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RESEARCH ARTICLE

Increase in slow-wave vasomotion by hypoxia and ischemia in lowlanders and highlanders

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Salvi P, Faini A, Castiglioni P, Brunacci F, Montaguti L, Severi F, Gautier S, Pretolani E, Benetos A, Parati G. Increase in slow-wave vasomotion by hypoxia and ischemia in lowlanders and highlanders. *J Appl Physiol* 125: 780–789, 2018. First published June 21, 2018; doi:10.1152/japplphysiol.00977.2017.—The physiological relevance of slow-wave vasomotion is still unclear, even though it has been hypothesized that it could be a compensatory mechanism for enhancing tissue oxygenation in conditions of reduced oxygen supply. The aim of our study was to explore the effects of hypoxia and ischemia on slow-wave vasomotion in microcirculation. Peripheral oxygen saturation and forearm microcirculation flow (laser-Doppler flowmetry) were recorded at baseline and during postocclusive reactive hyperemia in the Himalaya region from 8 European lowlanders (6 men; aged 29–39 yr) at 1,350, 3,400, and 5,050 m and from 10 Nepalese male highlanders (aged 21–39 yr) at 3,400 and 5,050 m of altitude. The same measurements were also performed at sea level in 16 healthy volunteers (aged 23–61 yr) during a short-term exposure to normobaric hypoxia. In lowlanders, exposure to progressively higher altitude under baseline flow conditions progressively increased 0.06–0.15 Hz vasomotion amplitude [power spectral density % was expressed as geometric means (geometric standard deviation) = 14.0 (3.6) at 1,350 m; 87.0(2.3) at 3,400 m and 249.8 (3.6) at 5,050 m; $P = 0.006$ and $P < 0.001$ vs. 1,350 m, respectively]. In highlanders, low frequency vasomotion amplitude was similarly enhanced at different altitudes [power spectral density % = 183.4 (4.1) at 3,400 m vs. 236.0 (3.0) at 5,050 m; $P = 0.139$]. In both groups at altitude, it was further increased after ischemic stimulus ($P < 0.001$). At baseline, acute short lasting normobaric hypoxia did not induce low frequency vasomotion, which was conversely induced by ischemia, even under normal oxygenation and barometric pressure. This study offers the demonstration of a significant increase in slow-wave vasomotion under prolonged hypobaric-hypoxia exposure at high altitude, with a further enhancement after ischemia induction.

NEW & NOTEWORTHY This study offers the demonstration in humans of the occurrence of enhanced slow-wave vasomotion in microcirculation induced by exposure to hypobaric hypoxia, ischemia, and their combination. This phenomenon, where vasomotion can be hypothesized to behave as a "peripheral heart," may represent a compensating adaptive change aimed at improving peripheral flow and tissue oxygenation in conditions of reduced oxygen supply, such as altitude-induced hypobaric hypoxia and postocclusion ischemia.

altitude; hypoxia; laser-Doppler flowmetry; microcirculation; vasomotion

INTRODUCTION

The main function of microcirculation is to supply an adequate amount of nutrients and oxygen to peripheral tissues. Small arteries and arterioles have an important role in the circulatory homeostasis to guarantee the redistribution of blood flow to different tissues as required by the changing needs of various organs.

The microvascular perfusion of peripheral tissues undergoes rhythmic flow variations. Periodic oscillations in microcirculatory flow (6, 12, 13, 15, 26, 34, 35) occur at different frequencies and are influenced by heart rate, respiratory rate, myogenic and neurogenic activity, including a resonance in the baroreflex loop (25), and vascular endothelium activity modulation, the last mediated by nitric oxide or by the endothelium-derived hyperpolarizing factors. In this context, the functional meaning of low frequency vasomotion, i.e., the slow-wave rhythmic fluctuation of blood flow with frequency between 0.06 and 0.15 Hz (myogenic vasomotion), remains controversial. Among other hypotheses, it has been suggested that low frequency fluctuations of microvascular flow might ensure better tissue oxygenation than that obtained with a steady blood flow (23).

Our explorative study was aimed at studying the effects of hypoxia and ischemia, as well as their combination, on microcirculation. In particular, we aimed at investigating the occurrence of slow-wave vasomotion in conditions associated with reduced tissue oxygenation. At present, the microvascular circulation can be easily examined through laser-Doppler flowmetry (2, 6, 12, 13, 34). We have therefore assessed the microcirculatory flow in the forearm by this technique, combined with a noninvasive assessment of peripheral oxygen saturation (SpO_2) by pulse oximetry, both at baseline and during postocclusive reactive hyperemia (PORH). This was done under different conditions of tissue oxygenation, namely normobaric hypoxia, ischemia in normoxia, normobaric hyp-

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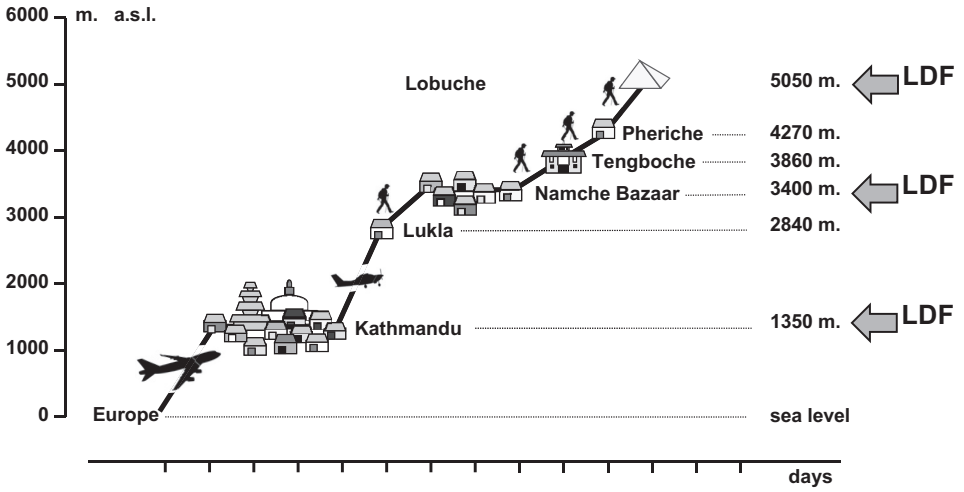

Fig. 1. Timing, altimetry, and sites of the expedition at high altitude. The arrows indicate where the laser-Doppler flowmetry (LDF) was performed: in Kathmandu [1,350 m above sea level (a.s.l.)], at Namche Bazaar (3,400 m a.s.l.), and at Lobuche, at the Pyramid laboratory (5,050 m a.s.l.).

oxia-ischemia combination, hypobaric hypoxia, and hypobaric hypoxia-ischemia combination. While exploring the effects of hypobaric hypoxia at altitude, we also compared the microcirculation response of lowlanders and highlanders.

MATERIALS AND METHODS

Data collection under prolonged hypobaric hypoxia condition. This study was performed during a scientific expedition in the Himalayas, including 8 European lowlanders (6 men and 2 women, aged from 29 to 39 yr) and 10 Nepalese porters (highlanders group, all men aged from 21 to 39 yr). Lowlanders were Europeans of Caucasian ethnicity permanently living nearly at sea level, whereas highlanders were Nepalese individuals of Rai ethnicity, born and living in the Khumbu Valley between 3,400 and 4,930 m (on average at 4,007 ± 583 m) above sea level (a.s.l.).

Lowlanders traveled by air from Europe (6 from Milan, Italy; 2 from Paris, France) to Kathmandu (Nepal, 1,350 m a.s.l.) where they stayed for 3 days (Fig. 1). They were then brought by air transportation to the Lukla airport (2,840 m a.s.l.), trekking the next day to the village of Namche Bazaar (3,400 m a.s.l.), where they stayed for 2 full days for acclimatization. From Namche Bazaar, both lowlander and highlander groups trekked over 3 days to Lobuche (5,050 m a.s.l.), at the base of the Nepali side of mount Everest, where they remained a few days for data collection in the Italian National Research Council (Consiglio Nazionale delle Ricerche) Pyramid International Laboratory. Measurements were performed as follows:

1) at sea level, in lowlanders (only clinical and blood parameters);
2) at Kathmandu (1,350 m a.s.l.), in lowlanders on the second day of permanence at this altitude (the same parameters as at sea level with addition of atrial natriuretic factor, plasma norepinephrine and epinephrine, 3,4-dihydroxyphenylalanine, and endothelin-1; forearm laser Doppler flowmetry);
3) at Namche Bazaar (3,400 m a.s.l.), in both lowlanders and highlanders, starting 24 h since arrival (same data collection as in Kathmandu);
4) at the Pyramid International Laboratory in Lobuche (5,050 m a.s.l), in both lowlanders and highlanders, starting 24 h hours since arrival (same data collection as in Kathmandu).

Data were collected at Kathmandu and Namche Bazaar in hotel rooms and at Lobuche in the Pyramid International Laboratory. Barometric pressure was recorded by a microclimatic station at the time of each study. Ambient temperature was similarly recorded and kept constant throughout the study. Examinations were performed in the morning after at least 18 h of physical rest and after a stay of at least 1 h in a room with a constant temperature of 19 ± 1°C.

Functional evaluation of the microcirculation was carried out by means of Microvascular Laser-Doppler Blood Flow Monitor MBF3D (Moor Instruments, Devon, UK) that emits light at 780 nm, with 1 mW power, delivered by 2 optical-fiber probes (P10A, Moor Instruments) placed on the forearm at 70 and 120 mm from the elbow fold. Flow signals were sampled at 40 Hz, expressed in arbitrary units, and stored in a personal computer. Measurements were performed in supine position after 30 min of rest in a quiet room with stable temperature. Each recording session included 15 min in baseline condition, followed by 3 min of forearm ischemia for administration of the ischemic test (see below) and 9 min after ischemia release.

SpO_2 was checked daily (finger 7845 Pulse Oximeter Kontron). Blood-cell count was carried out by means of MicroDiff 18 Coulter IL (Hialeah, Florida), determining white blood cells (WBCs) and their distribution, red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume, red blood cell distribution width,

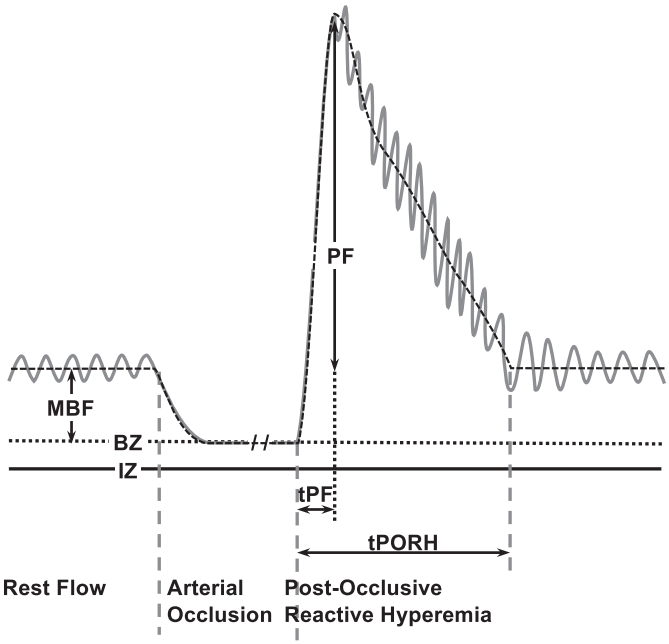

Fig. 2. Parameters evaluated during the ischemic test. Microcirculation flow is expressed in arbitrary units. BZ, biological zero; IZ, instrumental zero; MBF, mean basal flow; PF, peak flow; tPF, time to peak flow; tPORH, duration of postocclusive reactive hyperemia.

platelets, mean platelet volume, plateletcrit (Pct), and platelet distribution width. Plasma epinephrine, norepinephrine, dopamine, and natriuretic atrial peptide were assessed at Kathmandu (only in lowlanders) and at 5,050 m the day after arrival at the high-altitude laboratory (in all subjects). Plasma catecholamines were measured by high-performance liquid chromatography. Blood pressure and heart rate were measured by means of a validated oscillometric blood pressure recorder (Dinamap, model 1846 SX, Critikon, Tampa, Florida) with cuff placed on the left arm.

The study protocol was approved by the Ethics Committee of the Health Care Public Unit of Cesena, Italy. All participants gave their written informed consent to the study procedures.

Ischemic test. No meal, caffeine, or smoking was allowed within 3 h before measurements. Speaking and sleeping were not allowed during measurements. A few minutes after the start of skin blood flowmetry (baseline subperiod), the brachial artery was occluded for 3 min using a pneumatic cuff inflated to 200 mmHg, or at least to an air pressure 30 mmHg greater than the subjects' usual systolic blood pressure. The PORH (29) was quantified during 3 min after the postischemic peak flow (PF) following the pneumatic cuff release.

Parameters evaluated by skin blood flow changes induced during the ischemic test were (Fig. 2): the biological zero (i.e., the flow values recorded by the laser-Doppler when the arterial occlusion stops the blood flow); the mean basal flow (MBF) (average flow minus biological zero) during the baseline recording, preceding the cuff occlusion; the highest PF during PORH, expressed as absolute value and as percent change compared with basal flow; the time-to-PF; and the duration of postischemic hyperemia.

Slow-wave vasomotion spectral analysis. Powers of vasomotion components were evaluated by spectral analysis of Doppler flow signal during baseline subperiods and immediately after removal of brachial artery pneumatic occlusion during the ischemic test. Preliminary to spectral analysis, the blood flow signal of each recording was calibrated by setting the average flow in the baseline subperiod equal to 1 and the average flow during blood flow occlusion equal to 0 and detrended by least-square polynomial fitting. The analysis was performed with a high-resolution autoregressive spectral method (Burg algorithm with model order equal to 256). The power of the highest spectral peak in the slow-wave vasomotion band (between 0.06 and 0.15 Hz) was calculated as the highest residue among those of all poles with frequency in this band (18) and expressed as fraction of total power in normalized units. Our spectral approach allows a detailed assessment of vasomotion dynamics both at baseline and after PORH. Thus, changes in vasomotion spectral power and/or in its central frequency may offer insights into the responsible mechanisms. The example of Fig. 3 illustrates the performance of our spectral estimator, in particular its high frequency resolution and robustness to noise.

Data collection under short lasting normobaric hypoxia condition. To verify the importance of time of exposure to hypoxia in determining changes in microcirculation, the laser-Doppler flowmetry was performed at sea level on 16 healthy volunteers (11 men and 5

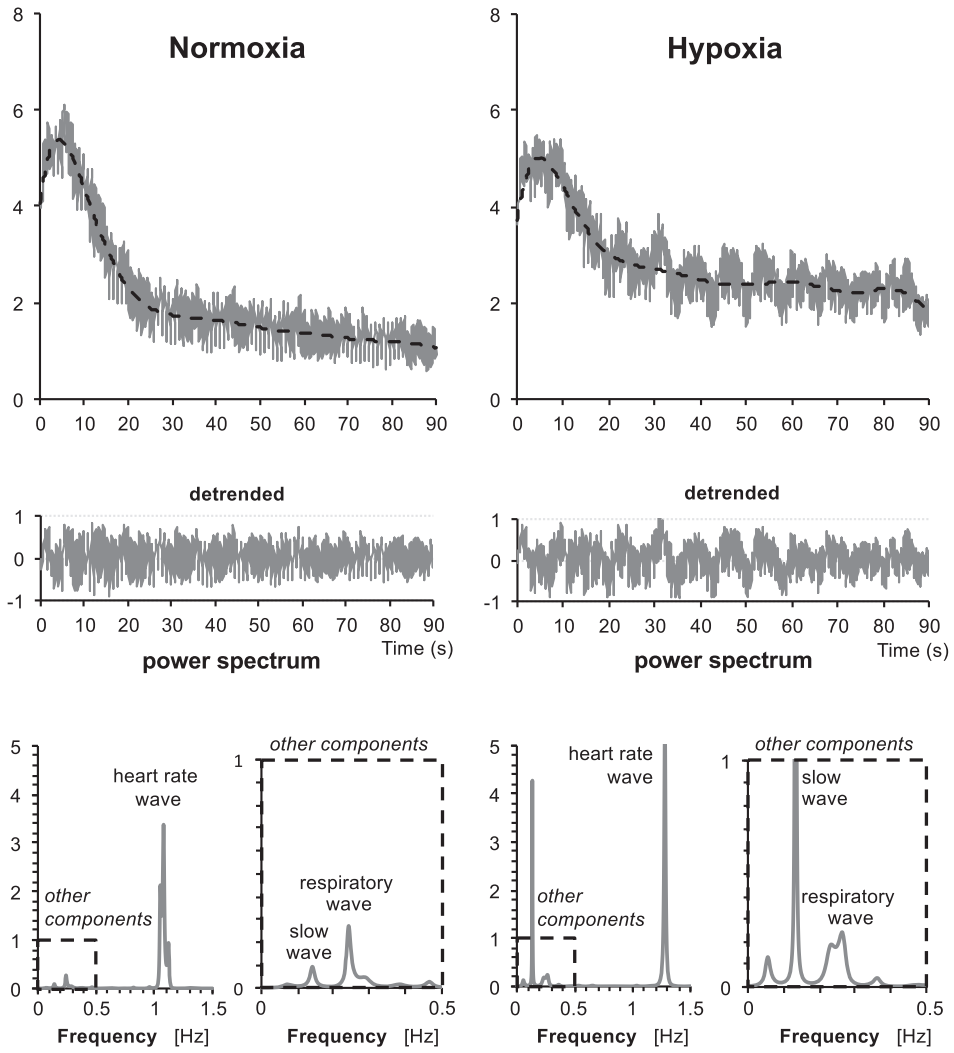

Fig. 3. Example of spectral analysis performed on the same volunteer during normoxia and during hypoxia at sea level after ischemic test. *Top*: 90-s segments of laser Doppler signals selected immediately after the start of the postocclusive reactive hyperemia; bold dashed lines represent the trend estimated by least-square polynomial fitting. *Middle*: detrended signals. *Bottom*: high-order autoregressive power spectra of detrended signals. Spectra show a dominant peak at the heart-rate frequency and other components at frequencies lower than 0.5 Hz; the enlargement of the frequency band up to 0.5 Hz is characterized by a respiratory component identified by the breathing rate measured on the ECG-derived respiration signal and by a slow-wave component amplified during hypoxia.

Table 1. *Anthropometric parameters of the study participants*

Parameters	Himalayan Expedition		Lowlanders vs. Highlanders (*P*)	Sea Level	Lowlanders vs.Healthy Volunteers (*P*)
	Lowlanders	Highlanders		Healthy Volunteers	
Sex, M/F	6/2	10/0		11/5	
Age, years	34.6 ± 3.5	28.5 ± 6.6	0.060	37.9 ± 12.2	0.915
Weight, kg	76.8 ± 7.6	51.2 ± 2.5	<0.001	69.9 ± 9.1	0.039
Height, cm	173.4 ± 6.3	159.6 ± 6.9	<0.001	174.9 ± 6.6	0.590
BMI, kg/m^2	25.5 ± 1.8	20.2 ± 1.7	<0.001	22.8 ± 2.5	0.008
BSA, m^2	1.91 ± 0.12	1.51 ± 0.06	<0.001	1.84 ± 0.14	0.121

Values are means ± SD. BMI, body mass index; BSA, body surface area; F, females; M, males.

women, aged from 23 to 61 yr) during a short-term exposure to normobaric hypoxia, both at baseline and during PORH. Subjects were studied in a quiet environment in supine position after 15 min of rest. Subjects were asked to breathe through a mouthpiece connected in series with a two-way, nonrebreathing valve while wearing a nose clip. Electrocardiogram, SpO_2 by pulse oximetry (NPB-295, Nellcor Puritan Bennett Inc, Pleasanton, CA) and noninvasive beat by beat blood pressure at the finger artery (Nexfin device, Edwards Lifesciences/BMEYE B.V., Amsterdam, the Netherlands) were continuously monitored and recorded. An ECG-derived respiratory signal was obtained as beat-to-beat changes of the area of the QRS complex. The laser-Doppler signal was acquired by a PeriFlux System 5000 (Perimed AB, Järfälla, Sweden) with probes placed on the forearm at 70 mm from the elbow fold. The microcirculatory flow was expressed in arbitrary units.

The experimental session included three consecutive phases, and an ischemic test (see above) was administered in each of the first two phases. In *phase 1* (12 min), participants spontaneously breathed room air. This phase consisted of 3 min of baseline rest followed by 3 min of forearm ischemia (ischemic test through upper arm cuff inflation using a pneumatic cuff inflated to 200 mmHg, or at least to an air pressure 30 mmHg greater than the subjects' usual systolic blood pressure) and 6 min after ischemia, following upper arm cuff release. At the end of *phase 1* the volunteers were switched from breathing ambient air to breathing a hypoxic gas mixture (10% O_2 in N_2, simulating the altitude of 5,800 meters a.s.l. at Himalaya latitude, according to the International Civil Aviation Organization standard atmosphere in standard conditions for temperature and pressure) through use of a bag reservoir by operating a three-way sliding valve (*phase 2*, normobaric hypoxic exposure of 27 min duration). *Phase 2* consisted of 15 min of rest, with the last 3 min being used as baseline for an additional ischemic test, followed by 3 min recording of forearm ischemia (upper arm cuff inflation) and by 9 min recording after ischemia release. At the end of *phase 2*, the sliding valve interrupted breathing of the hypoxic gas mixture, and participants were allowed to breathe room air for 6 min (recovery, *phase 3*).

This study protocol was approved by the Ethics Committee of the Istituto Auxologico Italiano (Milan, Italy). All participants gave their written informed consent to the study procedures.

Statistical analysis. Continuous data are presented as means ± standard deviation, categorical data as absolute numbers, and power spectra as geometric means and geometric standard errors.

Analysis of clinical and hemodynamic parameters at different altitudes was performed with Friedman's test followed by two-tailed Wilcoxon tests (paired where required). Slow-wave vasomotion power was log-transformed to achieve Gaussian distribution. Slow-

Table 2. *Changes in clinical and hemodynamic parameters with altitude*

Parameters	Lowlanders					Highlanders	
	Sea level	1,350 m a.s.l.	3,400 m a.s.l.	5,050 m a.s.l.	Friedman *P* Value	3,400 m a.s.l.	5,050 m a.s.l.
SpO_2, %	96 ± 1	96 ± 2	92 ± 3*	80 ± 7*	<0.001	93 ± 6	86 ± 4*†
WBC, μl/l	6.74 ± 0.96	7.02 ± 1.08	6.17 ± 0.63	6.45 ± 0.58	0.105	10.02 ± 1.98††	9.59 ± 1.97†
RBC, ×10^3 μl/l	4.85 ± 0.29	4.87 ± 0.28	4.46 ± 0.13*	5.03 ± 0.41	0.003	5.25 ± 0.36††	5.24 ± 0.29
Hb, g/dl	14.2 ± 0.7	14.5 ± 0.7	14.1 ± 0.8	15.5 ± 1.3*	0.005	16.8 ± 0.7††	16.4 ± 0.7
Hct, %	42.7 ± 1.6	43.6 ± 2.1	39.5 ± 1.9*	45.1 ± 4.0	0.002	47.6 ± 2.2††	47.5 ± 1.9
MCV, fl	88.2 ± 3.0	89.7 ± 2.9	88.6 ± 3.1	89.5 ± 2.5	0.092	90.8 ± 3.1	90.9 ± 3.3
RDW, %	12.8 ± 0.6	13.1 ± 0.6	13.2 ± 0.8	13.7 ± 0.7*	<0.001	13.4 ± 0.6	13.5 ± 0.5
Plt, ×10^3 μl/l	256 ± 39	260 ± 32	236 ± 28	318 ± 41*	<0.001	327 ± 71†	434 ± 126*†
MPV, fl	7.9 ± 0.6	8.2 ± 0.4	8.5 ± 0.5	8.3 ± 0.6	0.065	7.9 ± 0.7	7.4 ± 0.6*†
Pct, %	0.20 ± 0.04	0.21 ± 0.03	0.20 ± 0.03	0.26 ± 0.03*	<0.001	0.26 ± 0.05†	0.32 ± 0.08†
PDW, %	15.9 ± 0.8	15.9 ± 0.3	15.8 ± 0.4	15.9 ± 0.6	0.875	16.1 ± 0.5	15.9 ± 0.3
SBP, mmHg	112 ± 9	113 ± 4	116 ± 6	119 ± 6	0.0145	114 ± 6	114 ± 10
DBP, mmHg	73 ± 7	67 ± 5	70 ± 8	71 ± 7	0.048	72 ± 6	73 ± 8
MAP, mmHg	86 ± 6	82 ± 4	85 ± 7	87 ± 7	0.102	86 ± 5	87 ± 8
HR, beats/min	65 ± 8	68 ± 10	72 ± 17	77 ± 7	0.093	65 ± 10	70 ± 12
RR, breaths/min	10.5 ± 0.5	10.1 ± 0.6	12.4 ± 1.6	14.0 ± 2.1*	<0.001	11.3 ± 0.7	12.2 ± 0.6*†
ANF, pmol/l		13.3 ± 7.6		14.8 ± 7.8			13.6 ± 9.0
Norepinephrine, pg/ml		23.9 ± 20.4		197.4 ± 77.8*			414.7 ± 167.7†
Epinephrine, pg/ml		9.4 ± 6.9		58.2 ± 108.6			119.8 ± 117.2
DOPA, pg/ml		5 ± 7		42 ± 103			50 ± 95
Endothelin-1, pg/ml		0.0 ± 0.0		0.5 ± 0.9			0.2 ± 0.6

Data are mean ± standard deviation. ANF, atrial natriuretic factor; a.s.l., above sea level; DBP, diastolic blood pressure; DOPA, 3,4-dihydroxyphenylalanine; fl, femtoliter; Hct, hematocrit; Hb, hemoglobin; HR, heart rate; MAP, mean arterial pressure; MCV, mean corpuscular volume; MPV, mean platelet volume; Plt, platelets; PDW, platelet distribution width; Pct, plateletcrit; RBC, red blood cell; RDW, red blood cell distribution width; RR, respiratory rate; SBP, systolic blood pressure; SpO_2, peripheral oxygen saturation; WBC, white blood cell.Wilcoxon paired test, *$P < 0.05$ vs. baseline (sea level for lowlanders and 3,450 m for highlanders), †$P < 0.05$, ††$P < 0.001$ highlanders vs. lowlanders at the same altitude.

wave vasomotion power and central frequency were tested by linear mixed-effects models with contrasts a posteriori accounting for repeated measurements, fitting the models by maximizing the restricted log likelihood. The effect of ethnicity was assessed by a likelihood ratio test comparing the full model, including ethnicity as a covariate, versus the alternative (restricted) model without ethnicity, considering the two altitudes common to lowlander and highlander groups (3,400 m and 5,050 m a.s.l.). The relative importance of altitude levels, ischemic test, and ethnic group were evaluated comparing the Akaike's information criterion of all possible combinations of these predictor variables. For multiple comparison, the false discovery rate correction adjusted the significance levels. The distribution of variables in terms of proximity to normal and homogeneity of their variances was evaluated by Shapiro-Wilk and Bartlett tests, respectively. The α level was set at 0.05 for all tests. Analyses were performed using R Core Team (2017) software (Vienna, Austria).

In our study, measurements were performed on all the participants in our scientific expedition (including the guides and all the porters). Given the unique opportunity offered by our expedition and the difficulty in recruiting volunteers, our study should be considered as an explorative pilot, thus with no calculation of sample size. Moreover, the absence in previous studies of data concerning variations of slow vasomotion recorded by laser-Doppler on the skin of the forearm in conditions similar to those of our study was another reason that did not allow us to reliably calculate a suitable sample size. Despite the small group of subjects recruited in our study, however, the important differences in slow-wave vasomotion recorded at various altitudes led to significant results.

RESULTS

Tests in hypobaric hypoxic condition. Table 1 compares the anthropometric parameters of lowlander and highlander groups ascending to high altitude and those of the heathy volunteers studied at sea level. Acute mountain sickness symptoms were frequent among lowlanders when exposed to altitude. Six out of eight lowlanders reported a short lasting headache above 4,000 m of altitude, three had moderate dyspnea at rest, and one subject experienced vomiting at 5,000 m. At the arrival at 5,050 m, 1 lowlander showed signs of moderate cerebral edema (ataxia, headache, vertigo, and vomiting), which rapidly regressed with oxygen and high dose corticosteroids (data of this subject were excluded from the analysis). No symptoms linked to high altitude were observed in highlanders.

SpO_2 significantly decreased after acute exposure to very high altitude in both lowlanders and highlanders (Table 2). Nevertheless, oxygen saturation at 5,050 m a.s.l. was significantly higher in highlanders than in lowlanders. The respiratory rate increased with altitude in both groups, with lower breathing frequency in highlanders than in lowlanders at 5,050 m. In lowlanders, Hb, red blood cell distribution width, platelets, and Pct increased significantly at 5,050 m compared with sea level.

In lowlanders, Hct and RBCs tended to increase from sea level to 5,040 m, with a temporary reduction at 3,400 m, even if no significant difference in Hct and RBCs was observed between 1,300 m and acute exposure at 5,050 m. a.s.l. No significant difference in Hct and RBCs was observed between highlanders and lowlanders at 5,050 m. a.s.l., likely because of the relatively small number of subjects considered, in spite of a clear tendency for these values to be higher in the former group. No significant correlation was found between Hct and power spectral density at different altitudes and in the two ethnic groups. Similarly, at 3,400 m, Hb, WBCs, and Pct were lower in lowlanders than in highlanders. WBCs and Pct also remained lower in lowlanders than in highlanders at 5,050 m. Norepinephrine increased significantly in lowlanders at 5,050 m, yet it was significantly lower than in highlanders. Epinephrine showed the tendency to increase with altitude in lowlanders ($P = 0.059$).

Visual inspection of laser-Doppler flow traces did not reveal the periodic oscillations of slow-wave vasomotion (0.06 to 0.15 Hz) at 1,350 m in European lowlanders. Slow-wave vasomotion appeared at 3,400 m and at 5,050 m in all European participants, except in the subject with acute cerebral edema. Figure 4 shows an example of basal and postocclusive laser-Doppler flow and the corresponding power spectra in a lowlander at different altitudes.

Slow-wave vasomotion spectral powers increased with altitude both in lowlanders and in highlanders either in basal or

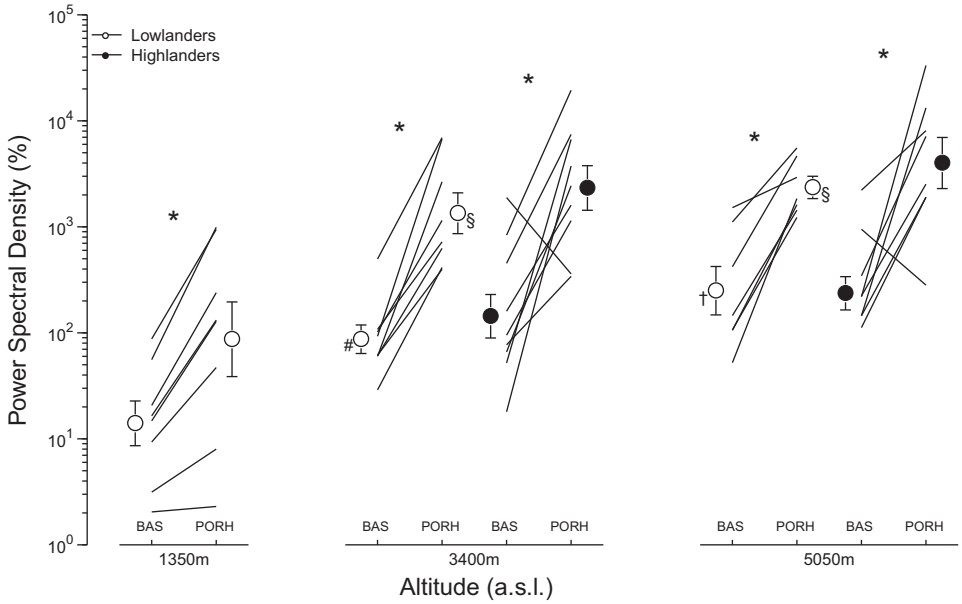

Fig. 4. Changes in power spectral densities of slow-wave vasomotion at different altitudes in lowlanders (open circles) and highlanders (closed circles) during baseline laser-Doppler flowmetry recordings (BAS) and after ischemic test [postocclusive reactive hyperemia (PORH)]. Power spectral density (*y*-axis, in logarithmic scale) is expressed in arbitrary units (a.u.). Power spectral density variables are expressed as percentage and presented as geometric means and geometric standard error. *$P < 0.001$ BAS vs. PORH; †$P < 0.001$ vs. BAS 1,350 m; #$P = 0.006$ vs. BAS 1,350 m; and §$P < 0.001$ vs. PORH 1,350 m.

postischemic periods, although in highlanders these changes did not reach levels of statistical significance ($P < 0.001$ and $P = 0.139$ for lowlanders and highlanders, respectively; Fig. 5). For lowlanders, the power in each condition was significantly greater at the higher altitudes (3,400 m and 5,050 m a.s.l.) than at 1,350 m a.s.l. The vasomotion spectral power was similar in lowlanders and in highlanders for the same altitude. An evident significant increase in vasomotion wave powers ($P < 0.001$) was observed during the postischemic period, both in lowlanders and highlanders. This increase was particularly pronounced when ischemic tests were performed at high altitude. By contrast, no significant differences were found between lowlanders and highlanders, neither in baseline measures ($P = 0.391$ at 3,400 m and $P = 0.923$ at 5,050 m a.s.l.) nor in measures after brachial artery occlusion both at 3,400 m and at 5,050 m ($P = 0.392$ at 3,400 m and $P = 0.389$ at 5,050 m a.s.l.).

The interaction between altitude and condition (baseline and after arterial occlusion) was not significant either for lowlanders or highlanders ($P = 0.151$ and $P = 0.826$, respectively) (Table 3). No effect of ethnic group was found comparing the model, which includes the ethnicity as a covariate with the model without ethnicity ($P = 0.360$). A multivariate analysis, aimed at exploring the relative importance of each tested variable in modulating microcirculation, showed that the ischemic test (reference variable) was the most important factor, followed by altitude (0.88 of the reference), explaining differences in vasomotion wave powers. The frequency of the vasomotion spectral peak did not change significantly with altitude neither in lowlanders nor in highlanders, either at baseline or after ischemic test.

In lowlanders, mean baseline microcirculation flow and PF during PORH decreased significantly from 1,350 m to 3,400 m (Table 4). Mean baseline flow was significantly lower and time

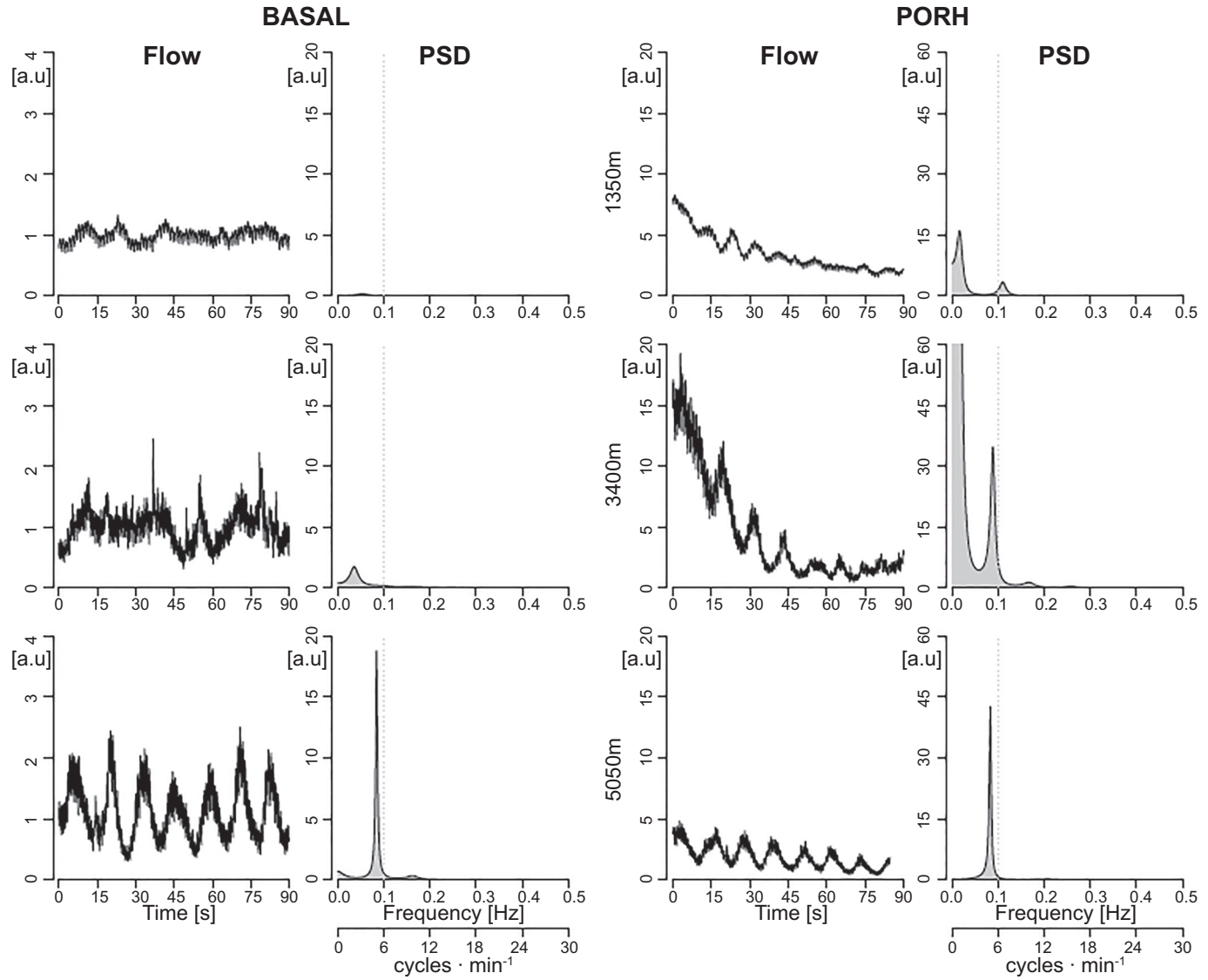

Fig. 5. Example of laser-Doppler flowmetry analysis in a lowlander. Recordings obtained at 1,350, 3,400, and 5,050 m above sea level are shown separately. First and third columns illustrate representative recordings with 90-s segments of laser-Doppler flow [(arbitrary units (a.u.)] at baseline (BASAL) condition and during postocclusive reactive hyperemia (PORH). Second and fourth columns illustrate the corresponding power spectral density (PSD).

Table 3. *Vasomotion power spectral density in lowlander and highlander groups at different altitudes*

Group	Altitude, m a.s.l.	Condition		*P* Value		
		Baseline	PORH	Altitude	Condition	Altitude × Condition
Lowlanders	1,350	14.0 (3.6)	87.0 (8.5)	<0.001	<0.001	0.151
	3,400	87.0 (2.3)#	1,343.3 (3.2)*			
	5,050	249.8 (3.6)*	2,353.2 (1.8)*			
Highlanders	3,400	143.4 (4.1)	2,325.3 (3.9)	0.139	<0.001	0.826
	5,050	236.0 (3.0)	4,000.7 (4.3)			

Data are expressed as percentage and presented as geometric means (geometric standard deviation). PORH, postocclusive reactive hyperemia. $P < 0.001$ PORH vs baseline for all altitudes, #$P = 0.006$ vs. 1,350 m a.s.l., *$P < 0.001$ vs. 1,350 m a.s.l.

to PF was significantly longer in highlanders than in lowlanders; moreover, peak flow was lower in highlanders at 3,400 m. However, at 5,050 m, the flow increase (in percent of baseline value) after arterial occlusion was significantly higher in highlanders than in lowlanders. Postocclusive reactive hyperemia was absent in the subject affected by acute cerebral edema at 5,050 m. Skin temperature was reduced slightly, but significantly, in lowlanders from 1,350 m to 3,400 m and to 5,050 m a.s.l., in parallel with a reduction in MBF. On the contrary, skin temperature increased in highlanders from 3,400 m to 5,050 m a.s.l., paralleled by an increase in MBF (Table 4). No significant difference in skin temperature was found at 5,050 m a.s.l. between lowlanders and highlanders. Moreover, no relationship between changes in power spectral density and changes in skin temperature was found, either in highlanders or in lowlanders.

Tests in normobaric hypoxic condition. Hypoxia induced a dramatic fall of blood oxygen saturation and a significant increase in resting heart rate and systolic blood pressure. No significant changes were observed in spectral powers of slow-wave vasomotion, neither at baseline nor during PORH ($P = 0.726$; Fig. 6 and Table 5). On the contrary, vasomotion-related low-frequency spectral power significantly increased during PORH, both while breathing room air and hypoxic gas mixture ($P < 0.001$). No significant interaction between O_2 level and condition (baseline and PORH) was found ($P = 0.612$) (Table 6). The frequency of the spectral peak representing vasomotion activity increased slightly, but significantly, from room air breathing to the normobaric hypoxic condition at baseline and during PORH. Time to PF and time of hyperemia did not significantly change during hypoxic gas mixture breathing (from 11 ± 3 s to 12 ± 4 s and from 124 ± 36 s to 166 ± 49 s, respectively).

DISCUSSION

Our study provides several interesting results. *1*) The most important finding of our study is the progressive increase in the amplitude of slow-wave vasomotion under baseline conditions at rest and under acute exposure to high altitudes in lowlanders. Indeed, in European lowlanders, vasomotion waves were negligible at 1,350 m, identifiable at 3,400 m, and evident over 5,000 m a.s.l. *2*) Slow-wave vasomotion power did not differ between lowlanders and highlanders when assessed at the same high altitude under baseline resting conditions, appearing therefore mainly related to high-altitude hypobaric hypoxia exposure, without any major influence by factors related to ethnicity or adaptation of high altitude. *3*) The increased amplitude of slow vasomotion waves (frequency between 0.06 and 0.15 Hz) during reactive hyperemia after a prolonged ischemic test, both at low and high altitude, highlights the important role of ischemia in determining the appearance of slow-wave vasomotion. *4*) Acute hypoxia in normobaric conditions by itself does not seem to be a sufficient stimulus to induce vasomotion.

The vasomotion activity during PORH has been clearly documented at all altitudes, both in lowlanders and in highlanders. In lowlanders, the vasomotion activity induced by the ischemic test further significantly increased at high altitudes compared with vasomotion induced by ischemia at low altitudes. Conversely, the increased vasomotion after the ischemic test in highlanders was not affected by the altitude at which the test was performed. Ischemia and hypobaric hypoxia at high altitudes are therefore important factors, independently affecting the appearance and magnitude of low frequency vasomotion in lowlanders. It is thus not surprising that the widest amplitude of low frequency vasomotion activity was reached in

Table 4. *Parameters from ischemic test*

Parameters	Lowlanders				Highlanders	
	1,350 m a.s.l.	3,400 m a.s.l.	5,050 m a.s.l.	Friedman *P* Value	3,400 m a.s.l.	5,050 m a.s.l.
Skin temperature, °C	31.8 ± 0.6	30.6 ± 1.0*	30.5 ± 1.0*	0.017	28.5 ± 1.6†	30.7 ± 0.8*
Mean basal flow, a.u.	39 ± 27	13 ± 4*	27 ± 10	0.008	7 ± 4†	10 ± 4††
Biological zero, a.u.	4 ± 1	4 ± 1	4 ± 1	0.147	2 ± 1†	2 ± 1†
Peak flow, a.u.	186 ± 71	101 ± 4*	104 ± 56	0.009	54 ± 32†	80 ± 30
Peak flow, %	607 ± 370	972 ± 387	363 ± 190	0.044	1,058 ± 623	1,142 ± 515†
Time to peak flow, s	8 ± 3	10 ± 4	12 ± 2	0.131	24 ± 13†	21 ± 8†
Time of hyperemia, s	130 ± 53	103 ± 38	104 ± 59	0.417	128 ± 39	138 ± 27

Peak flow was expressed as flow variation vs. basal flow. Data are ± standard deviation. a.s.l., above sea level; a.u., arbitrary unit.Wilcoxon paired test; *$P <$ 0.05 vs. baseline (1,350 m for lowlanders and 3,400 m for highlanders), †$P < 0.05$, ††$P < 0.001$ highlanders vs. lowlanders at the same altitude.

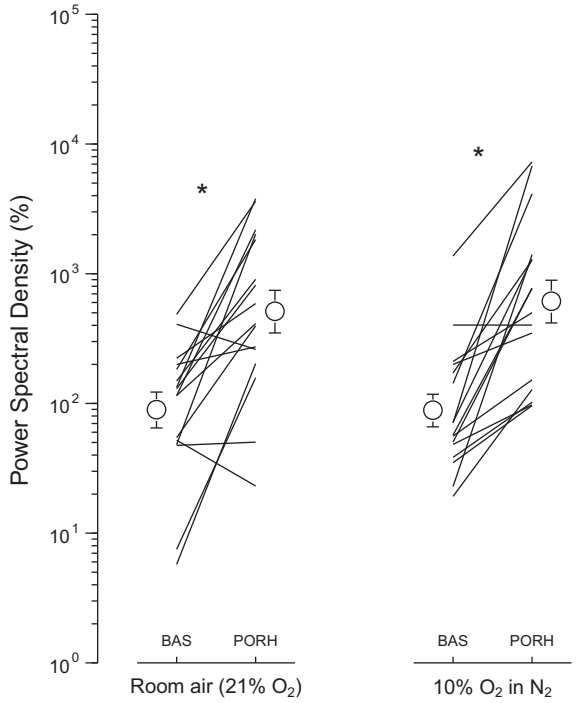

Fig. 6. Slow-wave vasomotion change recorded by laser-Doppler flowmetry in baseline condition (BAS) and during postocclusive reactive hyperemia (PORH) during room air breathing (*left*) and in normobaric hypoxic condition during breathing a mixture of 10% O_2 in N_2 (*right*). Power spectral density variables are expressed as percentage and presented as geometric means and geometric standard error. *$P < 0.001$ BAS vs. PORH.

these subjects after the ischemic test at the highest altitude. Conversely, altitude exposure did not affect microcirculation vasomotion amplitude at rest in highlanders, who also displayed the same vasomotion amplitude increase after ischemic test at different altitudes. It is interesting to note that in lowlanders, the amplitude of vasomotion activity induced by high altitude exposure under baseline conditions corresponded to the vasomotion amplitude induced by ischemic test at low altitudes.

The functional meaning of low frequency (0.06–0.15 Hz) vasomotion, likely because of rhythmic arteriolar diameter changes, remains controversial. It has been hypothesized that groups of smooth muscle cells working as peripheral pacemakers are present at the arteriolar bifurcations, originating the series of periodic arteriolar dilations and constrictions that move along the microvessels (8, 21, 32). According to this theory, vasomotion is generated from within the vessel walls and seems to be independent of heart rate, respiration, and neural influences (23, 32).

This flow oscillation, which could be regular or highly irregular, in normal conditions is generated by the intrinsic nonlinearity of smooth muscle control mechanisms and can be classified as "chaotic" (11). A regular and synchronous vasomotion may in fact not be present under normal physiological conditions (7, 31–33), but it seems to be prevalent under conditions of reduced perfusion (30). Actually, in studies involving experimental animals (rabbits, hamsters), vasomotion was induced by bleeding (32), hypotension (31, 32), and hypoxia (5). Experimental studies also showed that, in normal conditions, vasomotion is needed to ensure sufficient oxygen delivery in certain tissues where blood supply is critical (17). Moreover, vasomotion can be easily induced in forearm or leg skin after arterial occlusion in healthy subjects (2). Even if a physiological role of vasomotion is yet to be confirmed, at present it is considered as a possible compensatory mechanism aimed at allowing an improvement in tissue oxygenation in situations where oxygen supply is reduced (31, 36). The number of perfused capillaries was demonstrated to be related to perfusion pressure. Thus, a decrease of arteriolar pressure translates into a reduction of flowing capillaries (16). In this situation, vasomotion would represent a mechanism for creating adequate local arteriolar pressure, warranting an adequate perfusion through the capillary network (14). Therefore, this vasomotion pattern might be considered a "peripheral heart," aimed at compensating for a reduced peripheral oxygen and blood flow in the attempt to restore a normal tissue perfusion. This could be the functional meaning of vasomotion induced by hypobaric hypoxia exposure at high altitudes, as documented by the appearance and augmentation of low-frequency flow motion waves in our study conditions.

Ascent to high altitude induces an increased adrenergic activity, as highlighted by a number of previous studies (3, 4), and as indicated by the high levels of plasma norepinephrine found in our study. The adrenergic system activation is particularly important for the control of cardiac function and vascular tone in the first phases of adaptation to high altitude (24, 27). A modification of the number and function of beta-adrenergic receptors in subjects who live at a high altitude has been clearly shown (1). Nevertheless, adrenergic activation cannot be the only explanation for the activation and increase in low frequency vasomotion under acute exposure to high altitude, and the occurrence of changes in endothelial function in explaining changes in vasomotion during acute exposure to high altitude is still a matter of research (10) (22).

Our data collected at sea level, with the aim of investigating the role of acute exposure to hypoxia in normobaric conditions,

Table 5. *Clinical parameters in healthy volunteers undergoing normobaric hypoxic test*

Parameter	21% O_2	10% O_2	*t*-test
SpO_2, %	100 ± 3	64 ± 10	<0.001
SpO_2 min, %	99 ± 3	57 ± 12	<0.001
SBP, mmHg	133 ± 20	140 ± 22	0.016
DBP, mmHg	73 ± 12	74 ± 13	0.264
MAP, mmHg	95 ± 14	98 ± 16	0.103
HR, beats/min	69 ± 9	85 ± 10	<0.001
RR, breaths/min	13.4 ± 2.9	13.1 ± 2.6	0.456

DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; O_2, oxygen; RR, respiratory rate; SBP, systolic blood pressure; SpO_2, peripheral oxygen saturation; SpO_2 min, minimum SpO_2.

Table 6. *Vasomotion power spectral density under short lasting normobaric hypoxia condition*

	Condition		*P* Value		
O_2	Baseline	PORH	Condition	O_2	Condition × O_2
21% O_2	89.0 (3.4)	510.1 (4.3)	<0.001	0.726	0.612
10% O_2 in N_2	88.1 (3.1)	609.8 (4.3)			

Data are expressed as percentage and presented as geometric means (geometric standard deviation). N_2, nitrogen; O_2, oxygen; PORH, postocclusive reactive hyperemia. $P < 0.001$ PORH vs. baseline for both O_2 conditions.

showed the absence of an increased vasomotion after acute inhalation of a hypoxic gas mixture (10% O_2 in N_2) under baseline rest. This different behavior, compared with that observed at high altitude, has at least two possible explanations. First, the short time duration of induced hypoxemia in a laboratory condition may not be long enough to induce appearance of slow vasomotion waves. Second, hypobaric hypoxia, compared with normobaric hypoxia, might have a specific role in stimulating vasomotion. Actually, none of the participants in this study showed any clear synchronous vasomotor activity at low altitudes, and the induction of vasomotion at high altitudes was recorded in all subjects in the absence of any treatment for acute mountain sickness.

The results of our study provide further elements for the understanding of hypobaric hypoxia effects on microcirculation and of the mechanisms that regulate the adaptive capacity of the microcirculation at a high altitude. Previous studies (19, 20) demonstrated a significant increase in vessel density and a reduction in microcirculatory flow in relation to the progressive reduction in peripheral arterial oxygen saturation with altitude. Both these phenomena should be interpreted as adaptive responses to hypoxia. In fact, the decline in microcirculatory flow, leading to a longer erythrocyte tissue transit time, could improve the oxygen diffusion (19, 28). The activation of slow-wave vasomotion may constitute a further compensatory mechanism in improving tissue perfusion under critical hypoxia conditions. More recently, the Xtreme Everest 2 Research Group (9) demonstrated differences between Sherpa's and lowlander's microcirculatory response to sustained hypobaric hypoxia at high altitude. As demonstrated in previous studies (19), lowlanders showed a reduction in microcirculatory flow, whereas an increase in sublingual microcirculatory flow upon exposure to hypobaric hypoxia was shown in Sherpas. Both lowlanders and Sherpas showed an increase in capillary density, but the capillary network was 30% denser in Sherpas than in lowlanders. On the other hand, no difference was found between these two ethnic groups in normoxia. These findings seem to support the opinion that the peripheral microcirculation plays a key role in the process of long-term adaptation to hypoxia and in the extraordinary tolerance to hypoxia of Sherpa highlanders. The similarity in blood flow and capillary density in the two cohorts at baseline suggests the hypothesis that the most effective physiological adaptive mechanisms in highlanders to maximize microcirculatory blood flow at altitude are transient, hypoxia-dependent, and likely supported by genetic differences (9). In our study, at high altitudes, a similar activation of slow-wave vasomotion was shown in lowlanders and highlanders, suggesting that this functional phenomenon may be triggered by hypobaric hypoxemia, independently from genetic factors.

A limitation of our study is its relatively small sample size, imposed by the challenging conditions in which our study was carried out, up to an altitude of 5,050 m. However, given the univocal changes in vasomotion activity observed in our study, its relatively small number of participants did not prevent us from obtaining significant results in relation to the factors responsible for vasomotor changes under hypoxia and after ischemia. Nevertheless, our data should thus be considered as pioneering findings, which might stimulate additional studies to be performed in a larger group of subjects aimed at confirming our data and at exploring more in depth the mechanisms behind the low-frequency vasomotor activity induced by high altitude and ischemia.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

P.S., F.B., L.M., F.S., and E.P. conceived and designed research; P.S., P.C., F.B., L.M., F.S., S.G., E.P., and A.B. performed experiments; P.S., A.F., P.C., F.B., L.M., F.S., S.G., E.P., A.B., and G.P. analyzed data; P.S., A.F., P.C., F.B., L.M., F.S., S.G., E.P., A.B., and G.P. interpreted results of experiments; P.S. and A.F. prepared figures; P.S., A.F., P.C., and G.P. drafted manuscript; P.S., A.F., P.C., A.B., and G.P. edited and revised manuscript; P.S., A.F., P.C., F.B., L.M., F.S., S.G., A.B., and G.P. approved final version of manuscript.

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