placement between FMD and VD test due to distal and proximal cuff placement. ***** Lowlanders at acute (days 2-3) and chronic-high altitude (days 12-14) are significantly different from sea level, $P<0.001$; † Chronic-high altitude significantly different from acute-high altitude in lowlanders ($P=0.03$); ‡ Highlanders significantly different from lowlanders at sea level ($P=0.04$).

**Table 2:** Markers of oxidative stress and nitric oxide, at sea level and following acute (day 1) and chronic (7 days) exposure to high altitude in lowlanders.

| Metabolites | Sea Level (n=11) | Acute-HA (n=11) | Chronic-HA (n=7) |
|---|---|---|---|
| Lipid hydroperoxides (µM) | 1.87 ± 0.46 | 2.20 ± 0.67 ***** | 2.14 ± 0.75 † |
| Nitrite (nM) | 212 ± 77 | 283 ± 136 † | 304 ± 57 † |
| $\alpha$-tocopherol (µM) | 16.83 ± 3.91 | 15.56 ± 5.21 | 14.25 ± 3.12 |
| $\gamma$-tocopherol (µM) | 1.75 ± 0.86 | 2.37 ± 0.89 | 3.49 ± 2.32 |
| $\alpha$-carotene (µM) | 0.154 ± 0.092 | 0.141 ± 0.093 | 0.135 ± 0.119 |
| $\beta$-carotene (µM) | 0.465 ± 0.367 | 0.430 ± 0.404 | 0.320 ± 0.347 |
| Retinol (µM) | 1.77 ± 0.44 | 1.61 ± 0.45***** | 1.75 ± 0.86 |
| Lycopene (µM) | 1.00 ± 0.38 | 0.91 ± 0.45 | 1.14 ± 0.81 |
| Zexanthin (µM) | 0.054 ± 0.025 | 0.056 ± 0.026 | 0.044 ± 0.014 |
| Lutein (µM) | 0.199 ± 0.121 | 0.187 ± 0.136 | 0.357 ± 0.632 |
| $\beta$-cryptoxanthin (µM) | 0.084 ± 0.038 | 0.071 ± 0.032 | 0.054 ± 0.027 |

Values expressed as mean ± SD. HA, high altitude. * Acute-HA significant different from sea level (*n*=11, paired t-test: P≤0.03). † Acute- and chronic-HA significant different from sea level (n=7, one-way ANOVA: P≤0.05).

**Table 3:** The time course effect of normobaric hypoxia on cardiovascular variables and effect of $\alpha_1$-blockade.

| | **Inspirate** | **Normoxia** | **Hypoxia** | | | **Normoxia** |
|---|---|---|---|---|---|---|
| **Time (min)** | | 0 | 60 | 220 | 330 | 60 |
| SBP (mm Hg) | Placebo | 114 ± 11 | 121 ± 26 | 109 ± 9 | 109 ± 8 | 112 ± 13 |
| | $\alpha_1$-blockade | 109 ± 8 | 111 ± 8 | 116 ± 17 | 108 ± 12 | 113 ± 7 |
| DBP (mm Hg) | Placebo | 76 ± 10 | 71 ± 10 | 70 ± 10 | 74 ± 9 | 74 ± 9 |
| | $\alpha_1$-blockade | 72 ± 8 | 71 ± 10 | 72 ± 12 | 65 ± 12 | 70 ± 10 |
| MAP (mm Hg) | Placebo | 90 ± 9 | 88 ± 7 | 83 ± 8 | 86 ± 7 | 87 ± 9 |
| | $\alpha_1$-blockade | 83 ± 10 ‡ | 85 ± 9 | 79 ± 14 | 80 ± 8 | 84 ± 8 |
| HR (beats·min$^{-1}$) | Placebo | 59 ± 10 | 68 ± 15 | 72 ± 15 | 72 ± 15 *† | 60 ± 14 |
| | $\alpha_1$-blockade | 61 ± 9 | 66 ± 9 | 66 ± 8 | 77 ± 13 ‡ | 65 ± 10 |
| $SaO_2$ (%) | Placebo | 96 ± 2 | 72 ± 5 *† | 76 ± 7 *† | 80 ± 4 *† | 97 ± 1 |
| | $\alpha_1$-blockade | 96 ± 1 | 79 ± 7 | 81 ± 6 | 79 ± 7 | 97 ± 1 |
| ***FMD*** | | | | | | |
| Baseline diameter (mm) | Placebo | 4.5 ± 0.4 | 4.5 ± 0.5 | 4.7 ± 0.6 † | 4.5 ± 0.5 | 4.3 ± 0.5 |
| | $\alpha_1$-blockade | 4.4 ± 0.5 | 4.5 ± 0.6 | 4.5 ± 0.4 | 4.6 ± 0.6 | 4.4 ± 0.5 |
| Peak diameter (mm) | Placebo | 4.8 ± 0.5 | 4.8 ± 0.6 | 4.8 ± 0.5 | 4.7 ± 0.5 | 4.6 ± 0.5 |
| | $\alpha_1$-blockade | 4.7 ± 0.5 | 4.7 ± 0.6 | 4.8 ± 0.4 | 4.9 ± 0.6 | 4.8 ± 0.5 |
| Time to peak (s) | Placebo | 49 ± 17 | 46 ± 18 | 47 ± 20 | 54 ± 23 | 45 ± 12 |
| | $\alpha_1$-blockade | 56 ± 21 | 50 ± 18 | 54 ± 25 | 61 ± 23 | 54 ± 18 |
| $SR_{AUC}$ (AUC) | Placebo | 22727 ± 6813 | 17847 ± 7468 | 20745 ± 13011 | 20142 ± 11799 | 22543 ± 6110 |
| | $\alpha_1$-blockade | 23423 ± 11423 | 21498 ± 9124 | 22464 ± 11424 | 24043 ± 10701 | 25234 ± 11027 |

Values expressed as mean ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate; $SaO_2$, arterial oxygen saturation; FMD, flow mediated dilation; $SR_{AUC}$, shear rate area under the curve. * Significantly different from normoxia 0 min (baseline) in the placebo trial only. † Significantly different from normoxia 60 (recovery) in the placebo trial only ($P \leq 0.001$). ‡ Measurement time point in the $\alpha_1$-blockade trial significantly different from placebo trial ($P \leq 0.03$). Placebo vs. blockade at hypoxic time points 60 min and 120 min, $n$ =10.

**Figures and Legends**

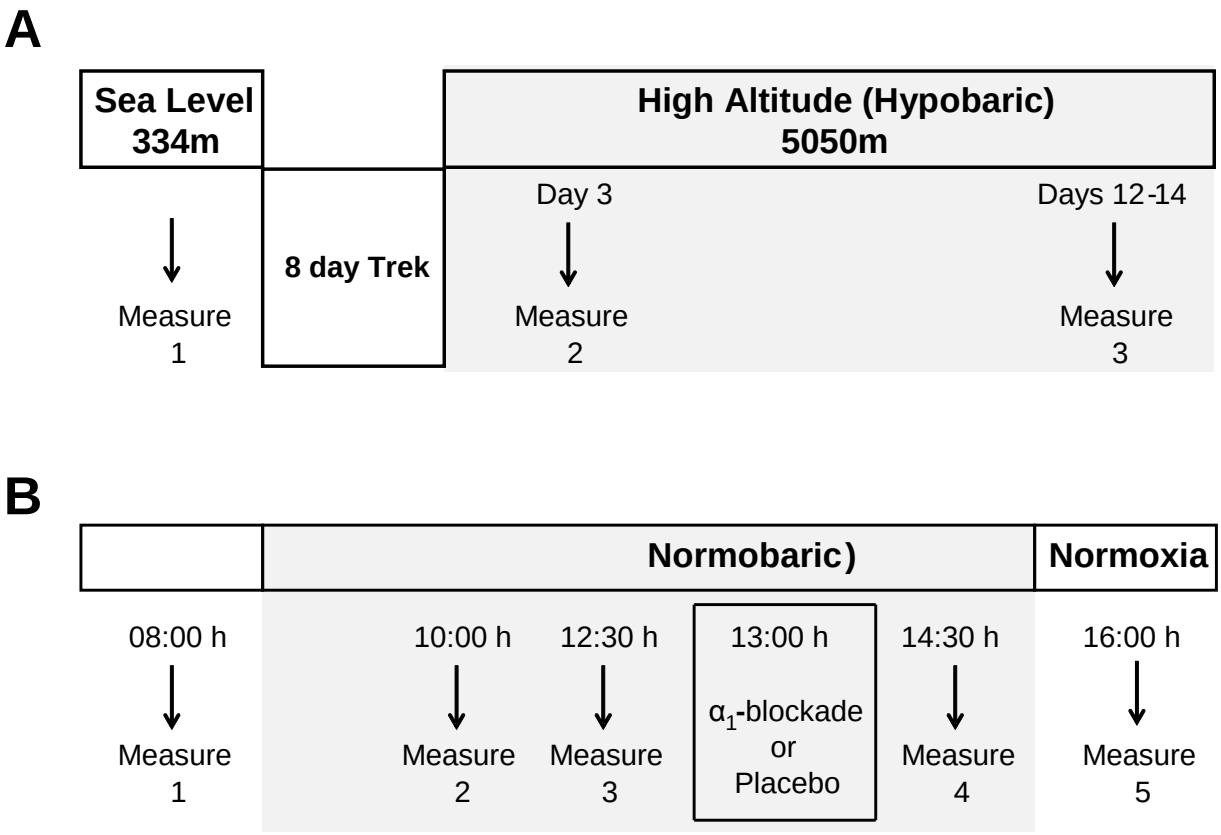


**Figure 1**: Schematic detailing experimental schedule for lowlander in study 1 (A) and study 2 (B). In study A, measures were completed at sea level, upon initial arrival at high altitude (5050 m; day 3) and following 12-14 days of partial acclimatisation at 5050 m. In the follow-up study B, measures were completed in normoxia, prior to a 6 hr exposure of normobaric hypoxia ($FIO_2$=0.11). Measures were repeated following 60 min, 210 min 330 min of hypoxia. Following 240 min of hypoxia (90 min prior to the last assessment in hypoxia [330 min]), participants orally consumed the $\alpha_1$-adrenoreceptor blocker, prazosin (1 mg/20 kg body mass) or placebo capsule. All measures were repeated in normoxia 60 min following the hypoxic exposure.

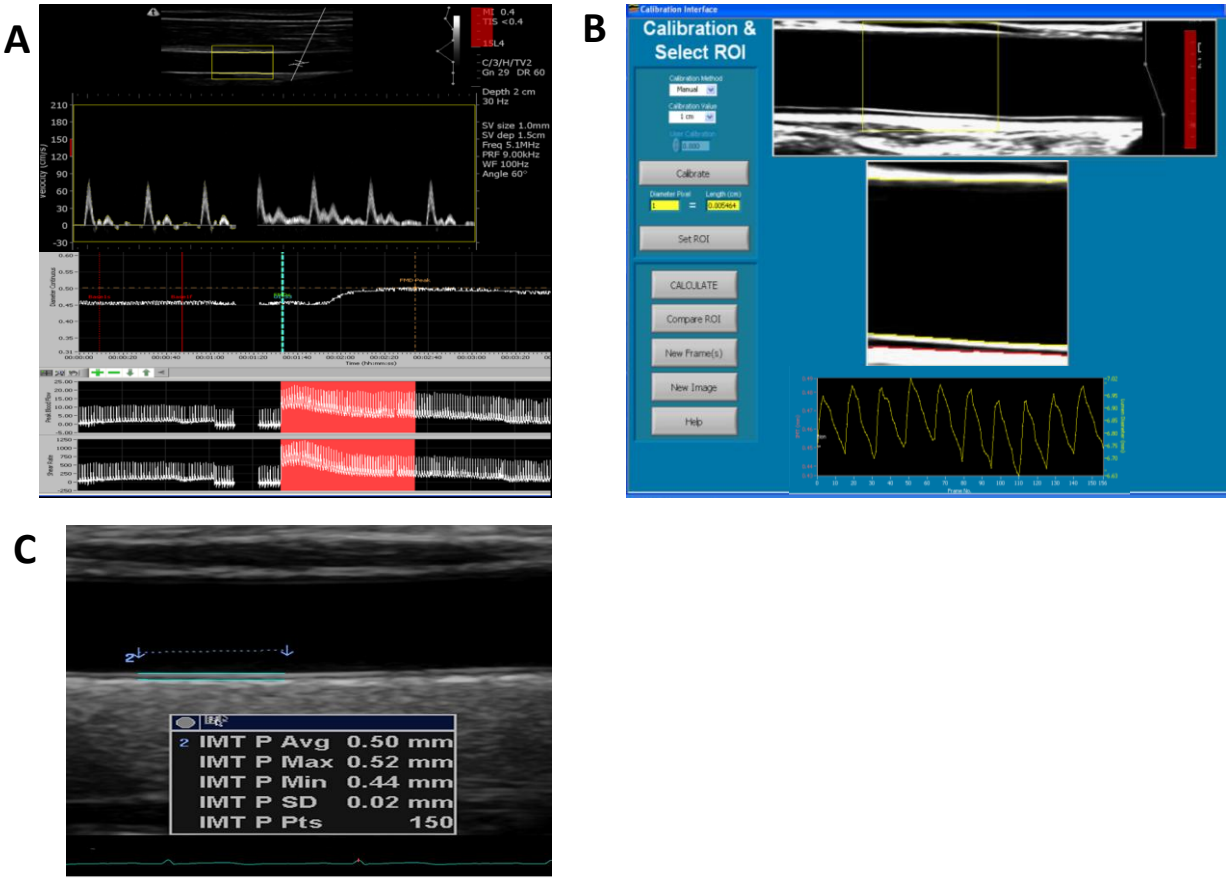


**Figure 2:** Illustration of ultrasound images and analysis techniques for: A, brachial flow mediated dilation (custom-designed edge-detection and wall-tracking software was used to obtain simultaneous measure of diameter and flow). B, beat-to-beat common carotid diameter (custom-designed edge-detection and wall-tracking software was used to obtain beat-to-beat diameter). C, common carotid intima-media thickness (CIMT: commercial edge-detection software (EchoPAC PC, GE Healthcare) was used to measure CIMT across consecutive cardiac cycles).

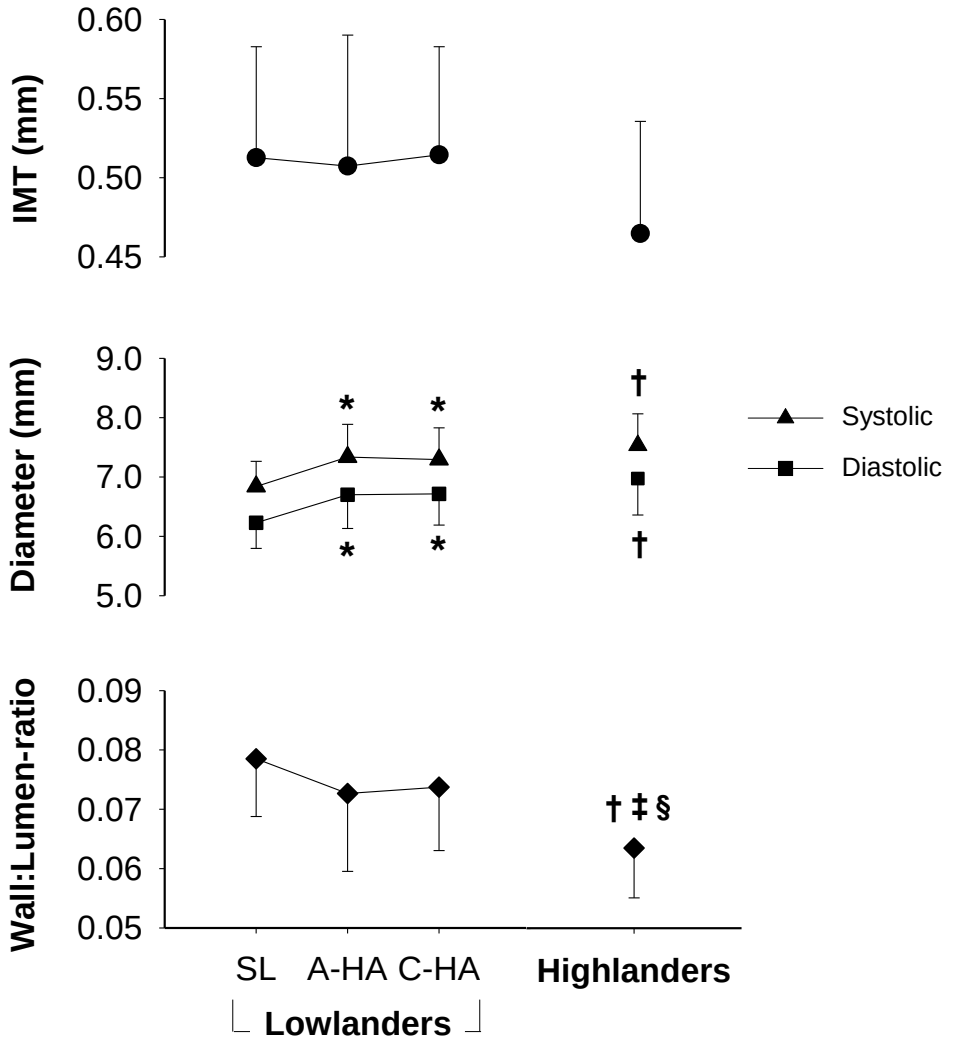


**Figure 3:** Carotid intima-medial thickness (IMT), carotid systolic and diastolic diameter, and carotid wall: lumen ratio in lowlanders at sea level (SL), acute-high altitude (A-HA) and following chronic-high altitude (C-HA), and comparison to highlanders at high altitude (5050 m). * Significantly different from sea level (P≤0.02). † ‡ § Highlanders were significantly different from lowlanders at sea level, acute-high altitude and chronic-high altitude, respectively (P≤0.04).

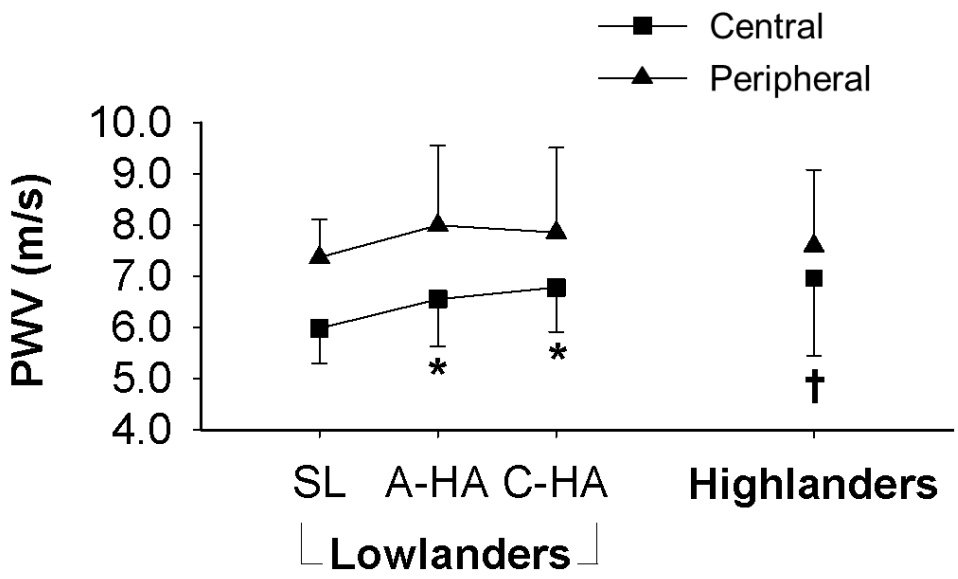


**Figure 4**: Central and peripheral pulse wave velocity (PWV) in lowlanders at sea level (SL), acute-high altitude (A-HA) and following chronic-high altitude (P-HA), and with comparison to highlanders at high altitude (5050 m). * Significantly different from sea level ($P \leq 0.04$). † Highlanders significantly different from lowlanders at sea level ($P = 0.05$).

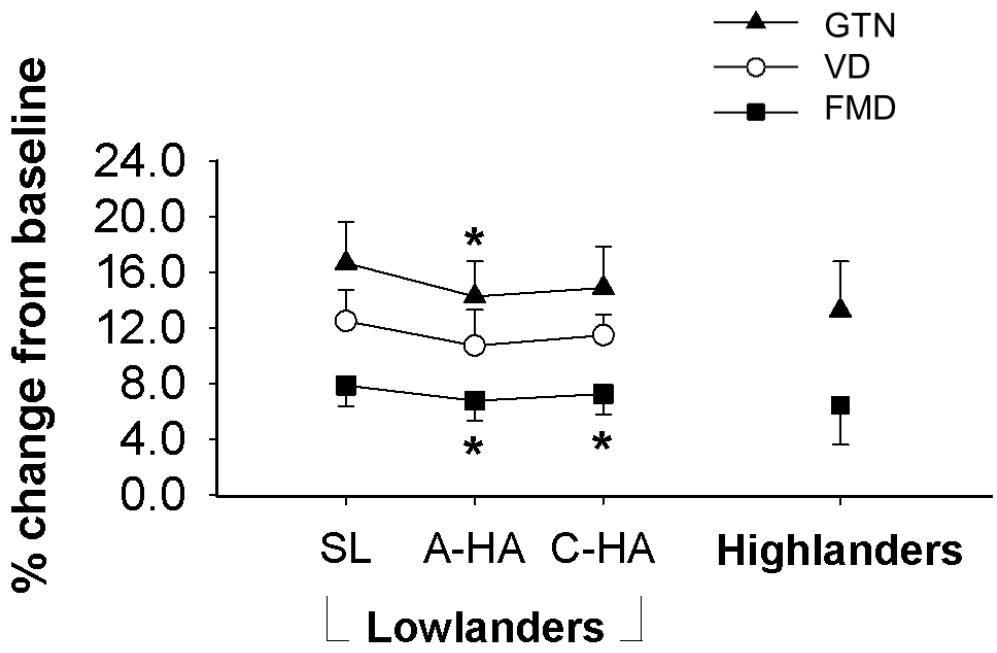


**Figure 5:** Brachial flow mediated dilation (FMD), vasodilator capacity (VD) and glyceryl trinitrate (GTN) in lowlanders at sea level (SL), acute-high altitude (A-HA) and following chronic-high altitude (C-HA), and with comparison to highlanders at 5050 m. * Significantly different from sea level ($P \leq 0.02$).

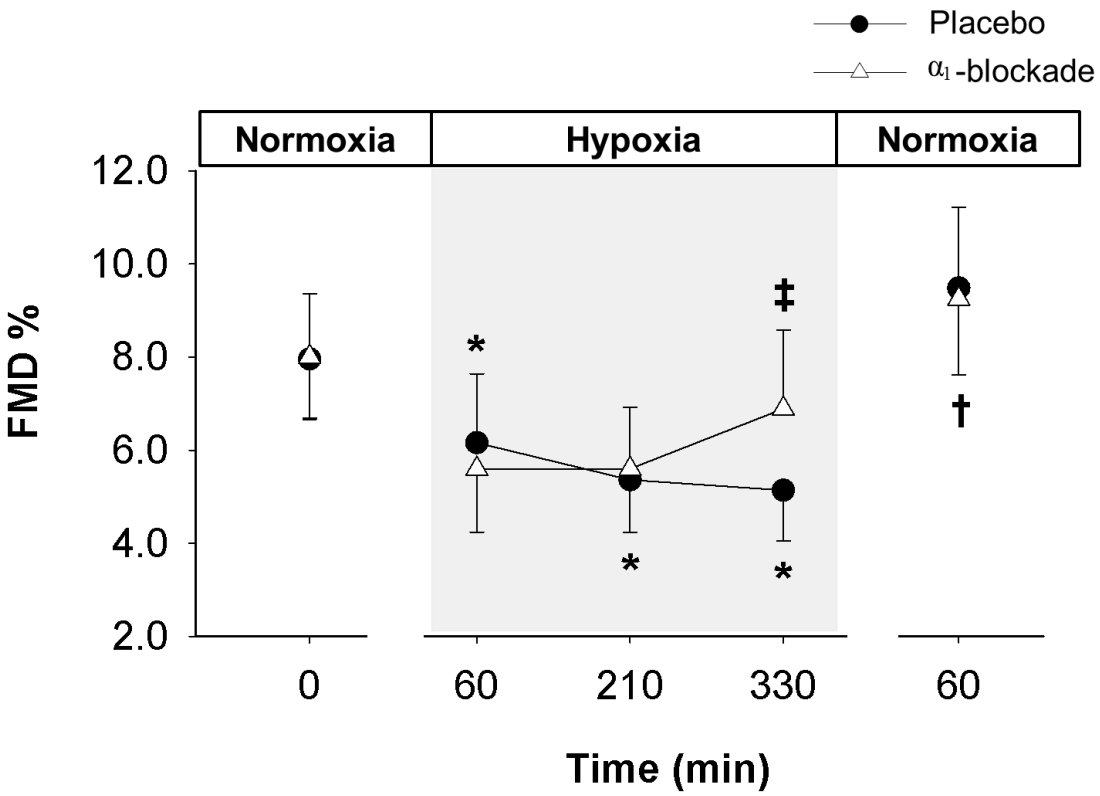


**Figure 6:** Changes in flow mediated dilation (FMD) during normobaric hypoxia and the effect of $\alpha_1$-blockade. * Significantly different from normoxia 0 min (baseline) in the placebo trial only. † Normoxia 60 (recovery) significantly different from hypoxic measures in the placebo trial only ($P \leq 0.001$). ‡ Measurement at 330 min time point in the $\alpha_1$-blockade trial significantly different from placebo trial ($P \leq 0.03$).