Renin–Angiotensin–Aldosterone System Is Not Involved in the Arterial Stiffening Induced by Acute and Prolonged Exposure to High Altitude

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Abstract—This randomized, double-blind, placebo-controlled study was designed to explore the effects of exposure to very high altitude hypoxia on vascular wall properties and to clarify the role of renin–angiotensin–aldosterone system inhibition on these vascular changes. Forty-seven healthy subjects were included in this study: 22 randomized to telmisartan (age, 40.3±10.8 years; 7 women) and 25 to placebo (age, 39.3±9.8 years; 7 women). Tests were performed at sea level, pre- and post-treatment, during acute exposure to 3400 and 5400-m altitude (Mt. Everest Base Camp), and after 2 weeks, at 5400 m. The effects of hypobaric hypoxia on mechanical properties of large arteries were assessed by applanation tonometry, measuring carotid–femoral pulse wave velocity, analyzing arterial pulse waveforms, and evaluating subendocardial oxygen supply/demand index. No differences in hemodynamic changes during acute and prolonged exposure to 5400-m altitude were found between telmisartan and placebo groups. Aortic pulse wave velocity significantly increased with altitude (P<0.001) from 7.41±1.25 m/s at sea level to 7.70±1.13 m/s at 3400 m and to 8.52±1.59 m/s at arrival at 5400 m (P<0.0001), remaining elevated during prolonged exposure to this altitude (8.41±1.12 m/s; P<0.0001). Subendocardial oxygen supply/demand index significantly decreased with acute exposure to 3400 m: from 1.72±0.30 m at sea level to 1.41±0.27 m/s at 3400 m (P<0.001), remaining significantly although slightly less reduced after reaching 5400 m (1.52±0.33) and after prolonged exposure to this altitude (1.53±0.25; P<0.001). In conclusion, the acute exposure to hypobaric hypoxia induces aortic stiffening and reduction in subendocardial oxygen supply/demand index. Renin–angiotensin–aldosterone system does not seem to play any significant role in these hemodynamic changes.


Key Words: altitude ■ altitude sickness ■ hemodynamics ■ pulse wave analysis ■ pulse wave velocity ■ renin–angiotensin system ■ vascular stiffness

The exposure to hypobaric hypoxia at high altitude (HA) is responsible for complex modifications in both systemic and pulmonary circulation. Activation of the peripheral chemoreflex leads to a sympathetic nervous system activation, which increases blood pressure (BP), heart rate, and cardiac output countering the direct systemic vasodilator effect of hypoxia.1,2 Previous studies have shown that systemic vascular dysfunction both in lowlanders during acute HA exposure3 and in native highlanders4,5 is related to increased sympathetic nervous system activity, increased oxidative stress, and decreased nitric oxide bioavailability. However, until now, there is no data supporting a role of the renin–angiotensin–aldosterone system (RAAS) in this setting. Hypoxia may also have a direct effect on RAAS, and retention of fluid and sodium has been proposed as one of the mechanisms involved in the pathogenesis of acute mountain sickness and possibly also of HA pulmonary edema.6 Although most available HA studies focused on pathophysiology of HA pulmonary hypertension,7 only few data are available on changes in cardiac function and in systemic circulation, and in particular, in arterial wall properties, primarily when explored in relation to changes in sympathetic and RAAS activity.

HIGHCARE Himalaya project (HA cardiovascular research) was designed to fill-in this gap, based on a randomized, double-blind, parallel group, placebo-controlled study design, and focusing on changes in several cardiovascular variables related to systemic circulation in response to acute
and prolonged exposure to hypobaric hypoxia at high and very HA, and on the possible modulation of these changes by angiotensin II receptor blockade.

In particular, among the aims of HIGHCARE Himalaya study was the investigation of the effects of hypobaric hypoxia at HA on elastic and muscular arteries, based on pulse wave velocity (PWV) assessment and on analysis of pulse waveform changes. An additional aim of the study was to clarify whether inhibition of RAAS may counteract the modifications induced by hypoxia on the vascular function at HA.

Methods

The HIGHCARE Himalaya is a randomized, double-blind, parallel group, placebo-controlled study examining the effects of telmisartan, a type-1 angiotensin II receptor blocker at HA. Healthy lowlander volunteers, without known cardiovascular disease, chronic cardiovascular therapy, diabetes mellitus or hypertension, and history of severe mountain sickness, were included in the study, provided that an exercise test immediately before the inclusion did not show evidence of myocardial ischemia. Professional athletes and recent exposure to altitudes >2000 m were additional exclusion criteria for this study.

On the basis of the wide literature available on this issue, we assumed that 20 subjects per group were sufficient to demonstrate with \( P<0.05 \) and a power of 0.8, a difference in PWV of 1.0 m/s between sea level (SL) and HA in a population 30 to 50 years of age, characterized by mean carotid–femoral PWV (cf-PWV)±SD of 7.10±1.08 m/s. A dropout rate of ≥20% was also expected. Based on these considerations, 50 subjects were recruited, randomly assigned to receive either telmisartan or placebo.

Subjects enrolled in the study were randomized in blocks of 4 and stratified by sex and age to receive either telmisartan (80 mg) or placebo (once) daily in the morning. Study drugs were distributed as identical capsules containing telmisartan (40 mg) or placebo. Randomization and distribution of drug containers were done by 2 people not otherwise involved in the study, and the randomization lists were kept secret to all participants and research team members throughout the study. Initially, the subjects were asked to take 1 capsule in the morning (ie, telmisartan [40 mg] or placebo). After 2 weeks, in subjects with no abnormalities in terms of plasma potassium and creatinine or sitting BP and without adverse events, the dose was increased to 2 capsules in the morning.

After 8 weeks of double-blind treatment, the participants travelled from Milan, Italy, 140 m above SL (ASL) to Kathmandu, Nepal (1355 m), where they stayed for 3 days. Then, they were brought within few hours by air transportation to Namche Bazaar (3400 m), where they stayed for another 3 days. From Namche Bazaar, they trekked >5 days to Mt. Everest Base Camp (BC; 5400 m), where they remained for 12 days (Figure 1). Study drugs were taken throughout the expedition until the last tests were completed, after performing the measurements planned on return to SL.

Study Design and Protocol

Tests were performed at 6 time points:
1. at SL baseline, pretreatment;
2. at SL baseline, post-treatment;
3. during acute exposure to HA at Namche Bazaar (Namche; 3400 m ASL);
4. during acute exposure to very high (5400-m ASL) altitude at Mt. Everest BC (BC1);
5. during prolonged exposure (2 weeks) to very high (5400-m ASL) altitude at Mt. Everest BC (BC2);
6. at SL, immediately after the return to Milan.

At Namche, all tests were performed in the morning, the first and the second day after arrival, in comfortable lodge rooms. At Everest BC, tests were performed in heated tents, in the morning, the first and the second day after arrival, and repeated after days 10 and 11 of permanence at this very HA.

Laboratory temperature and barometric pressure were recorded by means of a microclimatic station, both in Namche and at Everest BC.

A standardized questionnaire for the clinical assessment of acute mountain sickness (Lake Louise score\(^1\)) was completed daily. Participants were asked to take the study drugs until completion of all measurements (see below), after return to SL.

In each condition, peripheral BP and heart rate were measured 3x, at brachial artery level, in the supine position with a validated oscillometric device (AND UA-767P; AND Company Ltd, Tokyo, Japan). Measurements were spaced by 3-minute intervals. Data supporting the reliability of devices used at HA for measuring BP and PWV are shown in the online-only Data Supplement. Oxygen saturation was derived through a finger pulse oximeter (Ohmeda Tuff Sat with sensor OxylTip Finger 6051-0000-160; GE Healthcare, Finland).

The study was registered before the study start in EudraCT register (European Union Drug Regulating Authorities Clinical Trials; www.clinicaltrialsregister.eu) with EudraCT number 2008-000540-14.

Arterial Stiffness

Viscoelastic properties of large arteries were derived from the measurements of cf-PWV and carotid–radial PWV. At present, cf-PWV is considered the most reliable noninvasive measurement of aortic stiffness,\(^3\) and carotid–radial PWV reflects upper limb muscular arterial stiffness. A validated, easy-to-use, PulsePen device (DiaTecne srl, Milan, Italy)\(^4\) was used in this study. The PulsePen consists of a pocket-size, high-fidelity applanation tonometer, and an integrated ECG unit. PWV was measured by recording carotid and peripheral (femoral or radial) waveforms in rapid succession. PWV was defined as the distance between the measuring sites divided by the time delay between the distal pulse wave from the proximal pulse wave, using the ECG trace as reference. This method was previously described in detail.\(^5\) As recommended from an expert consensus document on the measurement of aortic stiffness,\(^6\) 80% of the direct distance from the 2 sites was used to calculate the PWV. The PulsePen device software did not validate measurements if the difference between BP or heart rate values taken at the time of carotid and peripheral (femoral or radial) artery recordings was >10%. Participants were asked to refrain from tobacco smoking and from drinking beverages containing caffeine at least 3 hours before measurements and to refrain from drinking alcohol since 10 hours before measurements. In each subject, PWV measurements were repeated twice, and their average was used for the analysis.

Central BP

PulsePen tonometer was also used to directly record the pulse waveform and central BP values at the common carotid artery site.\(^7\) Augmentation index (Alx) is a parameter that provides an indication of the contribution of reflected waves to the total pulse pressure and was defined as the difference between the second and first systolic peak on arterial pulse waveform and was expressed as a percentage of central pulse pressure.\(^8\) Because Alx is affected by heart rate, which is increased during hypobaric hypoxia exposure at altitude, Alx values were normalized for a theoretical heart rate of 75 bpm via a conventional formula.\(^9\)

The assessment of the degree of myocardial perfusion relative to left-ventricular workload was obtained through the quantification of subendocardial oxygen supply and demand ratio (subendocardial viability ratio, SEVR). This parameter has been previously described.\(^10\)\(^-\)\(^20\)

Briefly, SEVR was computed as the ratio between diastolic pressure–time index and systolic pressure–time index. Diastolic pressure–time index is considered as an estimate of myocardial oxygen supply and represents the area between the aortic and left-ventricular pressure curves in diastole: diastolic pressure–time index=(mean diastolic aortic pressure−mean diastolic left-ventricular pressure)/diastolic time). Left-ventricular mean diastolic pressure was estimated from the left-ventricular end-diastolic pressure value provided by echocardiography. Echocardiography was performed with a 3.5 MHz sector scanning electronic transducer, at SL by using Vivid 7 equipment (GE Healthcare), whereas at HA with a portable device (Vivid E; GE Healthcare). Details on the cardiac ultrasound examination are described elsewhere.\(^21\) Left-ventricular diastolic pressure was evaluated by echocardiography using the Nagueh formula, 1.24 E/e′+1.91.\(^22\) Systolic pressure–time index is considered as an estimate of myocardial needs of oxygen and represents the area under the aortic pressure curve in systole: systolic
pressure–time index=mean systolic aortic pressure (corresponding to left-ventricular mean systolic pressure)×left-ventricular ejection time. The area corresponding to isovolumic contraction time, assessed by echocardiography, was also taken into account in the evaluation of systolic pressure–time index. The isovolumic contraction period should also be considered as a cardiac work period and should thus be included in the heart demand period.

Exposure to HA hypoxia entails a significant decrease in arterial oxygen content (CaO₂). Thus, to more correctly evaluate the subendocardial oxygen supply, SEVR was multiplied by CaO₂ offering a global index of oxygen brought to the subendocardial muscle per minute (SEVR×CaO₂). Thus, to more correctly evaluate the subendocardial oxygen supply, SEVR was multiplied by CaO₂ offering a global index of oxygen brought to the subendocardial muscle per minute (SEVR×CaO₂). The CaO₂ was determined using the following formula: CaO₂=1.34×blood hemoglobin concentration (g/dL)×arterial oxygen saturation (%)+0.003×arterial pressure of oxygen (mmHg). Further details related to echocardiographic assessment and to the evaluation of SEVR and CaO₂ are reported in the online-only Data Supplement.

Biochemical Determinations
At each step, blood samples were collected for the determination of plasma angiotensin II (double antibody radioimmunoassay; Bühlmann Laboratories AG, Schonenbuch, Switzerland), plasma noradrenaline (high-performance liquid chromatography with electrochemical detection; Chromsystems Instruments & Chemicals GmbH, Munich, Germany), plasma renin concentration (Liaison Direct Renin Assay, chemiluminescent immunoassay; DiaSorin, Stillwater, MN), and serum aldosterone (Aldokt-2, radioimmunoassay; DiaSorin, Stillwater, MN) concentrations. Blood samples were always obtained in the morning after subjects had been sitting for at least 15 minutes. At SL, they were immediately sent to the laboratory for analyses, although during the expedition, they were immediately centrifuged and frozen in liquid nitrogen to be later shipped to laboratory in Milan.

Statistical Analysis
All analyses were performed using R software, version 2.15.3 (R Foundation for Statistical Computing). Continuous variables are reported as mean±SD. To assess the effect of both altitude level and treatment group, we used the linear mixed-effects models package (linear and nonlinear mixed-effects models) accounting for repeated measurements, with a compound symmetry covariance structure fitting the models by maximizing the restricted log likelihood. For multiple post hoc comparisons, we used the algorithm that controls the expected rate of false-positive results for all positive results (false discovery rate). An α level of 0.05 was used for all hypothesis tests.

The study protocol was approved by the Ethical Committee of the Istituto Auxologico Italiano, Milan, Italy. All subjects gave their written informed consent to the study procedures.

Results
Fifty subjects were randomized in the HIGHCARE study (33 men; age range, 26 to 62 years; mean±SD, 39.9±10.0 years). Three subjects (1 man) did not ascend to HA for personal reasons. Two subjects randomized in the telmisartan group (1 woman and 1 man) were excluded from the analysis because of scarce compliance to drug therapy. Thus, final data analysis was performed in 45 subjects (14 women), 20 randomized to telmisartan (7 women) and 25 randomized to placebo (7 women). All participants were whites. Only 3 volunteers (all included in the placebo group) were regular smokers. At baseline, there was no significant difference between the anthropometric characteristics of the 2 randomized groups (Table 1).

HIGHCARE Project: Effect of Type-I Angiotensin II Receptor Blocker
Table 2 shows the values of arterial oxygen saturation and of the main hemodynamic parameters in both randomized groups, for every step of the protocol.

Peripheral and central systolic BP in either placebo or telmisartan groups significantly increased with altitude, remaining elevated during the entire permanence at BC. The trend was similar for mean arterial pressure and diastolic BP. Altitude significantly affected all the hemodynamic and pulse wave parameters except for carotid–radial PWV. Changes in plasma noradrenaline, renin, serum angiotensin, and aldosterone levels in both treatment groups throughout the study have been shown in detail in a previous study of ours focusing on ambulatory BP. In brief, type-I angiotensin II receptor blocker induced, as expected, a significant increase in angiotensin II (P=0.001) and renin (P=0.003) plasma values at SL, although all these variables were significantly reduced under acute exposure to 5400 m (P<0.001), with a tendency to increase again after 2 weeks at this altitude. In the placebo group, angiotensin II, renin, and aldosterone were significantly

Figure 1. Time course of the HIGHCARE Himalaya Study (high altitude cardiovascular research) data collection. Altitude, locations, means of transportation between locations, and days of ascent and staying. a.s.l. indicates above sea level.
reduced at very HA (\(P<0.001\) for all). The exposure to hypobaric hypoxia was accompanied by a significant increase in plasma norepinephrine level proportional to the altitude reached (from 379.92±130.36 ng/L at SL baseline, post-treatment, to 950.65±324.10 ng/L at BC1; \(P<0.005\)). During prolonged exposure to hypobaric hypoxia, both in placebo and telmisartan groups, norepinephrine level did not change compared with acute exposure to this altitude (730.29±594.81 ng/L at BC2; \(P=\text{nonsignificant versus BC1}\)), suggesting a persistent and stable sympathetic activation.

The Lake Louise score was \(\geq3\) (considered as indicative of acute mountain sickness) in 9 subjects in Namche, in 22 subjects at BC1, and in 3 subjects at BC2. There were no between-group differences at HA in the Lake Louise score.

### Hemodynamic Changes With Altitude

Because no difference in hemodynamic parameters was found between telmisartan and placebo group, data acquired in all our subjects were taken into account to study the effects of hypobaric hypoxia on central pulse waveform and arterial stiffness. Thus 47 subjects were considered, recovering also data from those individuals excluded from the preceding analysis because of scarce compliance to the drug therapy.

Compared with SL, cf-PWV significantly increased when subjects reached 5400 m of altitude (Figure 2, top): from 7.41±1.25 m/s at SL baseline, pretreatment, to 7.70±1.13 m/s at 3400 m (+3.9%; +0.29 m/s; \(P=0.132\)), and to 8.52±1.59 m/s when just arrived at 5400 m (+15.5%; +1.15 m/s; \(P<0.0001\)), remaining elevated during prolonged exposure to this altitude (8.41±1.12 m/s; +13.8%; +1.02 m/s; \(P<0.0001\)), then quickly returned to baseline values on reentry to SL (7.64±1.17 m/s). On the contrary, no change in carotid–radial PWV at HA was observed. To assess whether altitude-related changes in arterial stiffness were simply a consequence of the BP and heart rate increase triggered by exposure to hypobaric hypoxia, all statistical analyses were performed with adjustment for heart rate and mean arterial pressure.

Alx normalized for a theoretical heart rate of 75 bpm did not show significant changes with altitude. Taking into account the well-known higher values of Alx in women than in men, \(P=0.002\) data are shown in Figure 2 (bottom) separately by sex (\(P<0.002\)).

SEVR values significantly decreased with acute exposure to HA (Figure 3). The minimum value of SEVR was recorded at acute exposure to 3400 m (1.34±0.45 at Namche versus 1.63±0.30 at SL; \(P<0.001\)); SEVR remained significantly reduced at arrival at 5400 m (1.47±0.32; \(P<0.001\)) and after prolonged exposure to this altitude (1.47±0.25; \(P<0.001\)).

Oxygen supply and demand ratio (SEVR×Ca\(_{O2}\)) fell after acute exposure to HA: from 31.4±6.7 at SL to 23.5±5 at 3400 m (\(P<0.001\)) to 25.1±7.6 at 5400 m (\(P<0.001\)) after prolonged staying at HA, this parameter gradually tended to return toward normal values (29.9±7; not significantly different versus SL values), in parallel with the increase in hemoglobin concentrations.

### Discussion

Our study provides important information on vascular changes at HA. First, exposure to very HA causes a significant increase in aortic stiffness, particularly evident with prolonged exposure, and our data suggest that such an increase in stiffness cannot be just explained by BP and heart rate variations with altitude. Second, subendocardial oxygen supply and demand ratio dramatically falls during acute exposure at HA, with a tendency toward a slow return to normal values after adaptation to altitude. However, the main, new finding of this study was that RAAS does not seem to play any significant role in the vascular changes occurring under acute and prolonged exposure to very HA.

#### Arterial Stiffness at Very HA

A first, interesting finding of our study is the observation of an increase in cf-PWV at very HA (5400 m). This was shown in a relatively large (in relation to the conditions where our study was performed) group of healthy volunteers investigated at rest in absence of recent physical exercise and in a condition of controlled ambient temperature. These changes remained significant after adjusting for mean arterial pressure and heart rate changes with altitude.

PWV can be affected by both artery structural and functional changes. Structural changes influence arterial stiffness through a steady alteration in the elastic–collagen fibers ratio and the organization of the extracellular matrix of the arterial wall. However, it is difficult to assume the occurrence of a structural alteration of the arterial wall in the relatively short time of a HA ascension. The quick return of PWV to baseline values after reentry to SL further contributes to make it unlikely that changes in PWV at altitude were because of structural changes in the arterial wall. Although they have less importance than structural changes on large artery distensibility, the role of functional changes in arterial wall modulation is also complex. Functional factors include left-ventricular performance, vascular smooth muscle tone, and the levels of mean BP, which may be modulated by the sympathetic nervous system and RAAS activity. In HIGHCARE study, we showed that exposure to HA hypoxia is accompanied by a significant increase in plasma noradrenalin proportional to the altitude reached.\(^{24}\) This sympathetic activation persisted throughout the time spent at HA. The muscular arteries are much more sensitive than elastic arteries to sympathetic nervous system activity, and, therefore, the weak and insignificant increase in carotid–radial PWV with altitude suggests that other factors, in addition to sympathetic activation, may...
Table 2. Hemodynamic Parameters in Both Randomized Groups for Every Step of the Protocol

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<th>SL-Post</th>
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<th>5400 m BC1</th>
<th>5400 m BC2</th>
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Table 2. Continued

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Pl and TS groups consisted of 25 and 20 subjects, respectively. Ax indicates augmentation index; Ax@75, augmentation index normalized for a theoretical heart rate of 75 bpm; BC1, Everest base camp: tests performed the first and the second day after arrival; BC2, Everest base camp: tests performed after 10 and 11 d of permanence at very high altitude; CaO₂, arterial oxygen content; cf-PWV, carotid–femoral pulse wave velocity; cPP, carotid pulse pressure; cr-PWV, carotid–radial pulse wave velocity; cSBP, carotid systolic blood pressure; DBP, diastolic blood pressure; DT, diastolic time; HR, heart rate; LV, left-ventricular ejection time; MAP, mean arterial pressure; O₂ sat, arterial oxygen saturation; PL, placebo group; pSBP, brachial systolic blood pressure; SEVR, subendocardial viability ratio; SL, sea level; SL-post, sea level post-treatment; SL-pre, sea level pretreatment; SL-return, sea level immediately after return; and TS, telmisartan group.

*P<0.001; †P<0.05; ‡P<0.01.

Influence the transient aortic stiffening at HA. Other effects of HA exposure, such as endothelial dysfunction,7,25 oxidative stress,26,27 hemococoncentration with consequent increase in blood viscosity,28 and interstitial edema29 of the arterial wall, may have an important role in determining the changes in arterial distensibility observed in this condition. Acute exposure to HA has indeed been reported to impair both endothelial30 and vascular smooth muscle–cell function,8 as shown in a previous study by our group which demonstrated a link between the altitude-induced increase in pulmonary artery pressure and an increase in plasma and urinary endothelin-1 levels.31 All these changes might contribute to explain the hypoxia-related increase in arterial stiffness at altitude, although their possible role in this regard is mostly based on speculative considerations which remain to be supported by further experimental studies.

In the HIGHCARE Alps Study,3 which we performed at the Capanna Regina Margherita (Monte Rosa; 4559 m ASL; Italian–Swiss Alps), no significant changes in cf-PWV were found. The very HA reached in the HIGHCARE Himalaya study (5400 m) and the longer time spent at very HA for acclimatization (≈12 days) may contribute to explain the significant change in PWV shown in this condition, which could not be seen during the short time exposure at the 4559 m altitude of the HIGHCARE Alps study. Our data are in line with the results of the recent study performed by Lewis et al2 in a small group of 12 lowlander volunteers, showing an increase of aortic stiffness during acute and chronic exposure at 5050 m ASL. Axl did not change in our healthy volunteers either with altitude or during treatment with telmisartan. The lack of change in Axl under altitude exposure might be explained by the interaction of several contributing factors that are known to undergo modifications under hypobaric hypoxia exposure.32 In particular, changes in the magnitude and variability of reflected waves, mainly in relation to changes in systemic vascular resistance, changes in volemia, in left-ventricular function and heart rate, and in PWV through the aorta and large arteries might have played a role. Therefore, given the contribution of all these factors to Axl magnitude, this parameter should not be considered as a pure index of arterial stiffening. Thus, it is not surprising that a significant increase in PWV with altitude was observed without an accompanying change in Axl.

Myocardial Oxygen Supply and Demand Ratio Changes With Altitude

SEVR estimates the balance between cardiac blood flow supply and demand and is, thus, a predictor of coronary flow reserve. Our study shows a sharp fall of SEVR and of the oxygen supply and demand ratio during acute exposure to HA. The observation of a decrease in SEVR with acute HA exposure is not a new and surprising finding. Actually, during the HIGHCARE Alps Study (Monte Rosa; 4559 m ASL), a significant reduction in SEVR and CaO₂-SEVR was also observed on arrival at very HA. In those conditions, these parameters returned to SL values after a 3-day permanence at that altitude only in a subgroup of subjects under treatment with acetazolamide, whereas in untreated individuals, SEVR and CaO₂-SEVR values remained reduced. In our HIGHCARE Himalaya article, we provide additional, new data on the behavior of SEVR under acute and prolonged exposure to high and very HA.
First, the greatest reduction in SEVR and CaO₂-SEVR was observed already under acute exposure at 3400 m, whereas BP and heart rate changes reached their peak when a higher altitude was later achieved (5400 m). Thus, the fall in SEVR values at altitude may be only partially explained by the concomitant increase in heart rate, BP, or arterial stiffness at HA, considering the different trends of the changes in these parameters during the progressive ascent to high and very HA. Our data suggest that, likely, the time of ascent and the duration of exposure to HA, more than the altitude level itself, represent critical factors in explaining the observed fall in blood flow supply and demand ratio.

Secondly, in our study, we have quantified not only the conventional SEVR, but also SEVR corrected for CaO₂ (CaO₂-SEVR). Whereas conventional SEVR remained significantly lower than at SL even after a prolonged exposure to very HA, CaO₂-SEVR values returned slowly toward SL values after 12 days of permanence at such very HA. Indeed, after a relatively long permanence at 5400 m, the reduced values of conventional SEVR seemed to be counterbalanced by the increase of CaO₂, mainly thanks to an increase in hemoglobin concentration. Moreover, during prolonged stay at HA, also adaptive respiratory phenomena, such as an improved alveolar diffusion capacity, contribute to an increase in arterial O₂ saturation, further improving the CaO₂-SEVR values.

These hemodynamic changes may become more relevant if we consider the severe hypoxemia caused by acute exposure to HA, which can further worsen subendocardial oxygenation, given the dependence of subendocardial viability not only on coronary blood flow but also on CaO₂. The current availability of transportation means for a rapid ascent to altitude (cable cars, cable railways, and chair lifts) allows every day also a large number of subjects with either manifest or subclinical ischemic heart disease to quickly reach moderate, high, or sometimes, even very high altitude. In these subjects, acute exposure to HA hypobaric hypoxia might, thus, lead to relative cardiac ischemia, with possible clinical manifestations. Thus, the reduction in subendocardial oxygen supply and demand ratio under exposure to HA hypobaric hypoxia could have clinical implications for subjects at risk for ischemic heart disease and with advancing age. Actually, we previously showed that under acute exposure to HA, arrhythmias, and ischemic ECG changes under stress testing may occur in association with SEVR fall.

RAAS and Hemodynamic Changes at HA

A final and novel finding of our study is related to the possible role of the RAAS in relation to changes in arterial stiffness at very HA. RAAS activation may affect vascular reactivity and structural vascular remodeling via genomic and nongenomic mechanisms. Clinical trials showed a reduction in arterial stiffness after long-term pharmacologic inhibition of the RAAS. However, studies focusing on the possible association between aldosterone/renin ratio and PWV did not provide unequivocal results.

The behavior of RAAS during exposure to HA has been extensively described in previous works because changes in this system induced by hypoxia and physical efforts can lead to alterations in sodium and fluid balance which, in turn, can favor the occurrence of acute mountain sickness. This possibility was suggested by the demonstration that prolonged exercise at SL results in sodium retention and peripheral edema because of the activation of RAAS, leading to hypothesize that also sustained exercise at HA might induce a similar fluid gain after a similar activation of RAAS. The effect of hypobaric hypoxia on plasma renin activity and plasma aldosterone concentrations may depend on the time spent at HA, on the altitude level, and on the physical activity degree. An acute exposure to hypoxia causes a decrease in angiotensin-converting enzyme concentration that seems to be protective against an increase in plasma aldosterone concentration and angiotensin II that could lead to...
an important vasoconstriction and sodium retention. This acute phase is also characterized by an increase in water and sodium excretion, probably occurring in response to RAAS suppression. During a prolonged stay at HA, plasma renin activity and plasma aldosterone concentration remain reduced compared with SL even if the level of angiotensin-converting enzyme returns to baseline value. In conclusion, the majority of the available data seem to indicate that the natriuretic effect of HA is mediated by a direct suppression of RAAS.

Our data agree with this hypothesis and offer the demonstration that, in fact, angiotensin II, renin, and aldosterone were significantly suppressed during acute exposure to very HA (BC1). Also in subjects treated with angiotensin receptor blocker, after the expected increase in their concentration at SL, angiotensin II and renin plasma values significantly fell at very HA.24 We have previously shown that during short exposure to lower altitudes (<3400 m) when RAAS at least partly retains its activity, the angiotensin antagonist telmisartan remains effective in BP lowering both in normotensive41 and in hypertensive subjects.41 Conversely, telmisartan lost its ability to reduce BP during prolonged exposure to very HA, likely because of the above reported suppression of RAAS.24 In line with these findings, we also observed no difference in parameters reflecting viscoelastic properties of large arteries, such as PWV and other variables obtained from pulse wave analysis, including subendocardial oxygen supply and demand ratio, between telmisartan-treated and untreated subjects at any altitude. Thus, our study provides unequivocal and novel evidence on the fact that RAAS does not play any relevant role in explaining arterial wall–property changes during acute and prolonged exposition at very HA.

**Perspectives**

The acute exposure of healthy volunteers to hypobaric hypoxia at HA and very HA induces an increase in aortic stiffness, proportional to the altitude reached and independent from the accompanying changes in RAAS activity, BP, and heart rate. It also produces a fall in subendocardial oxygen supply and demand ratio, already evident at HA and persisting at very

---

**Figure 3.** Changes in subendocardial oxygen supply and demand ratio with altitude. **Left**, changes in subendocardial viability ratio (SEVR) at different steps of the study. **Middle**, changes in SEVR×arterial oxygen content (CaO2) at different steps of the study. **Right:** top to bottom, changes in CaO2, arterial oxygen saturation, and hemoglobin at different altitudes. Data collected on the entire cohort (45 healthy volunteers). Data are expressed as mean±SD. BC1 indicates Everest base camp: tests performed the first and the second day after arrival; BC2, Everest base camp: tests performed after 10 and 11 d of permanence at very high altitude; Hb, hemoglobin; N-B, Namche bazaar; O2Sat, oxygen saturation; and SL, sea level. *P<0.001.
HA. These hemodynamic changes may have clinical implications in cases of exposure to altitude hypobaric hypoxia of subjects with subclinical cardiovascular diseases.

The demonstration provided in our study of the impact of HA exposure on wall properties of aorta and large arteries and on subendocardial oxygen supply and demand ratio in healthy subjects may be not only of theoretical but also of practical interest, on the background of the increasing number of people ascending to HA for either work or leisure. Every day, cable cars, cable railways, and chair lifts allow several thousand of subjects, including elderly individuals and subjects with known or subclinical coronary artery disease, to easily access HA locations. Although no harm has ever been reported in young healthy individuals in such conditions, in higher risk subjects with either manifest or subclinical cardiovascular problems, acute exposure to HA and to the related hemodynamic and arterial wall–property changes might lead to relative cardiac ischemia, with possible clinical manifestations. Further studies are needed to confirm our findings in different types of cardiovascular patients.

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Disclosures

P. Salvi is a consultant for DiaTecne srl, manufacturer of a pulse wave analysis system. The other authors report no conflicts.

References


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Renin-Angiotensin-Aldosterone System Is Not Involved in the Arterial Stiffening Induced by Acute and Prolonged Exposure to High Altitude

Short title: RAAS and arterial stiffness at high altitude

Miriam Revera,* Paolo Salvi,* Andrea Faini, Andrea Giuliano, Francesca Gregorini, Grzegorz Bilo, Carolina Lombardi, Giuseppe Mancia, Piergiuseppe Agostoni, Gianfranco Parati, on behalf of the HIGHCARE-Himalaya investigators

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Reliability of measurement acquired at high altitude

Blood pressure values (by electronic oscillometric sphygmomanometer)

Tests addressed to verify the reliability of blood pressure measurements with oscillometric devices were carried out during the HIGHCARE-ALPS project. In this study, multiple blood pressure measurements were obtained in 39 healthy volunteers (age: 36.4 ± 8.5 years, M/F: 21/18) at sea level and during acute exposure to high altitude (4559 m a.s.l., 437-439 Torr). Blood pressure measurements were compared using a mercury sphygmomanometer and 2 validated oscillometric devices (AND UA-767PC & AND TM2430). Blood pressure measurements with the different devices were performed sequentially on the same arm in random order. In this study, the absolute changes in the size of error between sea level and high altitude never exceeded 4 mmHg. The distribution of mean between-device differences within the group was consistent between sea level and high altitude. The conclusion of this study was that validated oscillometric devices commonly used at sea level remained reasonably accurate at high altitude. Consistent and clinically relevant changes in the accuracy of the tested devices caused by low barometric pressure at altitude was not found. These data are now being submitted for publication as a separate paper. These data agree with the results obtained by Cho K.W. et al. with Omron HEM-7201.

More recently, in an interesting editorial comment, WJ Verberk and S Mieke (Heart Asia 2016;8:52–53) demonstrated how, based on the technical aspects of oscillometric and mercury blood pressure monitors, there is no reason to assume that altitude and/or lower barometric pressure will have any effect on their accuracy.

Pulse wave velocity and pulse wave analysis (by PulsePen arterial tonometer)

Pulse wave velocity (PWV) estimation is commonly obtained as ratio of distance to pulse wave transit time. In our study the latter was determined by a time marker corresponding to the foot of the pulse wave captured by the PulsePen system.

Potentially two factors can affect the accuracy of the transit time measurement and they are:

- sampling frequency variability
- response time variability of the tonometric sensor

Sampling frequency

Signals capture occurs at intervals determined by a quartz oscillator whose stability depends on various environmental factors including the temperature that is the prevailing one.

The stability of the quartz in this system is ±30 ppm (parts per million) in the temperature range of -20°C to 70°C. It means a maximum deviation, in the whole temperature range, of 30 ns (nanosecond = 10⁻⁹ s) considering a sampling interval of 1 ms (millisecond = 10⁻³ s), which is absolutely negligible. Referred to the temperature dependency, the dependency on the ionizing radiations at high altitudes is about 4 orders of magnitude lower and the dependency on the atmospheric pressure is almost zero due to the fact that the quartz crystal is hermetically sealed in a metal case. The latter is also a protection against humidity.
**Response time of the tonometric sensor**

The tonometric sensor does not show response time variations related to the atmospheric pressure due to its differential type structure: indeed, the internal assembling includes a sensing foil element exposed on both sides to the same atmospheric pressure and for this reason it is always in equilibrium. The electric signal is proportional to the strain induced by the force applied on one face of the sensing foil through a little metallic cylinder that on the opposite end applies the same force on the patient’s skin (applanation tonometry). Operating temperature range is -40°C to +85°C.

**Arterial oxygen content \( \text{CaO}_2 \)**

The \( \text{CaO}_2 \) represents the amount of oxygen bound to hemoglobin plus the oxygen dissolved in plasma and was determined using the following equation:

\[
\text{CaO}_2 = 1.34 \times \text{blood hemoglobin concentration (Hb, g/dL)} \times \text{arterial oxygen saturation (O}_2\text{Sat, %)} + 0.0031 \times \text{partial pressure of oxygen in arterial blood (PaO}_2, \text{mmHg)}.
\]

The constant 1.34 is the amount of oxygen bound per gram of hemoglobin. The normal oxygen combining capacity is 1.39 ml/g, however due to abnormal forms of hemoglobin such as methemoglobin and carboxyhemoglobin this value is reduced to 1.34 ml/g. The constant 0.0031 is the solubility coefficient of oxygen in plasma at body temperature. Since the weight of the dissolved oxygen in plasma (0.0031 x PaO\(_2\)) is negligible, PaO\(_2\) was extrapolated from O\(_2\)Sat, calculated using the simplified Severinghaus equation.

Directly measured arterial oxygen saturation (SaO\(_2\)) requires invasive blood sampling by arterial puncture, however, non-invasive pulse oximeters is currently used in daily clinical practice since the 1970s and offering the clear advantages of availability of continuous monitoring and no discomfort for the patient. A pulse oximeter measures SaO\(_2\) based on the absorption of light by pulsating arterial blood at two specific wavelengths that correspond to the absorption peaks of oxygenated and deoxygenated hemoglobin. Although less accurate than invasive blood SaO\(_2\), the difference between the two methods is <2%, thus, usually, of no clinical significance. The reliability of SaO\(_2\) measured arterial oxygen saturation using the simplified equation of Severinghaus was recently confirmed by Collins JA et al.\(^6\) In a wide study of 3524 clinical specimens a remarkable accuracy of SaO\(_2\) values was found in patients with normal pH and SaO\(_2\) >70% (in our study mean values of SaO\(_2\) was 77.9±5.7% at BC1).

**Subendocardial viability ratio (SEVR)**

**SEVR invasively assessed**

Subendocardial viability ratio (SEVR) represents a useful index reflecting the subendocardial oxygen supply and demand ratio in the assessment of cardiac ischemic risk. SEVR was introduced by Buckberg and Hoffman at the beginning of the 1970s\(^7,8\) and originally defined by analyzing left ventricular and aortic pressure curves recorded during cardiac catheterization. The area under the left ventricular (or aortic) pressure waveform in systole (SPTI, systolic pressure-time index), from the onset of ventricular systole to the dicrotic notch, represents the left ventricular afterload and defines cardiac work. Thus, SPTI directly correlates with myocardial oxygen needs and mainly depends on left ventricular ejection time (LVET), ejection pressure, and myocardial contractility. The area between the aortic and left ventricular pressure curves in diastole represents the pressure that affects coronary blood flow and that maintains adequate subendocardial blood supply in the diastolic phase of cardiac cycle (DPTI, diastolic pressure-time index). Blood flow to subendocardial fibers layers is virtually absent in systole,
owing to the presence of extravascular compressive forces. During the diastolic phase, the degree of myocardial perfusion largely depends on DPTI, which in turn is a function of the coronary arterial diastolic pressure, i.e. the pressure gradient in diastole between coronary arteries and the left ventricle, and the duration of diastole. DPTI is obtained by subtracting the left ventricular diastolic pressure (LVDP) from the area, in diastole, of the aortic pressure curve. SEVR is computed as the ratio between DPTI and SPTI. A decrease of this ratio below a critical level was shown to be related to the occurrence of subendocardial ischemia. As originally calculated, the SEVR was obtained invasively, using cardiac catheterization, which was a major limitation for its application in a clinical setting (Figure S1).

**SEVR non-invasively assessed**

At present SEVR may be easily non-invasively assessed by the combined use of arterial tonometry and echocardiography.

Blood pressure waveforms recorded at the common carotid artery were taken as a surrogate for ascending aortic pressure waveforms. Direct application of tonometry to the carotid artery is considered an easy and reliable approach to estimate central blood pressure. Indeed, the carotid artery is generally well accessible and superficial, and good quality carotid waveforms can be easily obtained. Carotid pulse waves were recorded by means of validated arterial tonometer (DiaTecne, Milan, Italy).

In SEVR assessed by arterial carotid tonometry DPTI is obtained by subtracting the left ventricular mean diastolic blood pressure (LVDP) from the area, in diastole, of the aortic pressure curve. LVDP can be, substantially and successfully, replaced by an estimate of the left ventricular (or atrial) end-diastolic pressure, which is assessed by echocardiography. In our study Left ventricular diastolic pressure was evaluated by echocardiography using the Nagneh’s formula = 1.24 E/e’ +1.91. Pulse-Doppler left ventricular inflow recordings were performed in the apical 4-chamber view with the sample volume placed at the tip of the mitral valve. From the mitral inflow pattern E and A velocities as well as their ratio and the deceleration time of the E-wave were measured. Tissue velocities were obtained in standard way through TDI analysis from 4 chamber apical view. Early diastolic tissue velocities (e’) were measured at the mitral corner of septal and lateral walls.

As expected, the values of E/e’ and LVDP for all participants in the study were within normal range (E/e’ <8 and LVDP <12 mmHg), at SL (E/e’=4.93±1.13; LVDP=8.0±1.4 mmHg) this being the case also at high altitude (BC1) (E/e’=4.24±0.99; LVDP=7.2±1.2 mmHg).

Systolic pressure-time index (SPTI) represents the area under the aortic pressure curve in systole. However, in order to have a more reliable value of SEVR, the isovolumic period should be considered as a cardiac work period, thus STPI will be the sum of the area below the systolic phase of the pressure curve plus the area corresponding to the isovolumetric contraction time (Figure S2). DPTI is obtained by subtracting the left ventricular mean diastolic blood pressure (LVDP) from the area, in diastole, of the aortic pressure curve. LVDP can be, substantially and successfully, replaced by an estimate of the left ventricular (or atrial) end-diastolic pressure, which is more easily assessed, non-invasively, by echocardiography. Systolic pressure-time index (SPTI) represents the area under the aortic pressure curve in systole. Systolic time intervals were assessed based on pulsed Doppler acquisitions on left ventricular outflow tract. Isovolumic contraction time was calculated as the delay between the ‘R’ wave of the ECG and the start of the ejection period.
References


**Figure S1** - Subendocardial viability ratio (SEVR) obtained by cardiac catheterization. SEVR is computed as the ratio between diastolic pressure-time index (DPTI) and systolic pressure-time index (SPTI).

\[
SEVR = \frac{DPTI}{SPTI}
\]

**Figure S2** - Subendocardial viability ratio (SEVR) obtained by arterial carotid tonometry. SEVR is computed as the ratio between diastolic pressure-time index (DPTI) and systolic pressure-time index (SPTI).
Appendix

**HIGHCARE- Himalaya Investigators**

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