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Original Research

OCCUPATIONAL AND ENVIRONMENTAL LUNG DISEASES

Systemic Vascular Dysfunction in Patients With Chronic Mountain Sickness

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Background: **Chronic mountain sickness (CMS) is a major public health problem characterized by exaggerated hypoxemia and erythrocytosis. In more advanced stages, patients with CMS often present with functional and structural changes of the pulmonary circulation, but there is little information on the systemic circulation. In patients with diseases associated with chronic hypoxemia at low altitude, systemic vascular function is altered. We hypothesized that patients with CMS have systemic vascular dysfunction that may predispose them to increased systemic cardiovascular morbidity.**

Methods: **To test this hypothesis, we assessed systemic endothelial function (by flow-mediated dilation [FMD]), arterial stiffness, and carotid intima-media thickness and arterial oxygen saturation (Sao_2) in 23 patients with CMS without additional classic cardiovascular risk factors and 27 age-matched healthy mountain dwellers born and permanently living at 3,600 m. For some analyses, subjects were classified according to baseline Sao_2 quartiles; FMD of the highest quartile subgroup ($Sao_2 \geq 90\%$) was used as a reference value for post hoc comparisons.**

Results: **Patients with CMS had marked systemic vascular dysfunction as evidenced by impaired FMD (CMS, 4.6% ± 1.2%; control subjects, 7.6% ± 1.9%; $P < .0001$), greater pulse wave velocity (10.6 ± 2.1 m/s vs 8.4 ± 1.0 m/s, $P < .001$), and greater carotid intima-media thickness (690 ± 120 μm vs 570 ± 110 μm, $P = .001$). A positive relationship existed between Sao_2 and FMD ($r = 0.62$, $P < .0001$). Oxygen inhalation improved ($P < .001$) but did not normalize FMD in patients with CMS, although it normalized FMD in hypoxemic control subjects ($Sao_2 < 90\%$) and had no detectable effect in normoxemic control subjects ($Sao_2 \geq 90\%$).**

Conclusions: **Patients with CMS show marked systemic vascular dysfunction. Structural and functional alterations contribute to this problem that may predispose these patients to premature cardiovascular disease.**

Trial registry: **ClinicalTrials.gov; No.: NCT01182792; URL: www.clinicaltrials.gov**

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Abbreviations: CMS = chronic mountain sickness; FMD = flow-mediated dilation; hsCRP = high-sensitivity C-reactive protein; IMT = intima-media thickness; NO_2^- = plasma nitrite; PWV = pulse wave velocity; RSNO = S-nitrosothiol; Sao_2 = arterial oxygen saturation

Chronic mountain sickness (CMS) is a major public health problem in mountainous regions of the world, affecting many millions of high-altitude dwellers.[1,2] It is characterized by exaggerated chronic hypoxemia and erythrocytosis. While in more advanced stages, these patients often present with structural and functional changes in the pulmonary circulation, pulmonary hypertension, and right-sided heart failure,[3] little information is available on the systemic circulation. In patients with diseases associated with chronic hypoxemia at low altitude, systemic vascular function is altered,[4] and systemic cardiovascular morbidity and mortality are increased.[5,6] We hypothesized that in addition to pulmonary vascular dysfunction, patients with CMS have systemic vascular dysfunction that may predispose them to increased systemic cardiovascular morbidity and mortality.

To test this hypothesis, we assessed systemic endothelial function, arterial stiffness, and carotid intima-media thickness (IMT) in healthy mountain dwellers

and patients with CMS born and permanently living at 3,600 m. To provide insight into underlying mechanisms, we tested the effects of a 1-h oxygen inhalation on endothelial function in these subjects. Moreover, because erythrocytosis may alter vascular function,[7] we assessed the effects of hemodilution on vascular function in subjects with CMS.

Materials and Methods

Study Subjects and Protocol

Between August 2008 and August 2010, 23 male patients with CMS (mean ± SD age, 52 ± 11 y) and 27 control subjects (49 ± 10 y) without traditional cardiovascular risk factors or a family history of premature cardiovascular events who were born and had been permanently living in La Paz, Bolivia (3,600 m above sea level) were included in the study. Inclusion criteria for patients with CMS were excessive erythrocytosis (hemoglobin concentration > 20 g/dL) in the presence of normal pulmonary function and no history of smoking or working in the mining industry. None of the subjects was taking any medications or nutritional supplements that could have influenced vascular function. All had normal BP on 24-h ambulatory BP monitoring (≤ 130/80 mm Hg) (SpaceLabs 90217; Southwestern Biomedical Electronics Inc). All subjects had a typical Aymara surname; self-identified themselves as Aymaras, and had a similar socioeconomic background. The experimental protocol was approved by the Institutional Review Boards on human investigation of the University of San Andres, La Paz, Bolivia, and the University of Lausanne, Switzerland (IRB Lausanne, 89/06 and 94/10; IRB La Paz, CNB 52/04) and was registered as a clinical trial. All participants provided written informed consent. All studies were performed at the Instituto Boliviano de Biologia de Altura (3,600 m).

A complete clinical examination was performed, and the CMS score was determined on the basis of the following signs and symptoms: breathlessness and palpitations, sleep disturbance, cyanosis, dilatation of veins, paresthesia, headache, and tinnitus. A score between 0 and 3 was attributed for each item, with 0 indicating the absence of the symptom and 1 indicating mild symptoms; 2, moderate symptoms; and 3, severe symptoms. A score > 5 indicates CMS.[1]

For a more precise interpretation of the findings, we also examined vascular function in 22 healthy age-matched (47 ± 10 y) lowlanders in Bern, Switzerland. For some analyses, subjects were classified according to baseline arterial oxygen saturation (Sao_2) quartiles. Flow-mediated dilation (FMD) of the highest quartile subgroup ($Sao_2 \geq 90\%$) was used as the reference value for post hoc comparisons.

Assessment of Systemic Vascular Function

Systemic vascular function studies were performed after 15 min of rest in the supine position in a temperature-controlled room (22°C).

Endothelium-Dependent and Endothelium-Independent Vasodilation: Systemic conduit artery endothelial function was assessed by determining the increase of the brachial artery diameter evoked by reactive hyperemia using high-resolution ultrasonography and automatic wall tracking software according to international guidelines[8] and as previously described.[9] Briefly, the brachial artery was identified ~5 cm above the antecubital fossa with a high-resolution ultrasound device (Acuson Sequoia C512 [Acuson Siemens] or Esaote MyLab30 Gold [Esaote SpA]) and a high frequency (7-10 MHz) linear array probe. The ultrasound probe was then fixed in a stereotactic clamp with micrometer movement capabilities (AMC Vascular Imaging), and Doppler echocardiographic flow was recorded continuously throughout the study. After 1 min of baseline measurements, a pressure cuff placed around the forearm was inflated to 250 mm Hg for 5 min. After deflation of the cuff, the hyperemia-induced changes of brachial artery diameter and flow were continuously measured for 3 min. B-mode ultrasound images were analyzed with a validated system for automatic real-time measurement of the brachial artery diameter (FMD Studio; Computer Vision Group).[10,11] FMD was expressed as the maximal percentage change in vessel diameter from baseline. Endothelium-independent dilation of the brachial artery was assessed by measuring the increase of the brachial artery diameter evoked by oral glyceryl trinitrate (250 μg) (UCB-Pharma SA).[9]

Arterial Stiffness: Assessment of arterial stiffness was performed according to international guidelines.[12] Large artery stiffness was assessed noninvasively by measuring carotid-femoral pulse wave velocity (PWV) using the Complior device (Alam Medical).[12,13] Briefly, carotid and femoral artery waveforms were recorded simultaneously with mechanotransducers directly applied to the skin over the arteries. The mean wave transit time for 10 heart beats was calculated by the system software using the foot-to-foot method. To determine PWV, the surface distance between the recording sites was measured. Central pulse wave analysis was performed noninvasively using the SphygmoCor system (AtCor Medical Pty Ltd). The radial artery pressure waveform was recorded at the wrist using applanation tonometry with a high-fidelity micromanometer (Millar Instruments, Inc). Using a validated transfer function, the central (ascending aortic) pressure waveform was automatically derived from the peripheral pressure waveform.[14,15] The augmentation index, a measure of systemic arterial stiffness,[12] and the time-to-wave reflection were calculated automatically by the software. Because the augmentation index correlates with heart rate,[16] values were normalized for a heart rate of 75 beats/min.

Measurement of Carotid IMT: After identification of the carotid bulb, the segments of the right and left common carotid artery 1 to 2 cm proximal to the bulb were scanned to identify the optimal angle of incidence in accordance with current

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guidelines.[17,18] Carotid IMT was measured using radiofrequency signals with a 21-μm resolution (RF QIMT; Esaote SpA).[19] After scanning the vessel, each radiofrequency line was automatically analyzed forward and backward in real time by the ultrasonography device. Radiofrequency-based echo-tracking systems are considered reference techniques with very high precision and reproducibility.[12] IMT could not be assessed for technical reasons in three patients with CMS and three control subjects.

Sao_2, Pulmonary Function, and Diffusing Capacity of Lung for Carbon Monoxide

Transcutaneous Sa_{O_2} and heart rate were measured on a fingertip with a pulse oximeter (OxiMaxN-595; Nellcor Puritan Bennett LLC). Pulmonary function was assessed with a SensorMedics 2200 pulmonary function system (Bilthoven), and diffusing capacity of lung for carbon monoxide was measured with the single-breath technique following standard guidelines as previously described.[9,20]

Oxygen Administration

In a representative subgroup of 16 patients with CMS and 17 control subjects, the measurement of FMD was repeated after a 1-h administration of 100% oxygen by face mask.[21]

Hemodilution

In nine patients with CMS, we assessed the effects of isovolemic hemodilution on vascular function. After baseline measurements of vascular function, 500 mL of venous blood was withdrawn and replaced by 500 mL of normal saline infused over 30 min.[7] All manipulations were done on the left arm where no measurements were performed. Vascular function was reassessed 30 min after the end of volume replacement.

Metabolic Analyses

Blood samples were taken following a 12-h overnight fast and immediately centrifuged at 4°C, and the plasma was frozen at −80°C. Lipids and glucose were assessed with commercial kits at the Laboratory of the University Hospital Bern, Switzerland. High-sensitivity C-reactive protein (hsCRP) was measured by immunofluorescence (Kryptor; Brahms AG Pharmazeutische). Ozone-based chemiluminescence (OBC Model 280i Nitric Oxide Analyzer; Sievers) was used to measure plasma nitrite (NO_2^-) as previously described.[22] Briefly, samples (200 μL) were injected into triiodide reagent for the combined measurement of NO_2^- + S-nitrosothiol (RSNO) and 5% acidified sulfanilamide added and left to incubate in the dark at 21°C for 15 min to remove NO_2^- for the measurement of RSNO in a parallel sample. NO_2^- was subsequently calculated as $(NO_2^- + RSNO) - RSNO$. All calculations were performed using Peak Analysis software (OriginLab Corporation). The intraassay and interassay variability were 7% and 10%, respectively.

Statistical Analysis

Data were analyzed with the GraphPad Prism 5.0 software package (GraphPad Software Inc) using the paired and unpaired Student *t* test as appropriate. Relations between variables were analyzed by calculating the Pearson product moment correlation coefficients. FMD between subgroups of subjects classified according to baseline Sa_{O_2} quartiles was compared with repeated-measures analysis of variance and Dunnett post hoc tests. FMD of the highest quartile subgroup ($Sa_{O_2} \geq 90\%$) was used as a reference value for post hoc comparisons. Analysis of variance and the test for linear trend were used to calculate the effects of oxygen inhalation on FMD in these four subgroups. Unless otherwise indicated, data are presented as mean ± SD. $P < .05$ was considered to indicate statistical significance.

Results

The characteristics of the participants are shown in Table 1. As expected, Sa_{O_2} was markedly lower and hemoglobin and hematocrit levels significantly higher in patients with CMS than in control subjects. Arterial BP and lung function, lipid and glucose plasma concentration, WBC count, and hsCRP level were normal and comparable between the two groups.

Systemic Vascular Function

Baseline brachial artery diameter was greater in the patients than in the control subjects ($P = .02$), whereas baseline blood flow was similar in the two groups (Table 2). FMD was roughly 40% smaller in the patients than in the control subjects ($P < .0001$) (Fig 1A), whereas nitroglycerin-induced endothelium-independent dilation was similar in the two groups ($P = .76$) (Fig 1B). Reactive hyperemia was comparable in the two groups ($P = .47$), indicating a similar hyperemic stimulus. There existed no significant relationship between baseline brachial diameter and FMD ($r = -0.26$, $P = .22$). Moreover, when comparing subgroups of patients with CMS (n = 19) and control subjects (n = 24) with comparable baseline diameter (4.5 ± 0.5 mm vs 4.4 ± 0.5 mm, $P = .37$), the difference in FMD (patients, $4.6\% \pm 1.5\%$; control subjects, $7.6\% \pm 1.6\%$; $P < .0001$) was of similar magnitude as the one observed between the entire groups (patients, $4.6\% \pm 1.2\%$; control subjects, $7.6\% \pm 1.9\%$; $P < .0001$). There existed a significant positive relationship between Sa_{O_2} and FMD both in the whole population ($r = 0.62$, $P < .0001$) (Fig 2A) and in the patients ($r = 0.42$, $P = .04$). There was no relationship between FMD and plasma hemoglobin concentration in patients with CMS ($r = 0.22$, $P = .31$).

Carotid-femoral PWV was roughly 25% greater ($P = .0001$) (Fig 1C) in patients than in control subjects. In line with this finding, the augmentation-index normalized for a heart rate of 75 beats/min was significantly greater ($P = .003$), and the time-to-wave reflection was significantly shorter ($P = .01$) in the patients than in the control subjects.

Carotid IMT was significantly greater in patients than in control subjects ($P = .001$) (Fig 1D). In the whole population, there was an inverse relationship between Sa_{O_2} and both PWV ($r = -0.49$, $P < .001$) (Fig 2B) and IMT ($r = -0.44$, $P = .002$) (Fig 2C).

Finally, defective FMD was not limited to patients with CMS; it was also found in hypoxemic control subjects. When dividing the whole population in

Table 1—*Subject Characteristics*

Characteristics	CMS (n = 23)	Control Subjects (n = 27)	*P* Value
Age, y	52 ± 11	49 ± 10	.19
Sa_{O_2}, %	83 ± 3	90 ± 3	< .0001
CMS score	8 ± 3	1 ± 1	< .0001
FEV_1 % predicted	122 ± 18	121 ± 13	.91
FEV_1/FVC	0.79 ± 0.04	0.81 ± 0.06	.32
VA % predicted	115 ± 11	118 ± 5	.47
DLCO/VA, 1/min per mm Hg/L	6.1 ± 1.7	5.8 ± 1.0	.59
Heart rate, beats/min	70 ± 11	67 ± 10	.36
24-h ABPM			
SBP, mm Hg	115 ± 8	112 ± 7	.28
DBP, mm Hg	75 ± 4	71 ± 5	.15
Nocturnal dipping, %	13.3 ± 4.5	15.3 ± 5.4	.36
Hemoglobin, g/dL	21.5 ± 1.3	16.9 ± 1.1	< .0001
Hematocrit, %	66.3 ± (5.6	51.1 ± 3.1	< .0001
Leukocyte, $\times 10^9$/L	7.46 ± 0.70	7.59 ± 1.45	.59
hsCRP, μg/mL	2.50 ± 1.33	1.96 ± 1.42	.34
Glucose, mmol/L	4.73 ± 0.45	4.46 ± 0.49	.20
Total cholesterol, mmol/L	4.42 ± 1.15	4.79 ± 1.02	.35
HDL cholesterol, mmol/L	0.93 ± 0.40	0.84 ± 0.27	.49
LDL cholesterol, mmol/L	2.68 ± 0.83	2.91 ± 0.89	.48
Triglycerides, mmol/L	2.01 ± 1.07	2.29 ± 1.09	.51

Data are presented as mean ± SD. ABPM = ambulatory BP monitoring; CMS = chronic mountain sickness; DBP = diastolic BP; DLCO = diffusing capacity of lung for carbon monoxide; HDL = high-density lipoprotein; hsCRP = high-sensitivity C-reactive protein; LDL = low-density lipoprotein; Sa_{O_2} = arterial oxygen saturation; SBP = systolic BP; VA = alveolar volume.

quartiles by means of baseline Sa_{O_2}, the two lower quartiles ($Sa_{O_2} < 87\%$) included all patients with CMS and four control subjects, and the two higher quartiles ($Sa_{O_2} \geq 87\%$) included only control subjects. FMD in the highest quartile ($Sa_{O_2} \geq 90\%$) was significantly greater than in the three other subgroups ($P < .0001$) (Fig 3A). PWV (8.2 ± 0.9 m/s vs 8.4 ± 1.1 m/s, $P = .57$) and carotid IMT (600 ± 120 μm vs 580 ± 110 μm, $P = .56$) were not different between hypoxemic and normoxemic control subjects, respectively. Vascular function in these normoxemic high-altitude dwellers was comparable to the one observed in lowlanders (FMD, 7.8% ± 2.0%; $P = .78$; PWV, 8.4 ± 1.1 m/s; $P = .59$, lowlanders vs normoxemic highlanders).

NO_2^- was significantly lower in the patients than in the control subjects (148 ± 50 nM vs 207 ± 59 nM, $P = .01$). There was a positive relationship between NO_2^- and FMD ($r = 0.41$, $P = .04$) and NO_2^- and Sa_{O_2} ($r = 0.43$, $P = .03$).

Effects of Oxygen Inhalation and Hemodilution on Vascular Function

Oxygen administration significantly decreased heart rate, baseline artery diameter, and baseline blood

Table 2—*Systemic Vascular Function*

Variables	CMS (n = 23)	Control Subjects (n = 27)	*P* Value
Heart rate, beats/min	70 ± 11	67 ± 10	.36
Peripheral SBP, mm Hg	125 ± 13	125 ± 10	.99
Peripheral DBP, mm Hg	81 ± 8	79 ± 8	.38
Central SBP, mm Hg	116 ± 13	113 ± 10	.46
Central DBP, mm Hg	83 ± 9	80 ± 8	.23
Baseline artery diameter, mm	4.7 ± 0.5	4.3 ± 0.5	.02
Baseline blood flow, mL/min	15.4 ± 4.7	16.5 ± 7.6	.55
FMD, %	4.6 ± 1.2	7.6 ± 1.9	< .0001
Hyperemia, %	640 ± 190	680 ± 180	.47
GTN, %	12.5 ± 2.4	12.7 ± 2.3	.77
Aortic PWV, m/s	10.6 ± 2.1	8.4 ± 1.0	.0001
Augmentation index, %	19.1 ± 9.6	11.5 ± 7.1	.003
Time-to-wave reflection, ms	149 ± 15	161 ± 18	.01
Carotid IMT, μm	690 ± 120	570 ± 110	.001

Data are presented as mean ± SD. FMD = flow-mediated dilation; GTN = glyceryl trinitrate; IMT = intima-media thickness; PWV = pulse wave velocity. See Table 1 legend for expansion of other abbreviations.

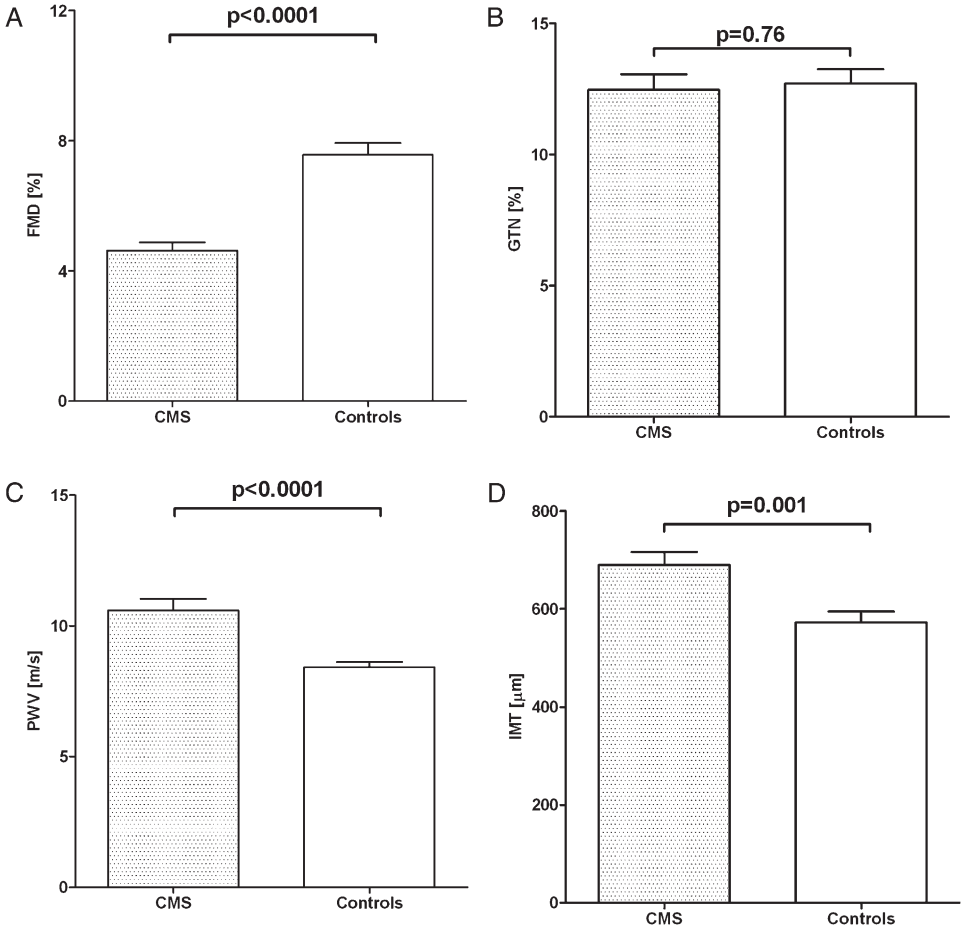

FIGURE 1. Data for 23 patients with CMS and 27 control subjects at 3,600 m. A, FMD. B, Nitroglycerine-induced endothelium-independent dilation. C, Carotid-femoral PWV. D, Carotid IMT. Data are presented as mean ± SEM. CMS = chronic mountain sickness; FMD = flow-mediated dilation; GTN = glyceryl trinitrate; IMT = intima-media thickness; PWV = pulse wave velocity.

flow in patients and control subjects (Table 3). Oxygen inhalation also significantly improved FMD in patients with CMS and hypoxemic control subjects (subgroups of the three lower quartiles of SaO_2), whereas it had no detectable effect in normoxemic control subjects (subgroup of the highest quartile of SaO_2) ($P = .02$) (Fig 3B). During oxygen inhalation, FMD remained significantly lower in patients with CMS than in control subjects (6.3% ± 2.2% vs 7.6% ± 1.9%, $P = .04$).

In patients with CMS, hemodilution that significantly decreased hemoglobin (from 21.4 ± 1.0 g/dL

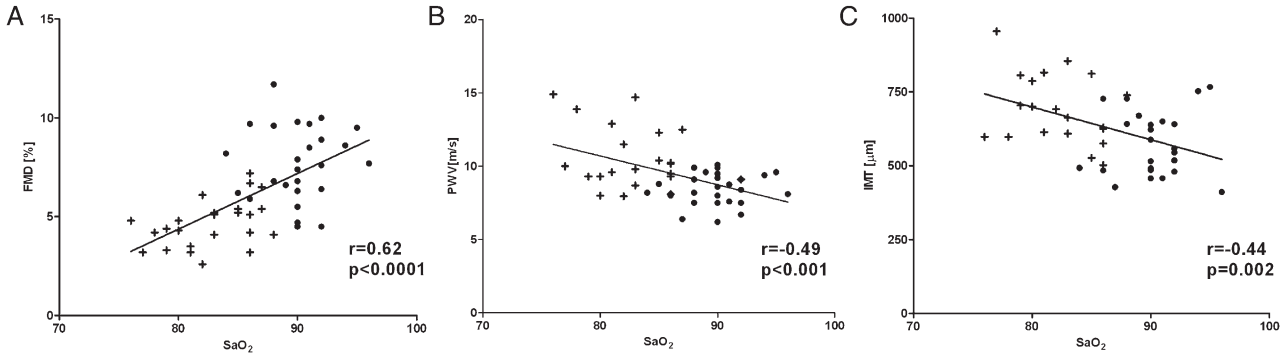

FIGURE 2. A, Relationship between SaO_2 and FMD. B, Carotid-femoral PWV and SaO_2. C, Carotid IMT and SaO_2 in 23 patients with CMS (+) and 27 control subjects (●) at 3,600 m. SaO_2 = arterial oxygen saturation. See Figure 1 legend for expansion of other abbreviations.

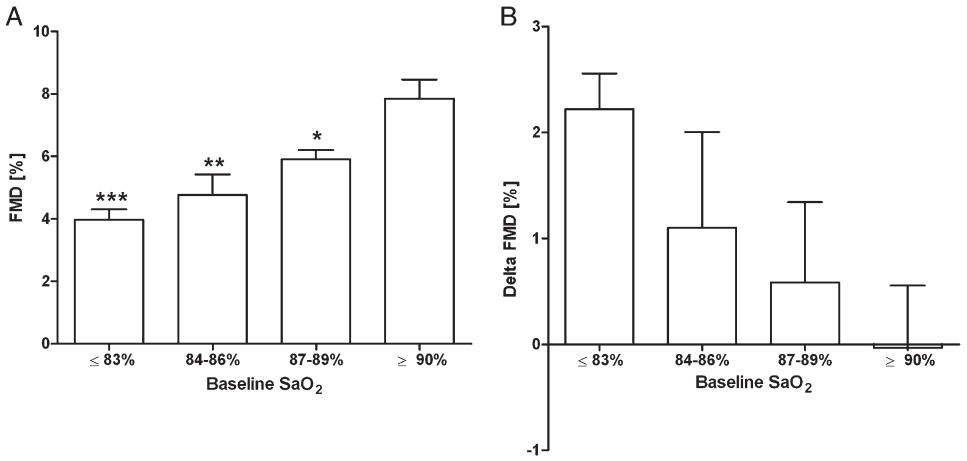

FIGURE 3. FMD (A) and effects of a 1-h oxygen inhalation thereon (B), depending on baseline SaO_2 in 16 patients with CMS and 17 control subjects at high altitude. Subjects were classified in quartiles according to SaO_2. The two lower quartiles included all subjects with CMS and four control subjects, whereas the two higher quartiles included only control subjects. A, FMD in the subgroup of the highest quartile was significantly greater ($P < .0001$) than in the three other subgroups. $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$ by Dunnett post hoc test. B, Oxygen inhalation significantly improved ($P = .02$, post test for linear trend $P < .01$) FMD in patients with CMS and hypoxemic control subjects (subgroups of the three lower quartiles of SaO_2), but it had no detectable effect in normoxemic control subjects ($SaO_2 \geq 90\%$). See Figure 1 and 2 legends for expansion of abbreviations.

to 20.4 ± 1.3 g/dL, $P = .005$) and hematocrit levels (from $66.6\% \pm 4.2\%$ to $62.3\% \pm 4.7\%$, $P < .001$) did not induce any significant change in FMD (from $4.0\% \pm 0.9\%$ to $4.3\% \pm 1.0\%$, $P = .28$).

DISCUSSION

CMS is a major public health problem in mountainous regions of the world, affecting many millions of high-altitude dwellers.[1,2] Although it is well established that this problem is associated with pulmonary vascular dysfunction and right-sided heart failure, there is little information on the systemic circulation. In the present study, we found that patients with CMS without additional cardiovascular risk factors have marked systemic vascular dysfunction as evidenced by impaired FMD, increased vascular stiffness, and increased carotid IMT. These vascular alterations are well-established independent predictors of cardiovascular risk and suggest that CMS may predispose to increased cardiovascular morbidity and mortality in the systemic circulation.

The impairment of FMD in patients with CMS was related to endothelial dysfunction because endothelium-independent vasodilation evoked by nitroglycerine was similar in the patients and control subjects. Chronic hypoxemia induces vasodilation in the systemic peripheral circulation.[23,24] In line with this concept, the baseline diameter of the brachial artery was significantly greater in patients with CMS than in control subjects, and oxygen inhalation caused a significantly larger decrease of the diameter in the patients. However, the impairment of FMD in patients with CMS does not appear to be related to the larger diameter of the brachial artery because when comparing subgroups of patients with CMS and control subjects with similar baseline brachial

Table 3—*Effects of 1-h 100% Oxygen Administration on Vascular Parameters*

	CMS (n = 16)			Control Subjects (n = 17)		
Variables	Ambient Air	Oxygen	*P* Value	Ambient Air	Oxygen	*P* Value
SaO_2, %	83 ± 3	97 ± 1	< .0001	90 ± 2	98 ± 1	< .0001
Heart rate, beats/min	70 ± 10	58 ± 6	< .0001	67 ± 9	54 ± 5	< .0001
Peripheral SBP, mm Hg	123 ± 11	125 ± 11	.20	123 ± 8	121 ± 11	.34
Peripheral DBP, mm Hg	79 ± 8	81 ± 8	.14	74 ± 7	73 ± 9	.44
Baseline artery diameter, mm	4.7 ± 0.5	4.5 ± 0.5	< .0001	4.0 ± 0.5	3.9 ± 0.5	< .001
Baseline blood flow, mL/min	15.9 ± 6.3	11.9 ± 3.8	.01	13.5 ± 5.5	9.4 ± 3.2	< .001
Hyperemia, %	585 ± 150	640 ± 140	.13	674 ± 115	700 ± 120	.21
FMD, %	4.3 ± 1.2	6.3 ± 2.2	< .001	7.0 ± 2.1	7.3 ± 1.9	.54

Data are presented as mean ± SD. See Table 1 and 2 legends for expansion of abbreviations.

artery diameters, the difference of FMD between these subgroups was of similar magnitude as the one observed between the entire groups. Moreover, there was no significant relationship between baseline brachial artery diameter and FMD.

In addition to endothelial dysfunction, stiffening of the vasculature also occurs during the development of atherosclerosis and represents an independent predictor of cardiovascular risk.[25] In the present study, we found that in patients with CMS, several proxies of arterial stiffness were significantly increased compared with control subjects. To further assess structural alterations of the systemic vasculature, we measured carotid IMT and found that it was significantly increased in patients with CMS. Taken together, these data demonstrate functional and morphological vascular alterations in patients with CMS that were of similar magnitude as those reported in asymptomatic subjects presenting with two to three classic cardiovascular risk factors.[26] Interestingly, there is evidence from uncontrolled studies that patients with CMS are at increased risk for systemic cardiovascular disease.[27]

An interesting finding of the present study was that vascular dysfunction in high-altitude dwellers was not limited to patients with CMS. Indeed, FMD also was impaired in hypoxemic control subjects ($Sa_{O_2} < 90\%$), whereas in normoxemic control subjects ($Sa_{O_2} \geq 90\%$), FMD was normal and comparable to age-matched control subjects living at low altitude in the present and in an earlier study,[28] suggesting that there exists a cutoff value for the detrimental vascular effects of chronic hypoxemia. In line with this speculation, a similar cutoff value discriminating between a physiologic and a pathologic response was reported for the chronotropic and respiratory effects of hypoxia.[29]

Oxygen inhalation improved FMD in hypoxemic subjects but had no detectable effect in normoxemic ($Sa_{O_2} \geq 90\%$) control subjects, suggesting that vascular dysfunction in patients with CMS and hypoxemic control subjects was, at least in part, functional and related to hypoxemia. In line with this speculation, a direct relationship existed between FMD and Sa_{O_2}.

In rodents, chronic hypoxia induces endothelial dysfunction[30] that is related to impaired nitric oxide bioavailability. Here, we found that NO_2^- concentration was lower in patients with CMS than in control subjects and that there was a significant relationship between NO_2^- concentration and FMD. These findings suggest that impaired nitric oxide bioavailability contributes to functional vascular dysfunction in humans with chronic hypoxemia.[23,24]

In patients with CMS but not in hypoxemic control subjects, FMD during oxygen inhalation remained significantly smaller than in normoxemic ($Sa_{O_2} \geq 90\%$) control subjects, and endothelial dysfunction was associated with increased arterial stiffness and carotid IMT, suggesting that the prolonged and more severe chronic hypoxemia associated with CMS may induce structural alterations of the systemic vasculature. Alternatively, it is possible that mechanisms other than hypoxemia contribute to alterations of the systemic circulation in patients with CMS. In this regard, erythrocytosis has been suggested to alter vascular function.[7] Here, we found that isovolemic hemodilution had no detectable effect on vascular function in patients with CMS, suggesting that erythrocytosis does not contribute importantly to vascular dysfunction. In line with this concept, there was no relationship between plasma hemoglobin concentration and endothelial function, and normalization of arterial oxygenation with oxygen inhalation significantly improved FMD in patients with CMS despite marked erythrocytosis.

In patients with COPD, inflammation, as evidenced by an increased WBC count and CRP level, has been suggested to contribute to vascular dysfunction.[31] In the present study, WBC count and hsCRP level were comparable in patients with CMS and control subjects.

Conclusions

To our knowledge, the present findings provide the first evidence that patients with CMS without additional cardiovascular risk factors have functional and morphological alterations of the systemic circulation. Some of these alterations also were found in hypoxemic high-altitude dwellers who did not experience CMS and were partially reversible during oxygen inhalation, suggesting that chronic hypoxemia may represent one of the underlying mechanisms.

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Dr Rimoldi: contributed to the clinical examinations, study design, data analysis and interpretation, and writing of the first draft of the manuscript.

Dr Rexhaj: contributed to the clinical examinations, data analysis and interpretation, and final writing of the manuscript.

Dr Pratali: contributed to the clinical examinations and approved the final draft of the manuscript.

Dr Bailey: contributed to the clinical examinations and data analysis and approved the final draft of the manuscript.

Dr Hutter: contributed to the clinical examinations and approved the final draft of the manuscript.

Dr Faita: contributed to the clinical examinations and approved the final draft of the manuscript.

Dr Salinas Salmòn: contributed to the clinical examinations and approved the final draft of the manuscript.

Dr Villena: contributed to the recruiting of patients and control subjects and the clinical examinations and approved the final draft of the manuscript.

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References

1. León-Velarde F, Maggiorini M, Reeves JT, et al. Consensus statement on chronic and subacute high altitude diseases. *High Alt Med Biol*. 2005;6(2):147-157.
2. Peñaloza D, Sime F. Chronic cor pulmonale due to loss of altitude acclimatization (chronic mountain sickness). *Am J Med*. 1971;50(6):728-743.
3. Penaloza D, Arias-Stella J. The heart and pulmonary circulation at high altitudes: healthy highlanders and chronic mountain sickness. *Circulation*. 2007;115(9):1132-1146.
4. Ip MS, Tse HF, Lam B, Tsang KW, Lam WK. Endothelial function in obstructive sleep apnea and response to treatment. *Am J Respir Crit Care Med*. 2004;169(3):348-353.
5. Huiart L, Ernst P, Suissa S. Cardiovascular morbidity and mortality in COPD. *Chest*. 2005;128(4):2640-2646.
6. Sidney S, Sorel M, Quesenberry CP Jr, DeLuise C, Lanes S, Eisner MD. COPD and incident cardiovascular disease hospitalizations and mortality: Kaiser Permanente Medical Care Program. *Chest*. 2005;128(4):2068-2075.
7. Giannattasio C, Piperno A, Failla M, Vergani A, Mancia G. Effects of hematocrit changes on flow-mediated and metabolic vasodilation in humans. *Hypertension*. 2002;40(1):74-77.
8. Deanfield J, Donald A, Ferri C, et al; Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. Endothelial function and dysfunction. Part I: methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens*. 2005;23(1):7-17.
9. Jayet PY, Rimoldi SF, Stuber T, et al. Pulmonary and systemic vascular dysfunction in young offspring of mothers with preeclampsia. *Circulation*. 2010;122(5):488-494.
10. Faita F, Masi S, Loukogeorgakis S, et al. Comparison of two automatic methods for the assessment of brachial artery flow-mediated dilation. *J Hypertens*. 2011;29(1):85-90.
11. Gemignani V, Faita F, Ghiadoni L, Poggianti E, Demi M. A system for real-time measurement of the brachial artery diameter in B-mode ultrasound images. *IEEE Trans Med Imaging*. 2007;26(3):393-404.
12. Laurent S, Cockcroft J, Van Bortel L, et al; European Network for Non-invasive Investigation of Large Arteries. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J*. 2006;27(21):2588-2605.
13. Asmar R, Benetos A, Topouchian J, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension*. 1995;26(3):485-490.
14. Chen CH, Nevo E, Fetics B, et al. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation*. 1997;95(7):1827-1836.
15. Pauca AL, O'Rourke MF, Kon ND. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension*. 2001;38(4):932-937.
16. Wilkinson IB, MacCallum H, Flint L, Cockcroft JR, Newby DE, Webb DJ. The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol*. 2000;525(pt 1):263-270.
17. Stein JH, Korcarz CE, Hurst RT, et al; American Society of Echocardiography Carotid Intima-Media Thickness Task Force; Endorsed by the Society for Vascular Medicine. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. *J Am Soc Echocardiogr*. 2008;21(2):93-111.
18. Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. *Cerebrovasc Dis*. 2007;23(1):75-80.
19. Paini A, Boutouyrie P, Calvet D, Zidi M, Agabiti-Rosei E, Laurent S. Multiaxial mechanical characteristics of carotid plaque: analysis by multiarray echotracking system. *Stroke*. 2007;38(1):117-123.
20. Stuber T, Sartori C, Schwab M, et al. Exaggerated pulmonary hypertension during mild exercise in chronic mountain sickness. *Chest*. 2010;137(2):388-392.
21. Keyl C, Schneider A, Gamboa A, et al. Autonomic cardiovascular function in high-altitude Andean natives with chronic mountain sickness. *J Appl Physiol*. 2003;94(1):213-219.
22. Ingram TE, Pinder AG, Bailey DM, Fraser AG, James PE. Low-dose sodium nitrite vasodilates hypoxic human pulmonary vasculature by a means that is not dependent on a simultaneous elevation in plasma nitrite. *Am J Physiol Heart Circ Physiol*. 2010;298(2):H331-H339.
23. Cosby K, Partovi KS, Crawford JH, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med*. 2003;9(12):1498-1505.
24. Maher AR, Milsom AB, Gunaruwan P, et al. Hypoxic modulation of exogenous nitrite-induced vasodilation in humans. *Circulation*. 2008;117(5):670-677.
25. Laurent S, Boutouyrie P, Asmar R, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension*. 2001;37(5):1236-1241.
26. Celermajer DS, Sorensen KE, Bull C, Robinson J, Deanfield JE. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J Am Coll Cardiol*. 1994;24(6):1468-1474.
27. Aparicio Otero O. *Texto de Medicina de Altura*. 1st ed. La Paz, Bolivia: GMC Artes Graficas; 2008.
28. Ghiadoni L, Penno G, Giannarelli C, et al. Metabolic syndrome and vascular alterations in normotensive subjects at risk of diabetes mellitus. *Hypertension*. 2008;51(2):440-445.
29. West JB; American College of Physicians; American Physiological Society. The physiologic basis of high-altitude diseases. *Ann Intern Med*. 2004;141(10):789-800.
30. Reboul C, Tanguy S, Gibault A, Dauzat M, Obert P. Chronic hypoxia exposure depresses aortic endothelium-dependent vasorelaxation in both sedentary and trained rats: involvement of L-arginine. *J Appl Physiol*. 2005;99(3):1029-1035.
31. Eickhoff P, Valipour A, Kiss D, et al. Determinants of systemic vascular function in patients with stable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2008;178(12):1211-1218.