Micro- and macrovascular function in the highest city in the world: a cross sectional study

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Summary

Background Since vascular responses to hypoxia in both healthy high-altitude natives and chronic mountain sickness (a maladaptive high-altitude pathology characterised by excessive erythrocytosis and the presence of symptoms—CMS) remain unclear, the role of inflammation and oxidative/nitrosative stress on the endothelium-*dependent* and -*independent* responses in both the micro- and macrocirculation, in healthy Andeans at different altitudes and in CMS patients, was examined.

Methods 94 men were included: 18 lowlanders (LL), 38 healthy highlanders permanently living at 3800 m (n = 21—HL-3800) or in La Rinconada, the highest city in the world (5100–5300 m) (n = 17—HL-5100/No CMS). Moreover, 14 participants with mild (Mild CMS) and 24 with moderate to severe CMS (Mod/Sev CMS) were recruited. All undertook two reactivity tests: i) local thermal hyperaemia (microcirculation) and ii) flow-mediated dilation (macrocirculation). Endothelium-*independent* function (glyceryl trinitrate) was also assessed only in La Rinconada.

Findings Conductance and skin blood flow velocity during the microcirculation test, as well as macrocirculation progressively decreased with altitude (LL > HL-3800 > HL-5100/No CMS). CMS also induced a decrease in macrocirculation (HL-5100/No CMS > Mild CMS = Mod/Sev CMS), while glyceryl trinitrate restored vascular function. Both oxidative stress and nitric oxide metabolites increased with altitude only. Principal component analysis revealed that increasing inflammation with altitude was associated with a progressive decline in both micro- and macrovascular function in healthy highlanders.

Interpretation Both micro and macrovascular function are affected by chronic exposure to hypoxia, the latter being further compounded by CMS.

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Keywords: Microcirculation; Flow-mediated dilation; Chronic mountain sickness; Altitude; Inflammation

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Research in context

Evidence before this study

Chronic Mountain Sickness (CMS), a condition afflicting 15–20% of high-altitude natives, and which manifests through excessive erythrocytosis (haemoglobin concentration [Hb] ≥21 g/dL for males) and several clinical symptoms, has first been identified nearly a century ago by Carlos Monge Medrano, a Peruvian physician. While central hypoventilation has traditionally been proposed as the principal mechanism explaining the accentuated hypoxemia, since CMS patients are prone to developing cardio- and cerebrovascular diseases, it is evident that this syndrome extends to the vascular function, of which flow-mediated dilation (FMD) is a landmark. We conducted a PubMed database search without language restriction for studies focusing on the effect of CMS on FMD (using "chronic mountain sickness" and "endothelial function" as the search terms) published between January 1st, 2012 and December 31st, 2022. The limited number of studies of interest revealed that FMD is decreased in CMS sufferers when compared with their healthy counterpart living at a similar altitude; however, the mechanisms seem multifaceted and have, therefore, not been clearly identified. Moreover, an extended search on CMS also revealed that the available body of research and the ensuing scientific consensus had never been conducted at altitudes beyond 4,300 m (Cerro de Pasco, Peru) where the hypoxic burden is considerably less significant than beyond 5,000 m above sea-level, where large groups of population, such as in La Rinconada (5100 m, Peru) are permanently living.

Added value of this study

The findings from the present study do not support the usual take home available from the literature which suggests a clear impairment in vascular function in CMS patients. Indeed, we report that i) in healthy indigenous populations, both micro- and macrovascular function were altered by life-long exposure to high-altitude, and ii) in CMS patient assessed at the extreme altitude of 5100 m, microvascular function (skin microvascular reactivity) was not compromised further, while the endothelium-*dependent* macrovascular function (FMD) appeared to be impaired. On another hand, endothelium-*independent* macrovascular function, assessed using glyceryl trinitrate (GTN) seemed to be preserved in CMS patients. These unique data also clearly point towards increased inflammation and potentially oxidative/nitrosative stress as a potential key mechanism to explain the reduced endothelium-*dependent* function at high-altitude, and in CMS patients.

Implications of all the available evidence

At extreme altitudes, the burden of hypoxia seems to be more prominent than other pathologies, such as CMS, on the observed vascular dysfunction.

The present demonstration of the role of oxidative/nitrosative stress and inflammation in vascular dysfunction offers therapeutic avenues to rectify it and in turns counteract the development of atherosclerosis and/or cognitive impairment.

Introduction

There is ample evidence in the literature showing that impaired conduit artery vasodilatory capacity (*e.g.*, flow-mediated dilation—FMD) is linked with an aggravated risk of cardiovascular disease.[1–4] Similarly, impaired microcirculation is a landmark for a range of pathological complications including diabetes, hypertension or obesity.[5] Increased inflammation and production of reactive oxygen species (ROS) interfere with the physiological balance between vasodilating and vasoconstricting substances, leading to the development of endothelial dysfunction and cardiovascular disorders in the general population.[6] Moreover, alterations in arterial O_2 partial pressure (PaO_2) also contribute to the observed changes in vascular tone notably through the aforementioned mechanisms. While the decrease in both macrovascular[7] and microvascular[8] reactivity has been well-documented in lowlanders exposed to hypoxia, far less is known about chronic exposure to hypoxia or indeed in high-altitude Indigenous populations.

It is generally admitted that high-altitude natives have developed physiological adaptive strategies and are, therefore, well-acclimatized to their hypoxic environment.[9] However, in the Andes, 15–20% of these highlanders do not thrive in their environment and develop a condition termed chronic mountain sickness (CMS),[10] which manifests through excessive erythrocytosis (EE, haemoglobin concentration [Hb] ≥21 g/dL for males), and several clinical symptoms.[11] An impairment of the brachial artery FMD[12,13] has recently been reported in this population. However, despite forays into understanding the mechanisms of these impairments,[6,13] since these are multifaceted, much remains to be elucidated.

Notably, the link between the microcirculation and the (mal)adaptation of the systemic endothelium during a life-long exposure to hypoxia remains poorly understood.[14] Moreover, the existence of a possible bidirectional cross talk and even more so of a possible causal relationship between the micro- and macrovasculature remains debated in the literature.[15] Nonetheless, from a mechanistic point of view, the increase in ROS and/or the decrease in nitric oxide (NO) bioavailability often observed in pathological conditions (*e.g.*, diabetes[15,16]) would contribute to the aforementioned vasomotor alterations.

We, therefore, hypothesized that endothelial function would be progressively impaired with increasing

altitude of residence (up to the highest city in the World, La Rinconada, Peru, 5100–5300 m) and with the severity of CMS, both in the micro- and macrovasculature. We further postulated, that increased oxidative/nitrosative stress and inflammation would be a mechanistic determinant in these functional alterations.

Methods

Ethical approval

This study was part of a larger research program (*Expedition 5300*) investigating the pathophysiological consequences of living permanently at high-altitude. The experimental protocol was approved by the ethics committee of the Universidad Nacional Mayor de San Marcos (CIEI-2019-002) and performed according to the Declaration of Helsinki. All participants were informed about the procedures and gave their written informed consent to participate in this study.

Participants

A total of 94 Andean men, aged 18 to 55, were included in this study: 18 lowlanders born at <1000 m and permanently living at sea level (Lima, Peru, 80 m—LL), 21 highlanders permanently living at 3800 m (Puno, Peru—HL-3800), and 55 highlanders permanently residing at 5100–5300 m (La Rinconada, Peru). The participants were tested at their current altitude of permanent residence. The highlanders were all Andean men native from high-altitude (≥3800 m); the gender bias is due to CMS essentially affecting males. Based on clinical interviews and examinations, participants with medical history of diabetes, respiratory and cardiovascular diseases were excluded. None of the highlanders reported a prolonged stay at low altitude (more than 5 days below 3500 m) over the past 3 months. All highlanders from La Rinconada were working in goldmine facilities. Recruitment was conducted on a voluntary basis through advertisement (e.g., flyers, radio) and word of mouth. Prospective participants reported to the laboratory for a general health check and after confirmation that they did not present any of the aforementioned contraindications, they were offered to take part in the study.

Following CMS evaluation according to the current recommendations,[11] all 21 highlanders from Puno were considered as CMS free. Among the La Rinconada dwellers, 17 did not present CMS (CMS score ≤5—HL-5100/No CMS), 14 had mild CMS symptoms (CMS score 6–10—Mild CMS), and 24 had moderate to severe CMS symptoms (CMS score ≥11—Mod/Sev CMS) (Table 1).

Study design

Each participant was assessed during a single experimental session conducted at his altitude of permanent residency. After inclusion, resting pulse oxygen saturation (SpO_2, mean value over 30 s; OxiMax N65, Medtronic, Dublin, Ireland) and blood pressure (measured twice 1-min apart; Digital Blood Pressure Monitor, A&D Medical, Sydney, Australia) were measured in supine position following at least 5 min at rest, according to the American Heart Association guidelines.[17] Subsequently, vascular explorations consisted in a series of tests aiming to assess endothelium-*dependent* micro- and macrovascular functions using local thermal hyperaemia (LTH) and post-occlusive hyperaemic response (flow-mediated dilation—FMD), respectively. The vasodilatory response to glyceryl trinitrate (GTN) was used to assess the endothelium-*independent* response for the macrocirculation in highlanders with or without CMS. Finally, a blood sample was taken from a forearm antecubital vein to assess various biomarkers and test the effects of plasma on endothelial cell activation. Participants were requested to avoid caffeine and alcohol intake within 12-h and to have their last meal at least 3 h before the evaluations.

	Effect of altitude		Effect of CMS		
	LL	HL-3800	HL-5100/ No CMS	Mild CMS	Mod/ Sev CMS
	n = 18	n = 21	n = 17	n = 14	n = 24
Anthropometrics					
Age (years)	29 ± 10	33 ± 11	42 ± 9$^{*,\$}$	43 ± 8	44 ± 7
Time at 5100 m (years)			13 ± 8	13 ± 10	17 ± 8
Weight (kg)	72.2 ± 16	70.0 ± 12	69.9 ± 6	72.8 ± 11	70.3 ± 10
Height (cm)	169 ± 5	168 ± 7	166 ± 6	166 ± 6	164 ± 5
BMI ($Kg{\cdot}m^{-2}$)	25.2 ± 5	24.8 ± 4	25.5 ± 2	26.6 ± 4	26.2 ± 3
Clinical					
CMS score	N/A	2 ± 2	4 ± 1$^{\$}$	8 ± 1$^{£}$	13 ± 2$^{£,\#}$
Haemoglobin ($g{\cdot}dL^{-1}$)	14.3 ± 2	18.6 ± 2*	22.2 ± 2$^{*,\$}$	22.2 ± 1	23.8 ± 2$^{£,\#}$
Haematocrit (%)	43.2 ± 5	54.9 ± 5*	69.2 ± 7$^{*,\$}$	70.2 ± 5	75.2 ± 5$^{£,\#}$
SBP (mmHg)	115.6 ± 9	116.5 ± 10	115.1 ± 12	115.5 ± 11	117.5 ± 17
DBP (mmHg)	70.1 ± 8	76.4 ± 7	79.1 ± 11*	80.3 ± 8	74.5 ± 9
MAP (mmHg)	85.2 ± 8	89.8 ± 6	91.1 ± 11	92.0 ± 8.0	88.9 ± 8
SpO_2 (%)	97.9 ± 1	93.0 ± 3*	83.7 ± 5$^{*,\$}$	82.6 ± 4	80.8 ± 6

Data are Mean ± SD. CMS, Chronic mountain sickness; LL, Lowlanders; HL-3800, Highlanders at 3800 m; HL-5100/No CMS, Highlanders without CMS at 5100 m; Mild CMS, Highlanders with mild CMS at 5100 m; Mod/Sev CMS, Highlanders with moderate to severe CMS at 5100 m; BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; MAP, Mean arterial pressure; SpO_2, Pulse oxygen saturation. An analysis of variance was used to detect the effect of altitude/CMS ($P < 0.05$). Bonferroni corrected post-hoc analyses were used where required and indicated in the table as follows: *, $P < 0.05$ vs. LL; $, $P < 0.05$ vs. HL-3800; £, $P < 0.05$ vs. HL-5100/No CMS; #, $P < 0.05$ vs. Mild-CMS.

Table 1: **Participants' characteristics.**

Measurements

A more detailed description of the protocol and methods is available in the online Supplement.

Briefly, cutaneous blood flow was measured on the forearm using single-point laser doppler flowmetry (Periflux System 5000, Perimed, Järfälla, Sweden), while macrocirculation was assessed using high-resolution Doppler ultrasound (Terason μSmart 3200t, Teratech,

United States) and a 10-MHz multifrequency linear array probe (15L4 Smart Mark, Teratech, United States).

The local thermal hyperaemia test was used to measure microvascular reactivity. Cutaneous blood flow was first recorded for 3 min, with skin temperature maintained at 33 °C, after which skin temperature was increased to 43 °C for 25 min. Macrovascular reactivity was assessed using either the flow-mediated dilation test (endothelium-*dependent* test) or following the administration of glyceryl trinitrate (GTN—endothelium-*independent* test). The former consists of concomitantly recording blood flow and diameter of the brachial artery, following a 5-min occlusion of the forearm.[2] During the endothelium-*independent* test, the same parameters were recorded over a period of 7 min following the administration of a set sublingual dose of 300 µg/metered dose of GTN. This test was only performed in the participants from La Rinconada.

A venous blood sample was then taken to measure haemoglobin, haematocrit, NO-related products and superoxide dismutase (SOD) activity, as well as an array of inflammatory markers (IL-6, IL-7, IL-8, IL-17, IFN-g, MCP-1, MIP-1b and TNF-α). Superoxide dismutase (SOD) is the major antioxidant defence system against superoxide anion and plays a critical role in inhibiting oxidative inactivation of nitric oxide, thereby preventing endothelial dysfunction (Fukai et al., Antioxid Redox Signal 2011). We selected a large panel of inflammatory markers, because each of these molecules has been shown to be involved in vascular and endothelial dysfunction in various disorders and/or to be modulated by hypoxic exposure.[18,19] Finally, the impact of plasma on endothelial cell activation was tested via the culture and treatment of human aortic endothelial cells (HAEC).[20] Flow Cytometry to measure E-selectin and ICAM-1 expression was also used on the HAEC.

Data analysis

A detailed description of the analysis and calculations applied to assess the local thermal hyperaemia, flow-mediated dilation, GTN-induced dilation as well as the scaling methods used for the macrovascular data is provided in the online Supplement.

Statistical analysis

Normality of the distribution was assessed visually and using the Shapiro–Wilk test. When data were not normally distributed, a natural-logarithm transformation was performed to meet the conditions of application of parametric tests (data concerned: microvascular and blood markers). A two-step analysis was used to answer our hypotheses. Firstly, the effect of altitude in healthy subjects (comparisons between LL, HL-3800 and HL-5100/No CMS) was challenged by an analysis of variance. The second step aimed at assessing the effect of CMS on micro- and macrovascular function in highlanders living at 5100 m by comparing the HL-5100/No CMS with the Mild CMS and Mod/Sev CMS groups. For both analyses, we applied a regression method (general linear model based on t-distributions) with the relevant covariates (e.g., age), followed by Bonferroni-corrected post hoc comparisons, when a main effect was identified. However, we did not account for the multiplicity of comparisons related to the high number of parameters that were analysed, and these results should be considered as exploratory. Detailed methods including the description of the exploratory principal component analyses (PCA) are provided in the online Supplement. Data are expressed as mean ± SD and significance level was set at $P \leq 0.05$. Statistical analyses were performed using SPSS software version 25.0 (IBM, New York, USA).

Role of the funding source

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Results

Participants

Participants' characteristics are presented in Table 1. There was no difference in the characteristics of our participants, except for age since HL-5100/No CMS were older than LL and HL-3800. Both haemoglobin and haematocrit progressively increased with altitude and were further elevated in the Mod/Sev CMS group compared to highlanders without CMS. Neither mean (MAP) nor systolic (SBP) blood pressure were altered by the progressively increasing altitude or severity of CMS. Only diastolic blood pressure (DBP) was 11% lower in LL compared to HL-5100/No CMS. Peripheral oxygen saturation progressively decreased with altitude, but this decrease was not significantly compounded by the presence of CMS in highlanders. Due to the observed difference in age between the dwellers from La Rinconada and the other participants, age has been used as a covariate in subsequent analyses.

Effect of altitude

Micro- and macrovascular reactivity

Local thermal hyperemia. Baseline CVC and velocity were reduced in highlanders compared to LL (Table 2). Both parameters were also reduced during the initial peak and plateau of the LTH, peak CVC even presenting progressive decrease with the altitude (HL-3800 > HL-5100/No CMS) (Fig. 1).

	Effect of altitude		Effect of CMS				
	LL	HL-3800	HL-5100/ No CMS	Mild CMS	Mod/ Sev CMS	P values	
	n = 18[a]	n = 21[a]	n = 17	n = 14	n = 24	Altitude	CMS
Microcirculation Cutaneous vascular conductance							
Baseline (mV/mm Hg)	0.17 ± 0.09	0.08 ± 0.03*	0.08 ± 0.03*	0.10 ± 0.05	0.09 ± 0.04	**0.007**	0.295
Peak (mV/mm Hg)	1.40 ± 1.11	1.15 ± 0.58	0.64 ± 0.36*,$	0.77 ± 0.42	0.58 ± 0.28	**0.007**	0.471
Plateau (mV/mm Hg)	2.13 ± 1.56	2.00 ± 1.16	1.09 ± 0.61*,$	1.26 ± 0.68	0.90 ± 0.33	**0.019**	0.379
Velocity[b]							
Baseline	0.34 ± 0.22	0.13 ± 0.05*	0.11 ± 0.04*	0.13 ± 0.06	0.11 ± 0.05	**<0.001**	0.441
Peak	2.79 ± 2.20	1.89 ± 0.90	0.86 ± 0.54*,$	0.98 ± 0.45	0.69 ± 0.35	**<0.001**	0.216
Plateau	4.12 ± 3.02	3.29 ± 1.76	1.46 ± 0.90*,$	1.61 ± 0.78	1.07 ± 0.43	**<0.001**	0.139
Macrocirculation Flow-mediated dilation							
Baseline diameter (mm)	4.0 ± 0.6	4.1 ± 0.7	5.1 ± 0.7*,$	5.3 ± 0.5	5.1 ± 0.8	**0.009**	0.655
Peak diameter (mm)	4.4 ± 0.6	4.4 ± 0.7	5.4 ± 0.7$	5.5 ± 0.5	5.3 ± 0.8	**0.013**	0.731
FMD (mm)	0.4 ± 0.1	0.3 ± 0.1*	0.3 ± 0.1*	0.2 ± 0.1£	0.2 ± 0.1£	**0.001**	**0.002**
FMD% (%)	9.8 ± 3.3	6.7 ± 2.0*	6.1 ± 1.5*	4.2 ± 2.4£	4.0 ± 1.6£	**<0.001**	**0.002**
$FMD\%_{corr}$ (%)	9.3 ± 0.5	6.4 ± 0.5*	6.8 ± 0.6*	4.2 ± 0.5£	4.0 ± 0.4£	**<0.001**	**0.002**
Time to peak (sec)	59.6 ± 14.6	66.2 ± 23.5	63.4 ± 21.3	82.9 ± 38.8	63.7 ± 27.6	0.603	0.112
Baseline blood flow ($ml{\cdot}s^{-1}$)	2.50 ± 0.96	1.18 ± 0.91*	1.80 ± 1.07*	2.30 ± 1.65	1.80 ± 1.92	**<0.001**	0.625
Peak blood flow ($ml{\cdot}s^{-1}$)	7.83 ± 2.50	5.46 ± 2.39*	5.95 ± 2.38*	6.51 ± 2.25	5.71 ± 2.89	**0.001**	0.655
Mean shear rate (s^{-1})	620.6 ± 256.8	406.9 ± 186.4*	238.4 ± 91.1*	234.3 ± 99.5	225.6 ± 101.4	**<0.001**	0.914
SR_{AUC} (s^{-1})	35,719 ± 12,621	24,713 ± 11,444*	15,453 ± 8710*	18,477 ± 10,270	13,472 ± 7636	**0.001**	0.240
FMD:SRAUC (u.a.)	0.00029 ± 0.0001	0.00031 ± 0.00012	0.00052 ± 0.00032*,$	0.00028 ± 0.00019£	0.00035 ± 0.0002£	**0.002**	**0.017**
GTN							
GTN baseline diameter (mm)			4.9 ± 0.5	5.3 ± 0.5	5.1 ± 0.8		0.364
Post GTN peak diameter (mm)			5.7 ± 0.5	5.8 ± 0.5	5.8 ± 0.7		0.797
GTN% (%)			14.5 ± 6.5	9.7 ± 4.8	14.0 ± 5.6		**0.042**
$GTN\%_{corr}$ (%)			15.0 ± 0.9	11.9 ± 1.1	15.0 ± 0.8		**<0.001**

Data are Mean ± SD. CMS, Chronic mountain sickness; LL, Lowlanders; HL-3,800, Highlanders at 3800 m; HL-5100/No CMS, Highlanders without CMS at 5100 m; Mild CMS, Highlanders with mild CMS at 5100 m; Mod/Sev CMS, Highlanders with moderate to severe CMS at 5100 m; FMD, absolute hyperemic response; FMD%, Flow-mediated dilation; FMD%corr, corrected Flow-mediated dilation; SRAUC, Shear rate area under the curve; FMD: SRAUC, FMD normalised to shear rate area under the curve; GTN, Glyceryl trinitrate. An analysis of variance was used to detect the effect of altitude/CMS ($P < 0.05$). These analyses were conducted on raw macrovascular data and on natural logarithm-transformed data for the microvascular function and blood markers. Bonferroni corrected post-hoc analyses were used where required and indicated in the table as follows: *, $P < 0.05$ vs. LL; $, $P < 0.05$ vs. HL-3800; £, $P < 0.05$ vs. HL-5100/No CMS. P values written in bold correspond to the significant tests. [a]Microvascular data was not complete for one subject in Lima, and two subjects in Puno. [b]Velocity was estimated by dividing skin blood flow by the hematocrit of the subject.

Table 2: **Effect of the altitude of residency or CMS on the vascular response.**

Flow-mediated dilation. There was an overall decrease in the vasodilatory response at altitude, materialized by a decrease in FMD% at HL-3800 (–32%) and HL-5100/No CMS (–38%) when compared to the LL group (Table 2, Fig. 1). Irrespective of the calculation methods (FMD, FMD% $FMD\%_{corr}$, FMD:SRAUC), this decrease can essentially be imputed to an increase in baseline diameter. The SRAUC response (which represents the stimulus for FMD) followed a similar trend to FMD%.

Blood markers

Among the circulating markers of inflammation that were studied, IL-17, IFN-γ and TNF-α were modified by life-long exposure to high-altitude, at 5100 m. Intercellular Adhesion Molecule *1* level but not E-selectin level was influenced by altitude with HL-3800 displaying higher levels than LL and HL-5100/No CMS. There was an overall increase in the antioxidant defense, assessed using SOD, as well as in the concentrations of both NOX and nitrites with altitude (Table 3).

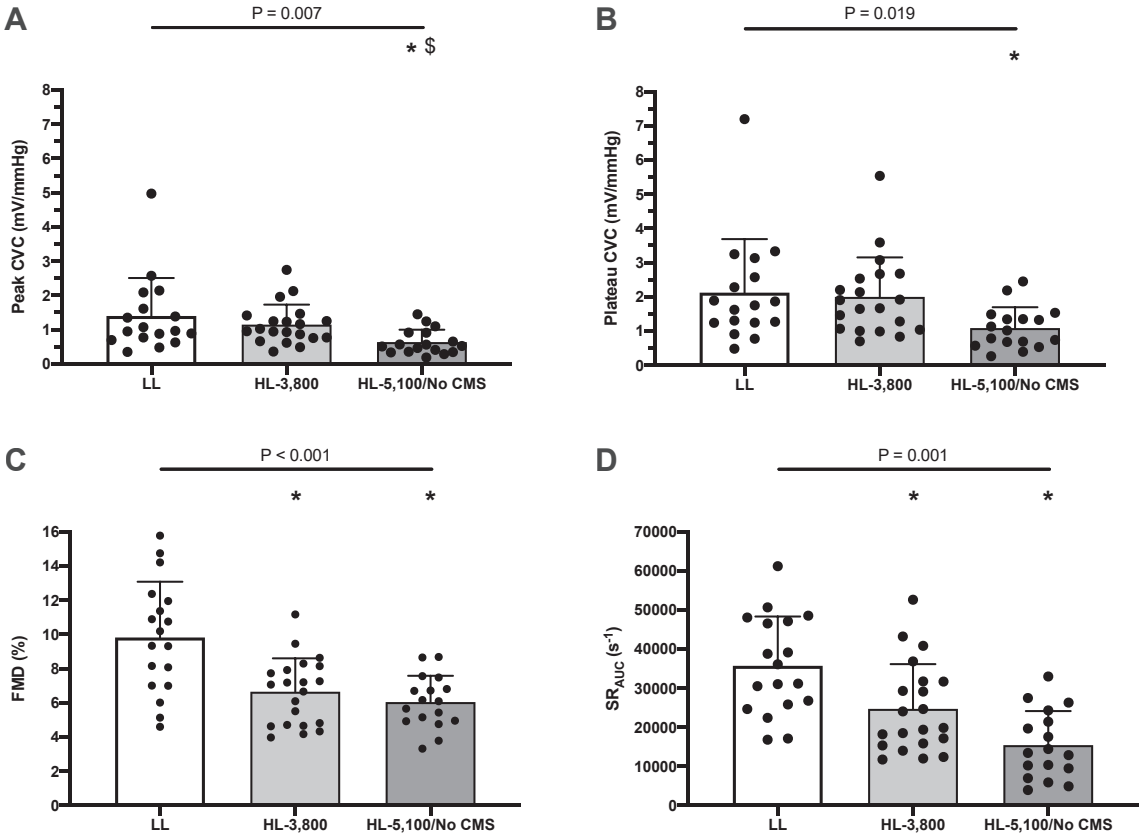

***Fig. 1:* Effect of altitude on micro- and macrovascular function.** Effect of the altitude of residence in healthy subjects on cutaneous vascular conductance during the initial peak (panel A) and plateau (panel B) of local thermal hyperemia, as well as on flow-mediated dilation (panel C) and shear rate area under the curve (panel D). List of abbreviations: CVC, cutaneous vascular conductance; FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve; LL, lowlanders living at sea level (0 m); HL-3800, healthy highlanders living at 3800 m; HL-5100/No CMS, healthy highlanders living at 5100 m. Data are expressed as Mean ± SD. An analysis of variance was used to detect any effect of altitude. These analyses were conducted on raw macrovascular data and on natural logarithm-transformed data for the microvascular function. Bonferroni corrected post-hoc analyses were used where required and indicated on the figure as follows: *, $P < 0.05$ vs. LL; $, $P < 0.05$ vs. HL-3800.

Relationships between micro-/macrocirculation and inflammation

Overall, IL-8, MCP-1, MIP-1β, and TNF-α levels were correlated with peak and plateau CVC as well as velocities. Moreover, NO metabolites were negatively correlated with both the peak and plateau velocities as well as with FMD%. On another hand, the chosen marker of oxidative stress (*i.e.*, SOD) was only associated with plateau velocity. FMD% itself was associated with Peak CVC response as well as both the peak and plateau velocities while SR_{AUC} was associated with FMD% but more interestingly with both markers of the peak and plateau phase of the LTH test (eTable 1).

Using PCA to determine the profiles of our populations, we noted that LL have a preserved micro- and macrovascular function and low inflammatory profiles, while HL-5100/No CMS display a pro-inflammatory profile with altered micro- and macrovascular function (Fig. 3). Conversely, HL-3800 have little inflammation and preserved microcirculation, while macrovascular function seems to be impaired (Fig. 3).

Effect of CMS

Endothelium-dependent *response*

Chronic mountain sickness had no effect on any of the microcirculation parameters measured during the LTH test (Fig. 1, Table 2). Irrespective of the method used to measure FMD (absolute, relative or corrected), CMS negatively impacted the vessels' capacity to dilate with a 31% and a 34% decrease in FMD% in Mild CMS and Mod/Sec CMS vs. HL-5100/No CMS, respectively (Table 2, and Fig. 2c). Unlike what was observed in the healthy participants, this decrease could not be attributed to a difference in baseline blood vessel diameter. Moreover, there was no effect of the worsening of the CMS score on FMD.

	Effect of altitude		Effect of CMS			P values	
	LL	HL-3800	HL-5100/ No CMS	Mild CMS	Mod/Sev CMS		
	n = 11	n = 10	n = 13	n = 11	n = 10	Altitude	CMS
Inflammation							
IL-6 (pg·mL^{-1})	1.7 ± 1.2	1.5 ± 1.2	1.4 ± 0.8	1.8 ± 1.4	2.4 ± 1.6	0.859	0.208
IL-7 (pg·mL^{-1})	1.0 ± 0.7	1.1 ± 0.4	2.2 ± 1.5	2.8 ± 2.2	3.1 ± 1.8	0.124	0.661
IL-8 (pg·mL^{-1})	3.5 ± 1.7	4.3 ± 3.5	11.1 ± 12.7	12.8 ± 10.1	25.0 ± 23.1	0.154	0.063
IL-17 (pg·mL^{-1})	0.17 ± 0.33	0.27 ± 0.31	0.78 ± 0.44$^{*,\$}$	0.76 ± 0.41	0.81 ± 0.48	**0.005**	0.996
IFN-γ (pg·mL^{-1})	1.4 ± 0.7	2.1 ± 0.8	3.5 ± 1.4*	4.9 ± 4.5	15.8 ± 33.2	**0.001**	0.078
MCP-1 (pg·mL^{-1})	15.0 ± 7.0	15.5 ± 7.1	33.4 ± 29.7	40.1 ± 29.8	72.2 ± 47.1	0.247	0.053
MIP-1β (pg·mL^{-1})	4.0 ± 2.3	5.1 ± 9.2	14.9 ± 22.4	14.9 ± 16.4	58.5 ± 82.4$^{£}$	0.380	**0.017**
TNF-α (pg·mL^{-1})	4.8 ± 1.6	4.8 ± 1.3	7.9 ± 3.2$	8.9 ± 3.4	12.0 ± 7.9	**0.032**	0.335
E-Selectin (MFI)	29,334 ± 2073	30,269 ± 1184	31,068 ± 5478	30,178 ± 3083	34,001 ± 5612	0.785	0.183
ICAM-1 (MFI)	108,537 ± 12,472	145,140 ± 20,301*	107,529 ± 7783$^{\$}$	113,942 ± 22,484	111,975 ± 16,397	**0.000**	0.725
NO metabolites and SOD activity							
SOD (μmol·mL^{-1}·min^{-1})	9.7 ± 2.0	13.0 ± 3.5	15.8 ± 5.8*	10.7 ± 4.6$^{£}$	14.6 ± 6.5	**0.021**	**0.031**
NOX (μmol·L^{-1})	28.0 ± 12.3	66.2 ± 54.4*	103.0 ± 66.2	86.2 ± 51.2	81.6 ± 44.8	**0.019**	0.876
Nitrites (μmol·L^{-1})	7.7 ± 3.3	21.2 ± 17.9*	39.5 ± 21.8*	32.5 ± 22.1	31.0 ± 18.3	**0.011**	0.664

Data are Mean ± SD. CMS, Chronic mountain sickness; LL, Lowlanders; HL-3,800, Highlanders at 3800 m; HL-5,100, Highlanders without CMS at 5100 m; IL-6/7/8/17, interleukin 6/7/8/17; IFN-γ, interferon γ; MCP-1, Monocyte chemoattractant protein 1; MIP-1β, Macrophage inflammatory protein-1; TNF-α, Tumor necrosis factor α; ICAM-1, Intercellular adhesion molecule 1; SOD, superoxide dismutase; NOX, Nitric oxide. LL: n = 7 for IL-17, n = 10 for NOX and Nitrites, n = 14 for E-Selectin and ICAM-1; HL-3800: n = 15 for SOD and n = 16 for NOX and Nitrites; No CMS: n = 12 for IL-17 and MIP-1b, n = 16 for SOD, NOX and Nitrites; Mild CMS: n = 17 for SOD, NOX and Nitrites; Mod/Sev CMS: n = 9 for MIP-1b, n = 17 for SOD, NOX and Nitrites. An analysis of variance was used on natural logarithm-transformed data to detect the effect of altitude/CMS (P < 0.05). Bonferroni corrected post-hoc analyses were used where required and indicated in the table as follows: *, P < 0.05 vs. LL; $, P < 0.05 vs. HL-3800; £, P < 0.05 vs. HL-5100/No CMS. P values written in bold correspond to the significant tests.

Table 3: **Blood markers.**

*Endothelium-*independent *response*

There was an overall effect of CMS on the endothelium-*independent* dilation (GTN% and GTN%$_{corr}$), with trends towards a smaller brachial artery dilation (GTN%) in those with Mild CMS when compared to either highlanders with no CMS (HL-5100/No CMS) (−33%, P = 0.07) or Mod/Sev CMS (−31%, P = 0.08) (Table 2).

Blood markers

Inflammation. MIP-1β was significantly increased by CMS, with similar trends observed for other inflammatory markers (e.g., MCP-1, IL-8, IFN-γ; Table 3). The effect of plasma on endothelial cell activation was not significantly different between CMS groups.

NO metabolites and SOD activity. Superoxide dismutase was significantly affected by CMS with lower activity measured in highlanders with Mild CMS. Nitric oxide and nitrite concentrations did not differ between CMS groups (Table 3).

Relationships between micro-/macrocirculation and inflammation

When considering all highlanders at 5100 m with or without CMS, there was a negative correlation between FMD% and CMS score (eTable 1). Similarly to what was observed when focusing the analyses on the effect of the altitude of residency, SR_{AUC} correlated strongly with markers of microcirculation (eTable 1).

Various markers of inflammation (IL-8, MCP-1 and MIP-1β) were associated with the CVC and velocity microcirculatory responses. None of the blood markers correlated with the endothelium-*dependent* response (FMD%). Only IL-7 and IL-17 were associated with Endothelium-*independent* response (GTN%) (eTable 1).

There was no association between oxidative/nitrosative stress and the measured markers of micro- and macrovascular function (eTable 1).

Discussion

The results presented herein constitute the first comprehensive appraisal of the effects of permanent life in hypoxia, up to the extreme altitude of 5100 m, and along the spectrum of CMS, on systemic vascular function. The primary novel findings were: i) both micro- and macrovascular function are altered by exposure to high-altitude; ii) at the altitude of 5100 m, only macrovascular function appears to be impaired in CMS patients; iii) markers of inflammation increased with altitude and to a lesser extent with CMS severity, while both oxidative stress and NO metabolites increased with altitude only; and iv) there were strong associations

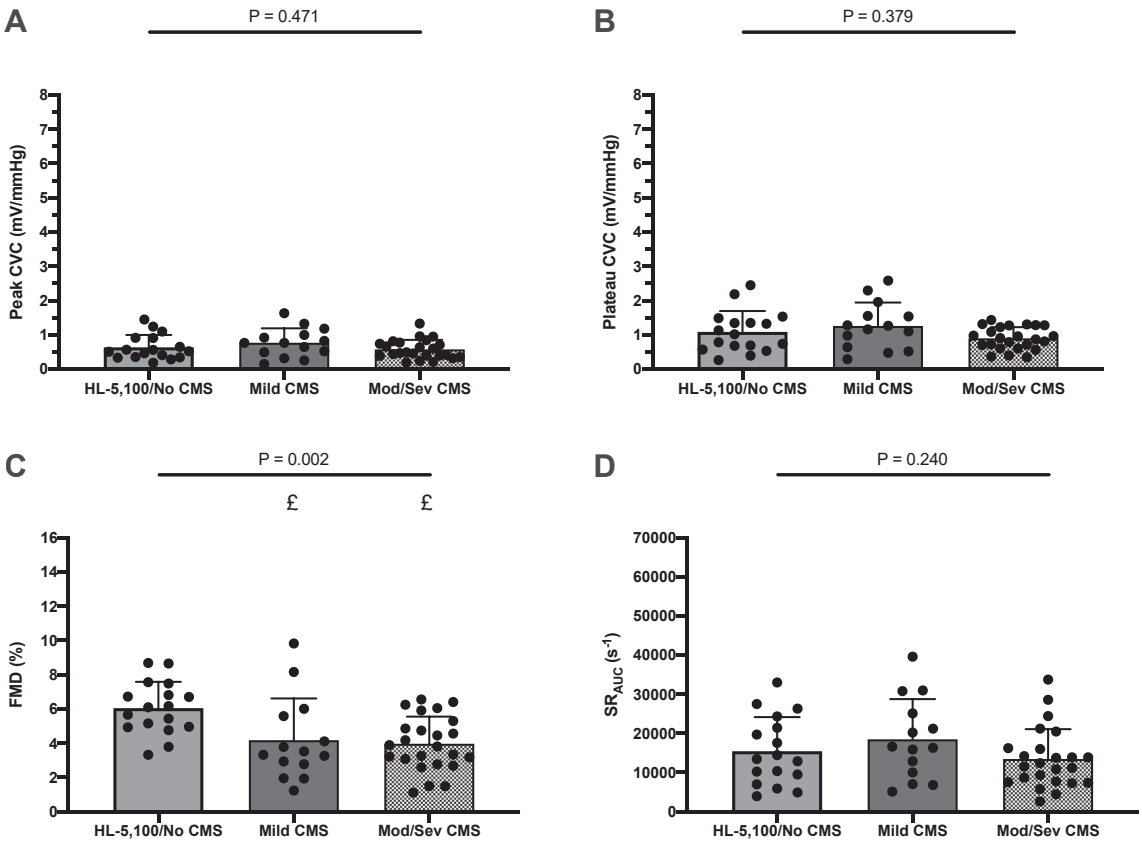

Fig. 2: **Effect of CMS on micro- and macrovascular function.** Effect of CMS in subjects living at 5,100 m on cutaneous vascular conductance during the Initial peak (panel A) and plateau (panel B) of local thermal hyperaemia, as well as on flow-mediated dilation (panel C) and shear rate area under the curve (panel D). List of abbreviations: CVC, cutaneous vascular conductance; FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve; CMS, Chronic Mountain Sickness; HL-5100/No CMS, healthy highlanders living at 5100 m; Mild CMS, highlanders with mild CMS at 5100 m; Mod/Sev CMS, highlanders with moderate to severe CMS at 5100 m. Data are expressed as Mean ± SD. An analysis of variance was used to detect any effect of CMS. These analyses were conducted on raw macrovascular data and on natural logarithm-transformed data for the microvascular function. Bonferroni corrected post-hoc analyses were used where required and indicated on the figure as follows: £: $P < 0.05$ vs. HL-5100/No CMS.

between the measured blood markers and both micro- and macrovascular function in the healthy participants, while these relationships only persisted in the microcirculation in CMS patients. Taken together, the latter two points offer a potential mechanistic link for the vascular impairments that were observed.

Clinical remarks

The unique model of permanent residency in "the highest city in the world" used in this study provided some unique findings in terms of the characterisation of the population. As expected, we observed an increase in Hb and haematocrit with altitude in the otherwise healthy participants. The mean increase of approximately 4 g dL^{-1} between Lima (0 m) and Puno (3800 m) seems in line with the model recently developed by Gassmann et al.[21] suggesting that in the Andes, Hb increases by 1 g dL^{-1}·1000 m^{-1}. However, the changes measured between Puno and La Rinconada (5100 m) largely exceeded this prediction, since we measured an average concentration >22 g dL^{-1} in non-CMS participants (vs. < 20 g dL^{-1} in the model).

Mean arterial blood pressure did not seem to be influenced by either altitude or CMS. Despite a lack of convincing data in the literature, this seems in line with the current consensus on blood pressure in Andean highlanders.[22] At such an extreme altitude, this is rather intriguing, in particular when one considers the aforementioned heightened Hb concentration and haematocrit, together with the concomitant elevation in blood viscosity[23] and hypervolemia,[24] presented elsewhere. The increases in the brachial artery resting diameter as well as in NOX and nitrite concentrations could account for this result. Nevertheless, neither of these parameters were exacerbated in CMS patients (*vs.* HL-5100/No CMS group), while haemoglobin, and even more so

haematocrit and blood viscosity[23] continued to rise in the CMS groups when compared to healthy La Rinconada dwellers. Taken together, the hemodynamic mechanisms sustaining the regulation of blood pressure in highlanders from the Andes remain unclear and, therefore, warrant further investigations.

Effect of altitude

The literature on the effect of the altitude of residence (*i.e.*, beyond chronic exposure of lowlanders) on vascular function is scarce, and thus, this physiological (mal) adaptation remains poorly understood. For instance, the vast majority of the available literature compares healthy highlanders with their diseased counterpart, essentially CMS,[6,12,13,19,25] but also dwellers with heightened cardiovascular risk factors.[26] On another hand, a few studies have used Caucasian lowlanders as their sea-level[6,19] and/or high-altitude point of comparison.[6] However, since it has been suggested that ethnic differences, at least in the regulation of blood pressure,[22] could appear, the use of Caucasian lowlanders as a control group could be hazardous. As a consequence, the closest proxy that could be identified in the literature, is a study published by Rimoldi et al.[12] who reported that, among non-CMS participants, only those presenting with hypoxemia had reduced FMD%. However, endothelial function in those with higher SpO_2 (>90%) seemed to be preserved compared to the other quartiles, at least when measurements were conducted at the altitude of La Paz (Bolivia, 3600 m). The fact that O_2 administration did not appear to modify FMD% in this latter subgroup, combined with the conclusions from another group proposing that endothelial function is preserved in pregnant Andeans highlanders[27] contributed to the current overall take-home message that, in healthy Andean highlanders, endothelial function is not compromised.[18] However, the present results seem to contradict this conclusion inasmuch as we observed a clear progressive decrease in FMD% with rising altitude. Likewise, microvascular function decreased with altitude. To the best of our knowledge, there is no previous study focusing on this aspect of microcirculatory function in high-altitude natives; however, a recent study by Treml et al.,[8] assessing microcirculatory cutaneous blood flow in lowlanders, observed that both acute and more prolonged (7 days) exposure to hypoxia led to an impairment in the blood flow response to reactive hyperaemia measured on the forearm.

The present study clearly shows enhanced inflammation at 5100 m. However, the large array of inflammatory biomarkers used in the present work can complicate the interpretation of the results. Indeed, the PCA identifies links between overall inflammation and both micro- and macrocirculation (Fig. 3) with a progressive worsening of the inflammation and as a consequence of the endothelial function with the altitude of residency. In addition, in the present study, the increase in SOD activity, a potent antioxidant,[28] with increasing altitude of residency indicates a rise in oxidative stress, which is in line with previous studies.[19,29] The reasons for the increased inflammation and oxidative stress at high-altitude are unknown but studies performed in polycythemic patients at sea level have reported increased levels of cell-free haemoglobin compared to healthy individuals,[30] the latter being known to promote the production of reactive oxygen species and of various cytokines by the endothelial and circulating cells, through the activation of several pathways such as NFκB or NLRP3.[31]

Our results also demonstrated increased NOX and nitrite levels in individuals living at high-altitude (HL-3800 and HL-5100) compared to lowlanders. This observation confirms previous studies in Tibetans, Peruvians and Indians, showing higher concentrations of nitrogen species in highlanders than in lowlanders.[32] The greater blood viscosity described in highlanders could partly be at the origin of the enhanced NO production, since eNOS activity is sensitive to wall shear stress.[33] Moreover, while enhanced oxidative stress should result in a decrease of NO bioavailability, enhanced inflammation could have promoted the production of NO through the activation of iNOS, as is the case in various chronic inflammatory syndromes.[34] Excess NO production through iNOS activation could be deleterious for the cardiovascular system since a large amount of NO could be converted to peroxinitrite.[35] However, this point of view is debated.[36] For instance, increased NO production by iNOS activation may contribute to the regulation of blood pressure, by counteracting the effects of potent vasoconstrictors, such as endothelin-1.[36] Whatever the reasons of the enhanced NOX and nitrite levels in the highlanders, it could be posited that the elevated baseline circulating concentrations of NO metabolites, while possibly explaining the higher baseline brachial artery diameter observed in the HL-5100/No CMS group, also impede further increases in NO production when the endothelium is challenged, such as during the reactive hyperaemia test. This hypothesis of an inability of the endothelium to produce additional NO is further reinforced by the fact that when GTN is used to measure endothelium-*independent* dilation capability, vascular function is restored in all of the groups from La Rinconada. Nevertheless, since the present study did not focus on these mechanisms, the roles of other vasodilators, such as endothelium-derived hyperpolarizing factor, ATP or prostaglandins cannot be discarded.[6]

Taken together, the present results seem to plead for a progressive impairment of vascular endothelial function with altitude, even in native highlanders permanently residing at high altitude. The physiological adaptations determining the balance between vascular resistance and vasodilation seem to indicate that a certain state of residual vasodilation predominates at

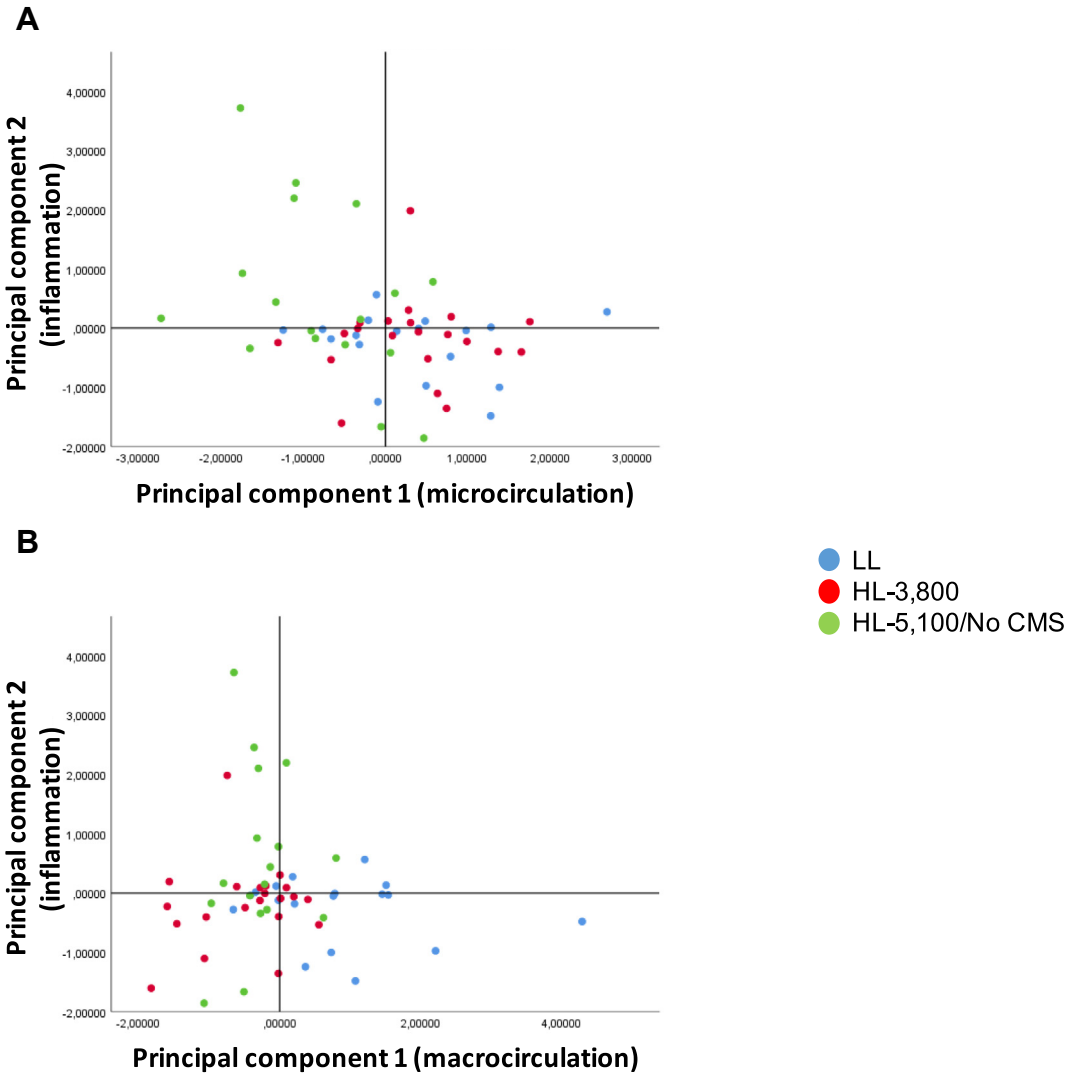

***Fig. 3:* Principal component analysis.** PCA explaining the role of inflammation on the microcirculation (panel A) and macrocirculation (panel B). IL-6/7/8 (interleukin 6/7/8) MCP-1 (Monocyte chemoattractant protein 1), MIP-1β (Macrophage inflammatory protein-1) and TNF-α (Tumor necrosis factor α) were highlighted as the main inflammatory components. Peak and plateau CVC (cutaneous vascular conductance) make up the microvascular parameters, while FMD% (flow-mediated dilation) is the chosen macrovascular parameter. LL, lowlanders living at sea level (0 m); HL-3800, healthy highlanders living at 3800 m; HL-5100/No CMS, healthy highlanders living at 5100 m.

rest, in turn decreasing the ability of the vasculature to dilate further when shear stress increases.

Effect of CMS

While the impact of residing at altitude in healthy participants characterised in the present study was rather clear, the effect of CMS on the systemic vasculature is harder to decipher. Nevertheless, the effect of CMS *per se* has been more broadly studied in the literature, albeit never at the extreme altitude of La Rinconada. The literature seems to agree that endothelial function is impaired in CMS patients when compared with healthy highlanders residing at the same altitude.[6,12,13,18,19] The present results confirm this consensus since CMS patients exhibited a lower FMD% than non-CMS participants from La Rinconada. However, we did not observe any progressive worsening of this response with the increased severity of the CMS symptoms, despite the clear correlation between CMS score and FMD%. On the other hand, we did not observe any association between CMS symptoms and microvascular reactivity, the latter remaining largely unaffected in CMS patients compared to the healthy highlanders. While the latter result goes against our original hypothesis, there is no

clear consensus in the literature regarding the direction (*i.e.* cause and effect relationship) of the potential cross talk between micro- and macrovasculature impairments, and it has even been recently suggested they could simply evolve concomitantly, such as in diabetes.[15] Heterogeneity between microvascular beds could also contribute to this observation.[15]

A recent review by Tymko et al.[18] summarizing the current state of the literature suggested that hypoxemia, oxidative-nitrosative stress and hyperviscosity are the main factors explaining the impairment in systemic vascular function observed in people with CMS. In the present study, highlanders with or without CMS at 5100 m exhibited similar SpO_2. Therefore, hypoxemia may not be a primary mechanism responsible for the disease exacerbation. Nevertheless, a previous study showed that the administration of 100% O_2 almost fully restored FMD.[12] However, there are some methodological questions arising from this study,[12] since breathing a hyperoxic gas mixture is a poor proxy to restored SpO_2, because hyperoxia itself can increase vascular tone and generate ROS,[37] meaning they could have misinterpreted the physiological response of their participants. Bailey et al. repeatedly demonstrated that oxidative-nitrosative stress is heightened in CMS patients in turn affecting both the cerebral[29] and the systemic[19] vasculature. However, we did not observe any clear worsening of inflammation with CMS, except for a very large increase in MIP-1β in the Mod/Sev CMS group. This chemokine is an indicator of early-phase atherosclerosis. Therefore, it could contribute to the decreased FMD% that was measured. However, MIP-1β is usually also associated with an increase in E-selectin and oxidative stress,[38] neither of which were clearly altered by CMS in the present work. Nevertheless, we did observe a decrease in the antioxidant defence (SOD), in the Mild CMS group, which could contribute to an overall increase in oxidative stress. Tremblay et al.[13] elegantly demonstrated the impact of blood viscosity on vascular function by using haemodilution to reduce Hb in CMS patients. This induced a clear improvement in FMD independently of any change in plasma NO. Blood viscosity is not presented in the present article, but another publication from our group concurs with these results since we noted a significant increase in blood viscosity in the participants with the most severe CMS.[23] The progressive increase in haematocrit and Hb that was measured in the Mod/Sev CMS group also seems to corroborate this previous observation. Moreover, at the extreme altitude of La Rinconada, it could be concluded that the basal vasodilation has reached its functional limit since no change in NOX, nitrites or indeed baseline diameter that could have compensated the increase in Hb or viscosity was observed.

The use of GTN to measure endothelial-*independent* function confirmed previous results from Rimoldi et al.[12] who observed that vascular function was preserved when assessed via sublingual nitroglycerin. Intriguingly, in the present study, the Mild-CMS group displayed a much lower response to GTN than either the HL-5100/No CMS or the Mod/Sev CMS groups. While there is no obvious explanation for this observation, it should be noted that the vasodilation induced by the GTN was still more than double that of the reactive hyperaemia test. It can therefore be concluded that the endothelium is impaired while smooth muscle seems to have remained intact, and that CMS does not cause any additional defect.

Clinical implications

While the alterations in either (or both) micro- and macrovascular function or the chronology of the appearance of their respective dysfunction remain elusive in the literature,[39] it is well-established that either constitutes an early sign of atherosclerosis,[16] the latter being a key player in the heightened risk of cardiovascular disease identified in CMS patients.[10] Microvascular dysfunction has also recently been recognized as a significant contributor to a worsening of cognitive performance.[40] Cerebrovascular dysfunction itself has also been associated with impaired cognition and depression in CMS patients.[29] Clinically, it is therefore of the utmost importance to understand the mechanisms underlying vascular impairments in high-altitude dwellers as well as in CMS patients, in order to create interventions designed to prevent this poor health prognostic. This extreme environment is also a pertinent model to better understand the consequences of lifelong exposure to hypoxia, as encountered by lowlanders in many pathologies.

Limitations

The main limitation of this study is that the HL-5100/No CMS group was significantly older than the other groups, while age is known to be associated with impaired vascular function.[18] As a consequence, we included age as a covariate in our statistical analyses.

Second, due to logistical limitations, we were only able to conduct the endothelium-*independent* vasodilation test (administration of GTN) in the participants from La Rinconada. Nevertheless, when the same test was conducted in highlanders residing at an altitude similar to Puno's, a similar response as in the present article was observed,[12] giving us a clearer insight into the response that could have been expected in the HL-3800 group.

Third, we opted for a stochastic recruitment strategy for the present study. While this approach may induce some bias, our groups, especially the highlanders from La Rinconada, are most likely highly representative of the local population since mining is the main activity meaning all inhabitants share the same socio-economical background.

Fourth, mining activity could have influenced the present results. For instance, silicosis and mercury contamination cannot be excluded since we do not, as yet, have data about these potential confounding factors. It could also be argued that the confined space of the mine could influence inspired the partial pressures in O_2 and CO_2. We have some preliminary (unpublished) data suggesting that the former is not decrease inside the mine.

Finally, the traditional definition of CMS has been established using data collected on patients essentially residing at altitudes between 2500 and 4300 m. However, in a recent publication from our group,[41] it was suggested that the Hb threshold traditionally used to define EE ([Hb] ≥21 g/dL for males and ≥19 g/dL for females), may not apply to the extreme altitude of La Rinconada. Moreover, it was also observed that EE was decorrelated from CMS symptoms raising the question to know whether one should focus on EE or CMS score. From a clinical standpoint one cannot ignore symptoms, which explains why we opted to focus on CMS rather than solely on EE. Altogether, these observations raised questions about the definition of CMS and the application of this definition beyond 5000 m of elevation.

At last, the present study design cannot determine whether the changes in vascular function observed in highlanders permanently residing at extreme altitude are i) reversible (e.g., following oxygen administration) or ii) specific compared to changes observed in lowlanders who would be acutely exposed to a similar altitude. The present results show, however, that despite highlander ancestries and permanent residency at high-altitude, physiological adaptations to chronic hypoxia do not normalize vascular function in highlanders compared to lowlanders.

Conclusion and perspectives

Despite the inherent limitations associated with field studies, the take-home message of this study is that both skin micro- and macrovascular function are diminished by altitude in high-altitude natives, along with increased systemic oxidative stress and inflammation. In addition, macrovascular function is further impaired by CMS but the mechanisms responsible for this worsening of the endothelial dysfunction with CMS do not seem as clearly related to this oxidative–inflammatory–nitrosative stress, and remain, therefore, to be elucidated.

In the constant "battle" between increases in vascular resistance due to heightened Hb and shear stress in the maintenance of oxygen delivery, the present data seem to plead for an altitude threshold (yet to be precisely determined) that makes the balance tip. The need to cope with the extreme hypoxic conditions and potential EE (*i.e.* vascular resistance) associated with life in the highest city in the world seems to be predominant and in turn the functional consequences of the shear stress in CMS patients residing at this extreme altitude are less obvious.

Contributors

All authors contributed to the conception or design of the work, acquisition or analysis or interpretation of data for the work, drafting the work or revising it critically for important intellectual content. JVB and SV verified the data and have access to the raw data. All authors approved the final version and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed and JVB had the final responsibility for the decision to submit for publication.

Data sharing statement

The deidentified data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of interests

None.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lana.2024.100887.

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