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# Viscosité sanguine et fonction cardio-vasculaire chez des résidents de haute altitude

Emmanuelle Loyrion

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UNIVERSITÉ GRENOBLE ALPES  
UFR DE MÉDECINE DE GRENOBLE

Année : 2018

**VISCOSITE SANGUINE ET FONCTION  
CARDIO-VASCULAIRE CHEZ DES RESIDENTS  
DE HAUTE ALTITUDE**

THÈSE  
PRÉSENTÉE POUR L'OBTENTION DU TITRE DE DOCTEUR EN MÉDECINE  
DIPLOME D'ÉTAT

**Emmanuelle LOYRION**

[Données à caractère personnel]

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***« Cela semble toujours impossible, jusqu'à ce que ce soit fait »***

*Nelson Mandela*



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# ABBREVIATIONS

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AT = right ventricular outflow acceleration time

CMS = Chronic mountain sickness

EDLA = End-diastolic left atrium

EDRA = End-diastolic right atrium

EDRV = End-diastolic right ventricular surface

EE = Excessive erythrocytosis

FBF = Forearm blood flow

FMD = Flow-mediated dilatation

[Hb] = Hemoglobin concentration

Ht = Hematocrit

[HbO<sub>2</sub>] = Oxyhemoglobin concentration

[HHb] = deoxyhemoglobin concentration

LV = Left ventricular

LVEF = LV ejection fraction

NIRS = Near-infrared spectroscopy

RV = Right ventricular

RVAC = Right ventricular area change

SpO<sub>2</sub> = Pulsed oxygen saturation

VO<sub>2m</sub> = Muscular oxygen consumption at rest

# RESUME

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Parmi les résidents permanents de haute altitude (> 2 500 m), 5-33% développent un mal chronique de montagnes (MCM). Les mécanismes conduisant à la maladie ne sont cependant pas complètement élucidés. L'objectif de cette étude était de préciser la viscosité sanguine et les fonctions cardiovasculaires des résidents de haute altitude souffrant ou non de MCM. 41 sujets résidant à 5 100 m, dont 15 résidents sains (concentration d'hémoglobine ([Hb]) < 21 g·dL<sup>-1</sup>), 13 résidents atteints de polyglobulie ([Hb] ≥ 21 g·dL<sup>-1</sup>) sans symptôme de MCM (Qingshai CMS score ≤ 5 ; MCM-) et 13 résidents avec polyglobulie et symptômes de MCM (Qingshai CMS score 6-14 ; MCM+) ont été comparés à 13 sujets résidant à 3 800m et 10 sujets résidant niveau de la mer. Après l'évaluation de la viscosité sanguine et des paramètres standard d'échographie cardiaque, la fonction vasculaire était évaluée par un test d'ischémie-reperfusion mesurée par spectroscopie dans le proche infrarouge. A 5 100 m, la différence d'[Hb] entre les résidents polyglobuliques et les résidents sains n'était pas associée à une différence de viscosité sanguine alors que les résidents sains à 5 100m avait une viscosité sanguine plus élevée que les résidents à 3 800 m pour un même niveau [Hb]. Chez les MCM+, la vasodilatation post-occlusive était augmentée par rapport aux résidents du niveau de la mer et de 3 800m. Tous les résidents de haute altitude avaient une oreillette droite dilatée comparé aux résidents du niveau de la mer, sans altération de la fonction cardiaque. Ces résultats suggèrent que les modifications de viscosité sanguine et cardio-vasculaires liées à l'exposition chronique à l'hypoxie de haute altitude pourraient être impliquées dans le développement du MCM.

# ABSTRACT

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While 5-33% of highlanders permanently living at high altitude (> 2,500 m) worldwide develop chronic mountain sickness (CMS), the underlying mechanisms of excessive erythrocytosis (EE) and CMS symptoms remain incompletely understood. The objective of this study was to characterize blood viscosity and cardiovascular function in highlanders with and without CMS. Forty-one Andean highlanders permanently living at 5,100 m, including 15 highlanders without EE (hemoglobin concentration [Hb] < 21 g·dL<sup>-1</sup>), 13 highlanders with EE ([Hb] ≥ 21 g·dL<sup>-1</sup>) without CMS symptoms (Qingshai CMS score ≤ 5; CMS-) and 13 highlanders with EE and CMS symptoms (Qingshai CMS score 6-14; CMS+) were compared to 13 Andean healthy highlanders living at 3,800 m and 10 Caucasian healthy lowlanders living at sea level. Whole blood viscosity, standard echocardiographic parameters and microvascular reactivity assessed by near-infrared spectroscopy after ischemic reperfusion stress were investigated. Blood viscosity was very high in all highlanders compared to lowlanders, but at 5,100 m, [Hb] difference between highlanders with and without EE was not associated with differences in blood viscosity, while highlanders without EE at 5,100 m had higher blood viscosity than highlanders at 3,800 m with similar [Hb]. CMS+ had larger post-ischemic vasodilatation compared to lowlanders and highlanders at 3,800 m. Compared to lowlanders, the right atrium was enlarged in highlanders at 3,800 m and in all highlanders at 5,100 m but the cardiac function was preserved. CMS+ only had higher pulmonary pressure compared to lowlanders but not compared to all other highlanders. These results highlight blood viscosity and cardiovascular responses to chronic hypoxic exposure that may allow tolerance to permanent high-altitude residence and underlie the development of CMS.

# INTRODUCTION

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Chronic hypoxic exposure is a physiologic challenge for about 140 million individuals permanently living at high altitude (> 2,500 m) worldwide. The adaptive responses to chronic hypobaric hypoxia aims to preserved oxygen delivery to the tissues (West, 2017). The mechanisms typically involved are an increase in red blood cells and hemoglobin concentration ([Hb]) to increase arterial oxygen content (Beall, 2007) and an increase in cardiac output and regional blood flow to enhance O<sub>2</sub> supply (Moore, 2017).

However, a significant proportion of highlanders (5-33%) develops signs of maladaptation generally known as chronic mountain sickness (CMS) (Villafuerte and Corante, 2016). Based on the current recommendations, CMS is a syndrome defined by an excessive erythrocytosis (EE) ([Hb]  $\geq$  21 g·dL<sup>-1</sup> for males and  $\geq$  19 g·dL<sup>-1</sup> for females), associated with various symptoms such as breathlessness, palpitations, sleep disturbance, cyanosis, dilatation of veins, paresthesia, headache and tinnitus (León-Velarde et al., 2005). The relative loss of ventilatory adaptation is known as the main mechanism accounting for CMS (León-Velarde and Richalet, 2006) but the exact pathophysiological mechanisms underlying EE and CMS symptoms are however debated (Gonzales et al., 2013; Rivera-Ch et al., 2007).

At high altitude, arterial pulmonary hypertension is a well-known consequence of chronic hypoxia which is frequently associated with cardiorespiratory diseases such as right or left heart failure (Penaloza and Arias-Stella, 2007). However, pulmonary hypertension is not a *sine qua non* part of the definition of CMS. Recent studies investigating cardiac function in permanent high altitude dwellers did not report any clinical symptoms or echocardiographic signs of right and left heart failure in CMS patients, despite obvious increase in pulmonary arterial pressure (Dedobbeleer et al., 2015; Maignan et al., 2009), suggesting the existence of

compensatory mechanisms. Hence, studies investigating the association between CMS symptoms, EE and cardiac function are still required.

While the effect of chronic hypoxic exposure on the pulmonary circulation has been studied in detail, only recent studies reported peripheral vascular function in highlanders chronically exposed to hypoxia, with contrasting results. High-altitude native Sherpa exhibited a greater post-ischemic increase in femoral artery blood flow velocity compared to acclimatized lowlanders at 5,050 m (Schneider et al., 2001; Tremblay et al., 2018), while impairments in endothelial function evaluated by flow-mediated dilation of the brachial artery was reported in Andeans residents at high altitude (Bruno et al., 2014; Lewis et al., 2014) and in highlanders suffering from CMS compared to healthy counterparts (Rimoldi et al., 2012). Thus, changes in systemic vascular function in response to chronic hypoxic exposure among apparently well-adapted highlanders and in highlanders with CMS remain to elucidate.

Prolonged exposure to hypoxia is known to increase hematocrit and to induce hematological changes (Beall, 2006). Both continuous and intermittent hypoxia induce increase whole blood and plasma viscosity in rat models (Kang et al., 2016; Pichon et al., 2012; Yelmen et al., 2011). In hematological diseases, such as polycythemia vera, abnormal production of formed elements of the blood is associated with the well-known hyper viscosity syndrome (Stone and Bogen, 2012). EE in the context of CMS in highlanders is thought to induce substantial increase in blood viscosity which would underlie symptoms and cardiovascular complications (León-Velarde et al., 2005). Any increases in blood viscosity should also affect the vascular resistance and the cardiovascular function (Naeije and Vanderpool, 2013). Despite the assumption of higher viscosity in highlanders with EE and CMS, very few data (Kametas et al., 2004) are however available regarding blood viscosity changes in high-altitude residents and the relationship between hematocrit, blood viscosity and cardiovascular function remains to be clarified within the context of permanent high altitude residency.

Hence, this study aimed to characterize blood viscosity and cardiovascular changes in highlanders with or without EE and CMS symptoms and to clarify the relationship between EE and CMS symptoms. We hypothesized that highlanders with EE and CMS symptoms should have increased blood viscosity, that would induce cardiac dysfunction and alterations in the peripheral vascular function compared to healthy highlanders and highlanders with EE but without CMS symptoms.

# MATERIALS AND METHODS

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## Study population

Between February and June 2017, 64 men, from 18 to 60 years, were included (Fig. 1): 10 Caucasian lowlanders living at sea level (Grenoble, France, 204 m), 13 Andean highlanders permanently living at 3,800 m (Puno, Peru), and 41 Andean highlanders permanently living at 5,100 m (5,100-5,300 m, La Rinconada, Peru). Of the 41 highlanders living at 5,100 m, 15 had no EE ( $[Hb] < 21 \text{ g}\cdot\text{dL}^{-1}$ ) and 26 had EE ( $[Hb] \geq 21 \text{ g}\cdot\text{dL}^{-1}$ ). Based on the Qingshai CMS score, at 5,100 m, 13 highlanders with EE were asymptomatic (CMS score  $\leq 5$ ) (CMS-) and 13 highlanders with EE reported CMS symptoms (CMS score  $> 5$ ; CMS+), from mild (CMS score 6-10,  $n = 8$ ) to moderate (CMS score 11-14,  $n = 5$ ) severity (León-Velarde et al., 2005). Smokers and subjects with medical history of diabetes, respiratory and cardiovascular diseases were excluded. None of the lowlanders at sea level were acclimatized to high altitude at the time of the tests (no sojourn above 2,000 m over the past 3 months) and none of the highlanders reported a prolonged stay at low altitude (more than 5 days below 3,500 m) over the past 3 months. The highlanders at 3,800 m and 5,100 m were all Andean natives from high-altitude ( $> 3,800\text{m}$ ). At 5,100 m, all highlanders were working in gold mine facilities. All subjects were informed about the procedure and gave their written informed consent to participate in this study. The study was approved by the local ethics committee and performed according to the Declaration of Helsinki.



## Study Design

Each patient was assessed during a single experimental session. The Qingshai CMS score was assessed based on the following criteria: symptoms of breathlessness and/or palpitations, sleep disturbance, cyanosis, paresthesia, headache, tinnitus, dilatation of veins and hemoglobin level (León-Velarde et al., 2005). Pulsed oxygen saturation (SpO<sub>2</sub>) (OxiMax N65, Medtronic, Dublin, Ireland) and blood pressure (Digital Blood Pressure Monitor, A&D Medical, Sydney, Australia) were measured after 5 min of rest. Hematological and blood viscosity evaluations were performed on a venous blood sample collected in a EDTA tube according to previous recommendations (Baskurt et al., 2009). The cardiac function was assessed by standard transthoracic echocardiography (see below). Finally, the systemic vascular function was assessed by an ischemia-reperfusion test. The post-ischemia endothelium-dependent vasodilatation was evaluated non-invasively using near-infrared spectroscopy (NIRS) on the right brachioradialis muscle, as previously described (Gerovasili et al., 2010; McLay et al., 2016). A pneumatic cuff (Lian nano, Spengler, Aix-en-Provence, France) was positioned proximally on the right arm. After completion of a 5-min baseline phase, the cuff was manually inflated to 50 mmHg for 30 s to create a venous occlusion. Three venous occlusions, separated by 1-min recovery periods, were successively applied. Then after 3 min of recovery, a complete arterial occlusion was performed for 5 min by inflating the cuff 50 mmHg above the systolic blood pressure (~180 mmHg). At the end of the ischemia, the cuff was rapidly (< 5 s) deflated and the reperfusion phase was monitored for a period of 5 min. Near-infrared spectrometry (NIRS) parameters (see below) were measured continuously throughout the ischemia-reperfusion test (Gerovasili et al., 2010).

## **Measurement and data analysis**

### ***Hematology and blood viscosity***

[Hb] was measured *in situ* with a HemoCue system (HemoCue®Hb201+, HemoCue AB, Ängelholm, Sweden) and the hematocrit (Ht) was assessed by microcentrifugation (Hemata STAT – II, Separation Technology Inc., Sandford, USA). Blood viscosity was measured at native hematocrit using a cone-plate viscometer (Brookfield DVII with CPE40 spindle, Ametek Brookfield, Middleborough, USA), according to the guidelines for hemorheological laboratory techniques (Baskurt et al., 2009). Blood viscosity was measured using a “loop” protocol: shear rate was increased from 11.25 s<sup>-1</sup> to 90 s<sup>-1</sup> and then decreased from 90 s<sup>-1</sup> to 11.25 s<sup>-1</sup> by changing shear rate every 20 seconds. Because the blood is a non-Newtonian rheofluidizing fluid, only viscosity values obtained at decreasing speed were kept for further analyses. Blood viscosity measurements were performed at room temperature (from 9°C to 21°C). In order to take into account the effect of temperature on blood viscosity, we performed additional viscosity measurements of blood samples conditioned at different temperatures in our laboratory and we calculated the blood viscosity – temperature relationship (linear relationship,  $r^2=0.98$ ) enabling the standardization of all blood viscosity values measured in the present study at the same temperature (17°C).

### ***Echocardiography***

Transthoracic echocardiography was performed with a portable ultrasound system (Vivid-I Portable Cardiac Ultrasound, GE Healthcare, Little Chalfont, UK), using a 1 to 3-Mhz cardiac probe. Recordings were stored for blinded offline analysis (EchoPac system, GE Healthcare, Little Chalfont, UK). Reported values represent the mean of three cycles. Left ventricular (LV) wall thickness, LV diameters and interventricular septum thickness were measured from the parasternal long-axis view with M-mode measurement. End-diastolic left and right atrium

(EDLA and EDRA respectively) and end-diastolic right ventricular surface (EDRV) were obtained using the biplane area-length formula from an apical 4-chamber view (Lang et al., 2005; Rudski et al., 2010). The LV function was assessed by LV ejection fraction (LVEF) calculated by Teicholz formula (Quinones et al., 1981). The right ventricular (RV) systolic function was assessed by measuring the right ventricular area change (RVAC) as previously described (Rudski et al., 2010). To assess left and right diastolic function, peak velocities of transmitral and transtricuspid early (E) and late (A) waves were measured using pulsed-wave Doppler technique (Nagueh et al., 2016; Rudski et al., 2010). The cardiac output was calculated from the velocity-time integral recorded by pulsed Doppler in the left ventricular outflow tract and was secondary adjusted to the body surface area to obtain cardiac index (Cheitlin et al., 2003). Tricuspid regurgitation velocity and the right ventricular outflow tract acceleration time (AT) was used to calculate systolic pulmonary artery pressure and as an index of pulmonary resistance respectively (Kitabatake et al., 1983).

### ***Near infrared spectroscopy (NIRS)***

Oxy- (HbO<sub>2</sub>) and deoxy- (HHb) hemoglobin concentration changes were measured using a two-wavelength (760 and 850 nm) NIRS device (PortaLite, Artinis Medical Systems, Nijmegen, Netherlands). Data were recorded continuously at 10 Hz. The three transmitters and the receiver were installed on the right forearm, on the *brachioradialis* muscle, with a interoptode distance of 30 mm, 40 mm and 45 mm. Theoretical and performance details of NIRS have been previously described (Ferrari et al., 2004; Gerovasili et al., 2010; McLay et al., 2016). Total hemoglobin concentration ([Hbtot]) was calculated as the sum of [HbO<sub>2</sub>] and [HHb] and reflects the tissue blood volume within the illuminated area. Ischemia-reperfusion NIRS response was characterized by changes in [HbO<sub>2</sub>] as a qualitative index of tissue oxygen consumption during the ischemic phase and by changes in [Hbtot] as a quantitative

index of changes in blood volume during the reperfusion phase. Figure 3A shows typical recordings and analyses of [Hbtot] and [HbO<sub>2</sub>]. The forearm blood flow (FBF) was assessed by the mean linear increase in [Hbtot] within the first seconds of the venous occlusions (Van Beekvelt et al., 2001). The muscular oxygen consumption at rest was evaluated by VO<sub>2m</sub>, i.e. the rate of [HbO<sub>2</sub>] reduction during arterial ischemia, and by the  $\Delta$ baseline-end [HbO<sub>2</sub>], i.e. the difference between the baseline value and the minimal value reached at the end of the ischemia phase (Van Beekvelt et al., 2001). The reperfusion phase was characterized by  $\Delta$ min-max [Hbtot], i.e. the difference between the [Hbtot] value at the end of the ischemia phase and the maximal [Hbtot] value reached during the reperfusion phase, and by the area under the curve (AUC [Hbtot]), i.e. the area under the [Hbtot] curve above baseline value during the first 3 min of the reperfusion phase (Le Roux-Mallouf et al., 2017).

### ***Statistical analysis***

Comparisons between groups were performed using a non-parametric Kruskal-Wallis test. When the analysis of variance revealed a significant difference ( $p < 0.05$ ), post-hoc Mann-Whitney U tests were applied to compare all groups to each other's with corrections for multiple comparisons. Relationships between haematological and cardiovascular parameters were evaluated by Spearman correlation coefficient ( $\rho$ ). Statistical analyses were performed using Statistica version 10 (Statsoft, Tulsa, OK, USA). All data are expressed as mean  $\pm$  SD.

# RESULTS

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## **Clinical and hematological characteristics (Table 1)**

Highlanders at 5,100 m were slightly older than lowlanders and highlanders at 3,800 m. The duration of residency at 5,100 m did not differ significantly between highlanders with or without EE and CMS symptoms. As expected, highlanders at 3,800 m and at 5,100 m had higher [Hb] and Ht and lower SpO<sub>2</sub> than lowlanders. While highlanders at 5,100 m had lower SpO<sub>2</sub> than highlanders at 3,800 m, no significant difference in SpO<sub>2</sub> was observed between highlanders with or without EE and CMS symptoms at 5,100 m. [Hb] and Ht did not differ significantly between highlanders at 3,800 m and highlanders without EE at 5,100 m, but was larger in highlanders with EE at 5,100 m. At 5,100 m, [Hb] and Ht did not differ significantly between highlanders with EE CMS- and highlanders with EE CMS+. There was no significant difference in heart rate, body mass index and systolic and diastolic blood pressures between groups.

## **Blood viscosity (Fig. 2)**

Due to technical reasons, blood viscosity could be measured at 5,100 m in only 11 highlanders without EE and 16 highlanders with EE (5 CMS+ and 11 CMS-, pooled together for analysis). At all shear rates, blood viscosity was significantly higher in highlanders at 3,800 m and highlanders at 5,100 m with or without EE, compared to lowlanders and in highlanders at 5,100 m with and without EE, compared to highlanders at 3,800 m. At 5,100 m, blood viscosity did not differ between highlanders with and without EE (Fig. 2).

### **Cardiac function (Table 2)**

At 5,100 m, echocardiography evaluations could be completed in only 11 highlanders without EE, 10 highlanders with EE CMS- and 12 highlanders with EE CMS+. Cardiac index did not differ between groups. There was neither difference in LV morphology regarding LV wall thickness, LV diameter, interventricular septum and EDLA volume, nor in LV function regarding LVEF and mitral E/A ratio. EDRA volume was significantly larger in highlanders at 3,800 m and highlanders at 5,100 m with and without EE compared to lowlanders. No significant difference was observed between groups in EDRV area, RVAC and tricuspid E/A ratio. Regarding pulmonary resistance, right ventricular outflow acceleration time was shorter in highlanders at 5,100 m, although difference was only significant between highlanders with EE CMS+ at 5,100 m and lowlanders only.

### **Vascular function (Fig. 3)**

Vascular evaluations could be assessed in all subjects at sea levels and at 3,800m, and in 11 highlanders without EE, 10 highlanders with EE CMS- and 12 highlanders with EE CMS+ at 5,100 m. No significant difference in FBF (Fig. 3B) was observed between groups. Based on  $VO_{2m}$  and  $\Delta_{\text{baseline-end}} [\text{HbO}_2]$  during the ischemic phase, muscular oxygen consumption at rest did not differ significantly between groups (Fig. 3C). During the reperfusion phase,  $\Delta_{\text{min/max}} [\text{HbTot}]$  was significantly larger in highlanders at 5,100 m with EE CMS+ compared to lowlanders and to highlanders at 3,800 m, while AUC  $[\text{HbTot}]$  was significantly increased only in highlander at 5,100 m with EE CMS+ compared to lowlanders (Fig. 3D). Lowlanders, highlanders at 3,800 m, highlanders at 5,100 m without EE and highlanders at 5,100 m with EE CMS- did not significantly differ. Similarly, no significant difference was observed between highlanders at 5,100 m with and without EE, and between highlanders with EE CMS+ and CMS-.

### **Correlations (Tables 3-4)**

When considering all subjects together (lowlanders and highlanders), SpO<sub>2</sub> was negatively correlated with [Hb], Ht and blood viscosity, and [Hb] and Ht were positively correlated with blood viscosity. Post-occlusive hyperemia assessed by NIRS was negatively correlated with SpO<sub>2</sub> and positively with [Hb] and blood viscosity especially at the highest shear rates (45 and 90 s<sup>-1</sup>). When considering highlanders at 5,100 m only, SpO<sub>2</sub> was negatively correlated with [Hb], Ht and the post-occlusive response ( $\Delta$ min/max [HbTot] only). Blood viscosity did not significantly correlate with SpO<sub>2</sub>, [Hb] and Ht but at the highest shear rates (45 and 90 s<sup>-1</sup>), it positively correlated with the post-occlusive response ( $\Delta$ min/max [HbTot] only). Among the cardiological parameters only EDRA was significantly correlated to [Hb] ( $r = 0.59$ ,  $p < 0.5$ ) and Ht ( $r = 0.55$ ,  $p < 0.05$ ) but not with blood viscosity and SpO<sub>2</sub>.

# DISCUSSION

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The aim of this study was to assess the cardiovascular function and blood viscosity features in apparently healthy highlanders and in highlanders experiencing EE with or without CMS symptoms at 5,100 m, to understand the mechanisms underlying the adaptative and maladaptive response to chronic hypoxic exposure at high altitude. Because this study is the first to investigate blood viscosity and cardiovascular parameters in a unique population living above 5,000 m in the highest city of the world (La Rinconada, Peru), healthy highlanders at 3,800 m and lowlanders at sea level were included to highlight specific mechanisms associated with permanent residence at such extreme altitude. The main results of this study are i) differences in blood viscosity between groups of highlanders are not associated with parallel changes in [Hb] and Ht, i.e. highlanders living at different altitude (3,800 m versus 5,100 m without EE) can have similar [Hb] and Ht but distinct blood viscosity, while highlanders living at identical altitude (5,100 m with or without EE) can have distinct [Hb] and Ht but similar blood viscosity, ii) despite profound effects of high altitude residence on [Hb] and Ht, highlanders exhibit relatively preserved cardiac function compared to lowlanders, and iii) highlanders with EE and CMS symptoms display a larger post-ischemic reperfusion assessed by NIRS compared to healthy highlanders and lowlanders. Altogether, these results highlight blood viscosity and vascular responses to chronic hypoxic exposure that may allow tolerance to permanent high-altitude residence but also underlie the development of CMS symptoms.



## **Blood parameters**

The diagnosis of CMS is currently based on threshold values of [Hb] and the presence of symptoms assessed by the Qingshai CMS score which allow the determination of the severity of the disease (León-Velarde et al., 2005). However, recent clinical observations suggest a dissociation between [Hb] levels and CMS symptoms, since many highlanders show high [Hb] but no symptoms and inversely others report symptoms without [Hb] over the defined threshold (Gonzales et al., 2013). A recent observational study conducted by our group (unpublished observations) in more than 1500 highlanders from La Rinconada highlights that individuals with EE report less severe CMS symptoms than individuals without EE and conversely, individuals reporting CMS symptoms have a lower average hematocrit than individuals without CMS symptoms. In the present population sample from La Rinconada, [Hb] and Ht between highlanders with and without symptoms (i.e. highlanders EE CMS+ and CMS-) did not significantly differ, confirming that the severity of the disease may not be related to [Hb]. These observations have led to consider EE as a specific pathophysiological entity or a preclinical form of CMS in individuals residing permanently at high altitude (Jiang et al., 2014; Vargas and Spielvogel, 2006). The definition of EE (e.g. [Hb]  $\geq$  21 g/dl for males and [Hb]  $\geq$  19 g/dl for females) provided by the recommendations for CMS diagnosis (León-Velarde et al., 2005) and based on previous observations in cities near 4,000 m of altitude is however debated (Gonzales et al., 2013; Rivera-Ch et al., 2007). Polycythemia is indeed a required adaptive response to long term hypoxic exposure to preserve tissue oxygen delivery and, secondly, threshold values to determine EE may depend on the altitude of residence since erythrocytosis is mainly simulated by hypoxemia, therefore depending on the altitude level. As already reported (Corante et al., 2018; De Ferrari et al., 2014), [Hb] and Ht were significantly associated with SpO<sub>2</sub> since a more severe hypoxic stress underlies larger erythropoietic response. Conversely, there was no significant difference in SpO<sub>2</sub> between

highlanders with EE CMS+ and CMS-, suggesting that the severity of CMS symptoms is not related to the severity of hypoxemia at 5,100 m.

Paradoxically, although high altitude residents are known to experience profound hematological changes with hypoxia-induced polycythemia, very few data are available regarding their blood rheological characteristics. Previous studies investigating blood viscosity in Andean women at 4,800 m (Kametas et al., 2004) and in Sherpa at 5,050 m (Tremblay et al., 2018) reported lower whole blood viscosity (4.2 – 5.6 mPa.s) than what we observed. The viscosity was however assessed at higher shear rates ( $128 \text{ s}^{-1}$  and  $225 \text{ s}^{-1}$ ) in both studies, making these results difficult to compare with the present data.

As expected, highlanders chronically exposed to hypoxia exhibited higher blood viscosity compared to sea levels individuals. Chronic hypoxic exposure in rat models was shown to increase [Hb], Ht and blood viscosity (Pichon et al., 2012; Yelmen et al., 2011). However, while it is commonly admitted that increased [Hb] and Ht are associated with higher blood viscosity at high altitude, the present results highlight a remarkable dissociation between [Hb], Ht and blood viscosity in Andean highlanders. At 5,100 m, highlanders with CMS experiencing higher [Hb] (i.e.  $\geq 21 \text{ g/dl}$ ) have similar blood viscosity than highlanders with lower [Hb] (i.e.  $19.1 \pm 1.2 \text{ g/dL}$ ). Conversely, healthy highlanders without polycythemia at 5,100 m had similar [Hb] but larger blood viscosity than individuals living at lower altitude (3,800 m). These findings strongly suggest an adaptive phenomenon to counteract the consequence of EE on blood viscosity and the presence of additional mechanisms than increased [Hb] and Ht responsible for changes in blood viscosity at high altitude.

Whole blood viscosity depends on Ht but also on the rheological properties of red blood cells (red blood cell deformability and aggregation) and plasma viscosity (Baskurt and Meiselman, 2003). Specific hemorheological changes associated with polycythemia at high altitude have been poorly studied. In rat models exposed chronically to hypoxia, greater blood viscosity and

Ht was associated with lower plasma volume, while red blood cell deformability and aggregation were not affected by hypoxia (Pichon et al., 2012). Increased plasma viscosity in rats chronically exposed to long-term intermittent hypoxia has also been reported (Yelmen et al., 2011). In Andean women permanently living at 4,370 m, higher blood viscosity was associated with increased plasma viscosity, which was partly caused by greater plasma total proteins concentration and higher fibrinogen concentration compared to women at sea levels (Kametas et al., 2004). Hypoxia, inflammation and oxidative stress are some of the mechanism able to underlie these changes, although this remains to be demonstrated. Conversely, recent studies suggested that EE might be balanced by various mechanisms to prevent hyperviscosity syndrome. In transgenic mice overexpressing erythropoietin (Vogel et al., 2003), thrombocythemia and greater deformability of red blood cells, possibly modulated by an increased proportion of juvenile erythrocytes, known to be more flexible, contributed to keep blood viscosity in the same range in mice with polycythemia and controls mice. In contrast, transgenic mice with low erythropoietin production have lower red blood cell deformability than control mice (Pichon et al., 2014). Low albumin concentration was also suggested as a compensatory response in pregnant women at high altitude to decrease blood viscosity compared to nonpregnant highlanders. The underlying mechanisms involved in changes in albumin concentration are debated (i.e. increased loss, catabolism or reduced protein intake) but there is evidence of increased urinary protein excretion due to changes in capillary permeability at high altitude (Kametas et al., 2004). Hence, further studies are required to understand the relationship between polycythemia and blood viscosity at high altitude. Other specific hemorheological parameters (i.e. plasma viscosity, proteins concentration, red blood cell rheological proprieties) should be investigated to elucidate the adaptive mechanisms involved in apparently healthy highlanders and in highlanders with CMS.

## **Pulmonary pressure and cardiac function**

Classically, chronic hypoxic exposure at high altitude induces pulmonary vasoconstriction and pulmonary artery remodeling leading to chronic increase in right heart load, right atrium enlargement, right ventricular hypertrophy and heart failure (Penaloza and Arias-Stella, 2007). Nevertheless, as already observed (Dedobbeleer et al., 2015; Huez et al., 2009; Maignan et al., 2009; Stembridge et al., 2014), we did not find any obvious signs of right and left heart failure in highlanders, even residing permanently above 5,000 m, i.e. the highest permanent population characterized by echocardiography. In accordance with previous echocardiographic studies in Andeans high-altitude permanent dwellers (Huez et al., 2009; Maignan et al., 2009), we observed a significant enlargement of the right atrium in highlanders at 3,800 m and at 5,100 m compared to lowlanders. It is currently admitted that changes in the right heart at high altitude are associated with increased pulmonary pressures due to the pulmonary hypoxic vasoconstriction (Penaloza and Arias-Stella, 2007). However, in our study, we did not measure a significant increase in pulmonary resistance (i.e. AT) in highlanders compared to lowlanders and enlarged right atrium at high altitude was not correlated with hypoxemia (i.e. SpO<sub>2</sub>). As already demonstrated in rat models chronically exposed to hypoxia (Schreier et al., 2014), right atrium enlargement was significantly correlated with [Hb] and Ht, confirming the independent contribution of blood parameters in right heart remodeling at high altitude.

An interesting observation in the present study is the relatively comparable AT in healthy highlanders at 5,100 m compared to Andean highlanders living at lower altitude between 4,000 m and 4,300m ( $116 \pm 16$  and  $127 \pm 21$  ms respectively) (Huez et al., 2009; Maignan et al., 2009). This result suggests that well-adapted Andeans highlanders don't have exaggerated hypoxic pulmonary vasoconstriction possibly by mechanisms such as increased local

production of nitric oxide as already observed in Tibetan dwellers permanently living at high altitude (Beall, 2007; Groves et al., 1993).

In Andeans experiencing CMS, previous studies reported an increase in artery pulmonary pressure, an enlarged right atrium and right ventricular in highlanders with EE and CMS symptoms compared to healthy highlanders at the same altitude (Dedobbeleer et al., 2015; Maignan et al., 2009; Pratali et al., 2013). The present results do not support these previous observations since we did not highlight significant difference in AT and cardiac function between highlanders suffering from EE and CMS symptoms and those without symptoms at rest. Hence, these data further support that high-altitude pulmonary hypertension and CMS are two different pathophysiological entities (León-Velarde et al., 2005; Rivera-Ch et al., 2007). However, Pratali and al. (Pratali et al., 2013) showed that pulmonary pressures were significantly higher during exercise in patients with EE and CMS than in healthy highlanders. Therefore, further studies should investigate pulmonary pressure during exercise and its cardiac consequences in highlanders with or without EE and CMS symptoms, both at rest and during exercise.

## **Vascular function**

While several earlier studies on high altitude populations addressed the effect of chronic hypoxic exposure on the pulmonary circulation, only some recent studies reported evaluations of peripheral vascular function in highlanders with contrasting results.

Blood pressure has been shown previously to be either well preserved in highlanders with or without CMS (Dedobbeleer et al., 2015; Moore et al., 2006; Rimoldi et al., 2012), either increased in highlanders experiencing CMS compared to healthy highlanders (Richalet et al., 2005). Despite very high [Hb], Ht and blood viscosity in highlanders with CMS, we did not observe any significant difference in blood pressure between groups.

Impaired endothelial function was reported in apparently well-adapted highlanders in Nepal in two studies showing a reduce flow-mediated dilatation (FMD) of the brachial artery compared to lowlanders at sea level (Bruno et al., 2014; Lewis et al., 2014) and in Andean CMS patients at 3,600 m compared to healthy highlanders at the same altitude, (Bailey et al., 2013; Rimoldi et al., 2012) suggesting a significant systemic vascular dysfunction. Conversely, studies in Himalayan dwellers (Schneider et al., 2001; Tremblay et al., 2018) showed that high-altitude native Sherpa have a greater post-ischemia femoral artery blood flow velocity increase at 5,050 m compared to acclimatized lowlanders. In our study, the post-ischemic vasodilatation was in the same range in healthy highlanders at 3,800 m, at 5,100 m and in lowlanders at sea levels. We observed however a larger increase in [HbTot] during the reperfusion phase in highlanders with EE and CMS symptoms at 5,100 m compared to lowlanders and highlanders at 3,800 m, suggesting a greater flow-mediated hyperemic response.

These contrasting results may be related to the use of various techniques to assess vascular function. Measuring the increase of the brachial artery diameter by ultrasound during a FMD protocol (Bailey et al., 2013; Bruno et al., 2014; Rimoldi et al., 2012) investigated the macrovascular system, while we assessed changes in the microvascular system by recording local changes in muscle [Hb] with NIRS. Even if previous results indicated a good correlation between FMD ultrasound and NIRS parameters (Kragelj et al., 2001) during an ischemia-reperfusion test, it has been recently suggested that microvascular dysfunction assessed by NIRS may be more sensitive to the cardiovascular risk (Gayda et al., 2015).

In the previous studies highlighting an impaired endothelial function in healthy highlanders (Bailey et al., 2013; Lewis et al., 2014) and in highlanders with CMS (Rimoldi et al., 2012), the vascular dysfunction was associated with an enlarged vessel diameter at baseline. It was suggested that highlanders and CMS patients may present a chronically vasodilated macrocirculation, possibly due to tonically elevated nitric oxide production and a

parasympathetic predominance in the sympatho-vagal balance, allowing an increased regional blood flow to compensate for the reduced arterial oxygen content (Erzurum et al., 2007; Gilbert-Kawai et al., 2014; Lewis et al., 2014). This chronic vasodilation may in turn reduce the reserve for vasodilation and therefore leads to a reduced post-ischemic FMD (Bruno et al., 2014; Lewis et al., 2014). In our study however, the local blood flow at rest did not differ between groups (Fig. 3B), which does not support this hypothetical mechanism.

The macrovascular vascular changes at high altitude are likely due to mechanisms triggered by hypoxemia. Oxygen inhalation improved FMD in hypoxemic highlanders and in patient with CMS at 3,600 m (La Paz) (Rimoldi et al., 2012) and a positive relationship between arterial oxygen content and FMD has been reported previously (Lewis et al., 2014; Rimoldi et al., 2012). Conversely, we observed a larger post-occlusive vasodilatation in the most hypoxemic subjects, i.e. highlanders with EE and CMS symptoms at 5,100 m. Post-ischemic  $\Delta_{\text{min/max}} [\text{HbTot}]$  was statistically negatively correlated with  $\text{SpO}_2$  in all subjects pooled together and in highlanders at 5,100 m only. In agreement with our results, Martin and al. (Martin et al., 2013) reported that lower tissue oxygen saturation, assessed by NIRS, during vascular occlusion was associated with a greater recovery slope during the reperfusion phase in lowlanders acutely exposed to hypoxia, suggesting an adaptive mechanism to counterbalance oxygen availability deficiency. These data suggest a coupling mechanism of metabolic and microvascular functions: a larger post-occlusive blood flow may support tissue oxygenation through an increase in oxygen supply, especially in hypoxemic highlanders with CMS symptoms.

Very few studies have assessed the effect of [Hb] changes on the post-occlusive response. In diabetic (Sonmez et al., 2010) and hypertensive (Maio et al., 2011) patients, higher [Hb] was correlated to endothelial impairment measured by FMD, while, in Andean highlanders with EE and CMS symptoms, the macrovascular dysfunction was not improved by isovolumic

hemodilution decreasing [Hb] and Ht (Rimoldi et al., 2012). To the best of our knowledge, no data are available regarding changes in [Hb] and Ht and the microvascular function assed by NIRS. In our study, the post-ischemia vasodilatation was weakly associated with [Hb] when considering all subjects together and did not correlate with the hematological parameters among highlanders at 5,100 m only, suggesting that the larger post-ischemic response observed in highlanders CMS+ was most probably independent of the large [Hb] and Ht values observed in subjects with EE.

One could suggest that the increase in vascular shear stress caused by the very high blood viscosity in highlanders with EE and CMS symptoms could be involved in the enlarged post-occlusive vasodilatation observed in these subjects. Shear stress depends on viscosity and is significantly correlated with FMD (Gnasso et al., 2001). Several studies have reported that any increase in blood viscosity could promote greater vasodilation due to its effects on vascular shear stress and nitric oxide production (Connes et al., 2009; Tsai et al., 2005). Unfortunately, we were not able to assess the blood viscosity separately in the subgroups of highlanders with and without CMS symptoms, but the post ischemic responses, measured by  $\Delta_{\text{min/max}} [\text{HbTot}]$ , was significantly correlated with blood viscosity at the two highest shear rates (i.e. 45 and 90  $\text{s}^{-1}$ ) in highlanders at 5,100 m. Even if the exact hemorheological alteration *in vivo* are difficult to predict by *in vitro* assessment (Baskurt et al., 2004), blood viscosity measured at high shear rates tends to reflect the hemorheological phenomena in the capillaries associated with red blood cells deformability (Baskurt and Meiselman, 2003). Moreover, due to axial migration of the erythrocytes in blood vessels, influenced by the red blood cells aggregation, the viscosity is known to be heterogeneous throughout the vasculature (Baskurt et al., 2004; Lipowsky and Firrell, 1986). Thus, biophysical properties of red blood cells (i.e. deformability and aggregation) could affect the microvascular adaptative response to chronic hypoxic exposure and the development of CMS symptoms.



Nitric oxide metabolism is probably one of the key factors involved in cardiovascular and hemorheological changes at high altitude. During hypoxic exposure, changes in nitric oxide metabolism, that plays an essential roles in vascular tone, has been described in both pulmonary vasculature (Beall et al., 2012) and systemic circulation (Bailey et al., 2013; Rimoldi et al., 2012). Additionally, the endothelial production of nitric oxide is regulated by a variety of factors including hypoxia, blood parameters and shear stress. Recently, an animal study reported a significant association between high-viscosity plasma and increased perivascular nitric oxide production (Tsai et al., 2005). Furthermore, nitric oxide has been reported to reduce red blood cells aggregation and increase deformability of erythrocytes (Starzyk et al., 1999). All together these data suggest a closed relationship between vascular function and hemorheological proprieties trough NO bioavailability in highlanders chronically exposed to hypoxia and in individuals developing CMS symptoms and EE. Future studies should consider NO metabolism and bioavailability together with hemorheological and vascular evaluations at high altitude.

## **Limitations**

Because of obvious logistical issues in remote territories at high altitude, performing hemorheological and cardiovascular investigations in the highest city of the world is challenging. As a consequence, the sample size was relatively small and therefore a lack of statistical power may have influenced some of our results.

Currently, the data available regarding the vascular function at high altitude are quite contrasting. The vascular function is a complex system tightly regulated along the vascular tree and in the various organs. Local microvascular impairment in the arm has been however described even though the endothelial function was preserved in the upper limb in Sherpas (Tremblay et al., 2018). Additionally, it is also well known that regional blood flow is

specifically modulated according to hypoxemia and local metabolism, e.g. in the brain (Ainslie and Subudhi, 2014). In our study, we only assessed the microvascular function in one area, i.e. the upper limb. To elucidate the vascular responses to chronic hypoxia in high-altitude dwellers, both the micro- and macrocirculations need to be carefully investigated simultaneously in various organs, e.g. brain, muscle and lungs, using high-standard techniques, associated with pharmacological modulation (e.g. NO donors, sublingual nitroglycerin and acetylcholine) to highlight the roles of the endothelium function and the sympathetic tonus in vascular impairments.

Finally, the potential impact of toxic exposure in mining facilities remains unclear and difficult to evaluate. Previous studies observed a significant association between polycythaemia and increase in Zinc, Lead and Cobalt blood concentration (Gonzales et al., 2011; Jefferson et al., 2002). Mining at altitude has also been reported to accelerate pulmonary disease, like silicosis and other pneumoconiosis, known to worsen hypoxemia and to affect the cardiopulmonary function (Vearrier and Greenberg, 2011). The pathogenic role of environmental factors in EE and CMS onset is probably insufficiently considered and should be investigated in future studies.

# CONCLUSION

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The present study investigated for the first time the concomitant changes in blood viscosity, peripheral vascular function and cardiac function in apparently healthy highlanders and highlanders with EE and CMS symptoms, chronically exposed to hypobaric hypoxia at high altitude. Strikingly, no direct relationship between changes in [Hb] and whole blood viscosity was observed when comparing groups of highlanders residing at distinct altitudes and with or without EE. Moreover, highlanders at 5,100 m with EE and CMS symptoms exhibited the largest post-ischemia hyperemic response assessed by NIRS, i.e. paradoxically suggesting an improved microvascular endothelial function. These results suggest that highlanders probably develop adaptative mechanisms enabling them to tolerate extremely high [Hb]. Further studies are required to clarify the relationships between chronic hypoxic exposure, hematological responses and cardiovascular changes in highlanders and in the context of CMS.


## CONCLUSION

Thèse soutenue par Emmanuelle LOYRION

### Viscosité sanguine et fonction cardio-vasculaire chez des résidents de haute altitude

While 5-33% of highlanders permanently living at high altitude (> 2,500 m) worldwide develop chronic mountain sickness (CMS), the underlying mechanisms of excessive erythrocytosis (EE) and CMS symptoms remain incompletely understood. The objective of this study was to characterize blood viscosity and cardiovascular function in highlanders with and without CMS. Forty-one Andean highlanders permanently living at 5,100 m, including 15 highlanders without EE (hemoglobin concentration [Hb] < 21 g·dL<sup>-1</sup>), 13 highlanders with EE ([Hb] ≥ 21 g·dL<sup>-1</sup>) without CMS symptoms (Qingshai CMS score ≤ 5; CMS-) and 13 highlanders with EE and CMS symptoms (Qingshai CMS score 6-14; CMS+) were compared to 13 Andean healthy highlanders living at 3,800 m and 10 Caucasian healthy lowlanders living at sea level. Whole blood viscosity, standard echocardiographic parameters and post-ischemia reperfusion assessed by near-infrared spectroscopy were investigated. At 5,100 m, [Hb] difference between highlanders with and without EE was not associated with differences in blood viscosity, while highlanders without EE at 5,100 m had higher blood viscosity than highlanders at 3,800 m with similar [Hb]. Regarding the vascular function, CMS+ had larger post-ischemic vasodilatation, compared to lowlanders and highlanders at 3,800 m. Compared to sea level, the right atrium was enlarged in highlanders at 3,800 m and in all highlanders at 5,100m but the cardiac function was preserved. CMS+ only had higher pulmonary pressure compared to lowlanders but not compared to all other highlanders. These results highlight hemorheological and cardiovascular responses to chronic hypoxic exposure that may allow tolerance to permanent high-altitude residence and underlie the development of CMS.

*Vu et permis d'imprimer*  
Grenoble, le 7/11/18

  
Le Doyen,  
Pr Patrice MORAND

Pour le Président  
et par délégation  
—  
Le Doyen de Médecine  
Pr. Patrice MORAND

Le Président de thèse,  
Pr Jean-Francois PAYEN



# **TABLES AND FIGURES**

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**Table 1. Clinical and hematological characteristics.**

	<b>Lowlanders at SL (n = 10)</b>	<b>Highlanders at 3,800 m (n = 13)</b>	<b>Highlanders without EE at 5,100m (n = 15)</b>	<b>Highlanders with EE CMS- at 5,100m (n = 13)</b>	<b>Highlanders with EE CMS+ at 5,100m (n = 13)</b>
<b>[Hb] (g·dL<sup>-1</sup>)</b>	14.3 ± 0.9	18.3 ± 1.3 *	19.1 ± 1.2 *	22.6 ± 1.5 * † †	23.5 ± 1.7 * † †
<b>Hematocrit (%)</b>	41.9 ± 2.5	57.0 ± 4.7 *	58.2 ± 4.9 *	69.8 ± 5.9 * † †	70.1 ± 7.8 * † †
<b>SpO<sub>2</sub> (%)</b>	98.1 ± 1.0	91.6 ± 1.4 *	83.8 ± 4.5 * †	83.1 ± 3.6 * †	80.1 ± 3.3 * †
<b>CMS score</b>	0.4 ± 0.7	0.5 ± 0.8	0.6 ± 0.8	3.2 ± 1.3 * † †	9.4 ± 3.0 * † † #
<b>Age (years)</b>	32.8 ± 7.2	28.8 ± 11.4	37.7 ± 10.1 *	35.1 ± 8.1	40.8 ± 7.2 †
<b>Duration of stay (years)</b>	-	-	11.6 ± 8.9	7.3 ± 6.9	10.3 ± 6.3
<b>BMI (kg·m<sup>-2</sup>)</b>	22.8 ± 1.7	26.5 ± 3.2	25.9 ± 3.1	25.6 ± 3.1	26.3 ± 2.9
<b>Heart rate (bpm)</b>	69.2 ± 13.0	71.9 ± 11.8	80.2 ± 8.6	81.3 ± 11.5	85.8 ± 9.5
<b>Systolic BP (mmHg)</b>	121.7 ± 9.2	110.8 ± 7.7	120.4 ± 10.7	116.5 ± 9.6	114.5 ± 11.3
<b>Diastolic BP (mmHg)</b>	70.4 ± 8.2	72.3 ± 8.7	81.5 ± 8.0	76.5 ± 9.8	76.2 ± 8.5
<b>Mean BP (mmHg)</b>	81.6 ± 8.0	85.1 ± 7.9	94.5 ± 8.5 *	89.8 ± 7.9	88.7 ± 8.9

Results are mean ± SD. BP, blood pressure; BMI, body mass index; CMS, chronic mountain sickness; EE, excessive erythrocytosis; [Hb], hemoglobin concentration; SL, sea level.

\* significantly different compared to lowlanders, † significantly different compared to highlanders at 3,800 m, + significantly different compared to highlanders without EE, # significantly different compared to EE CMS-

**Table 2. Echocardiographic measurements.**

	<b>Lowlanders at SL (n = 10)</b>	<b>Highlanders at 3,800 m (n = 13)</b>	<b>Highlanders without EE at 5,100m (n = 11)</b>	<b>Highlanders with EE CMS- at 5,100m (n = 10)</b>	<b>Highlanders with EE CMS+ at 5,100m (n = 12)</b>
<b>Cardiac Index</b> (L·min <sup>-1</sup> ·m <sup>-2</sup> )	2.4 ± 0.5	2.8 ± 0.6	3.1 ± 0.7	2.8 ± 0.7	3.1 ± 1.1
<b>LV ejection fraction</b> (%)	74.9 ± 7.9	69.5 ± 13.6	67.8 ± 6.3	64.7 ± 9.9	71.1 ± 7.7
<b>Interventricular septum</b> (cm)	1.1 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	1.2 ± 0.2
<b>LV wall thickness</b> (cm)	1.1 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	1.1 ± 0.2
<b>LV diameter</b> (cm)	4.5 ± 0.5	4.2 ± 0.6	4.2 ± 0.6	4.4 ± 0.6	4.5 ± 0.4
<b>EDLA volume</b> (mL·m <sup>-2</sup> )	30.1 ± 8.1	34.5 ± 13.1	30.1 ± 10.3	34.5 ± 16.2	28.6 ± 11.9
<b>Mitral E/A ratio</b>	1.6 ± 0.4	1.5 ± 0.4	1.4 ± 0.5	1.3 ± 0.2	1.4 ± 0.3
<b>EDRA volume</b> (mL·m <sup>-2</sup> )	25.2 ± 8.4	57.2 ± 19.2 *	59.6 ± 26.5 *	56.1 ± 16.2 *	63.8 ± 17.4 *
<b>EDRV area</b> (cm <sup>2</sup> )	17.7 ± 2.0	17.6 ± 3.0	18.3 ± 3.4	19.5 ± 3.3	18.5 ± 4.6
<b>RV Area Change</b> (%)	43.0 ± 5.2	46.5 ± 6.8	46.7 ± 8.1	40.9 ± 5.7	42.1 ± 5.6
<b>Tricuspid E/A ratio</b>	1.6 ± 0.6	1.7 ± 0.4	1.6 ± 0.5	1.4 ± 0.4	1.4 ± 0.3
<b>Acceleration time</b> (ms)	145.3 ± 9.4	130.4 ± 11.8	119.6 ± 22.4	118.2 ± 19.4	113.3 ± 16.4 *

Results are mean ± SD. EDLA, end-diastolic left atrium; EDRA, end-diastolic right atrium; EDRV, end-diastolic; LV, left ventricle; RV, right ventricular; see Table 1 for other abbreviations). \* significantly different compared to lowlanders, † significantly different compared to highlanders at 3800 m, + significantly different compared to highlanders without EE, # significantly different compared to highlanders with EE CMS-

**Table 3. Correlation between hematological characteristics, viscosity and post-occlusion hyperemia in all subjects**

	SpO <sub>2</sub> (%)	[Hb] (g·dL <sup>-1</sup> )	Ht (%)	Viscosity at 11.25 s <sup>-1</sup> (mPa.s)	Viscosity at 22.5 s <sup>-1</sup> (mPa.s)	Viscosity at 45 s <sup>-1</sup> (mPa.s)	Viscosity at 90 s <sup>-1</sup> (mPa.s)
SpO <sub>2</sub> (%)	-						
[Hb] (g·dL <sup>-1</sup> )	<b>-0.89</b>	-	-				
Ht (%)	<b>-0.87</b>	-	-				
Viscosity at 11.25 s <sup>-1</sup> (mPa.s)	<b>-0.73</b>	<b>0.70</b>	<b>0.70</b>	-	-	-	-
Viscosity at 22.5 s <sup>-1</sup> (mPa.s)	<b>-0.79</b>	<b>0.79</b>	<b>0.78</b>	-	-	-	-
Viscosity at 45 s <sup>-1</sup> (mPa.s)	<b>-0.80</b>	<b>0.83</b>	<b>0.81</b>	-	-	-	-
Viscosity at 90 s <sup>-1</sup> (mPa.s)	<b>-0.82</b>	<b>0.86</b>	<b>0.85</b>	-	-	-	-
AUC [Hbtot] (μmol·s)	<b>-0.42</b>	<b>0.40</b>	0.30	0.27	0.34	<b>0.41</b>	<b>0.47</b>
Δmin/max [Hbtot] (μmol <sup>-1</sup> )	<b>-0.53</b>	<b>0.45</b>	0.35	<b>0.36</b>	<b>0.44</b>	<b>0.52</b>	<b>0.56</b>

Results are Spearman correlation coefficient (rho). [Hb], hemoglobin concentration; Ht, Hematocrit; [Hbtot], total hemoglobin concentration. Bold values are statistically significant correlation (p < 0.05)



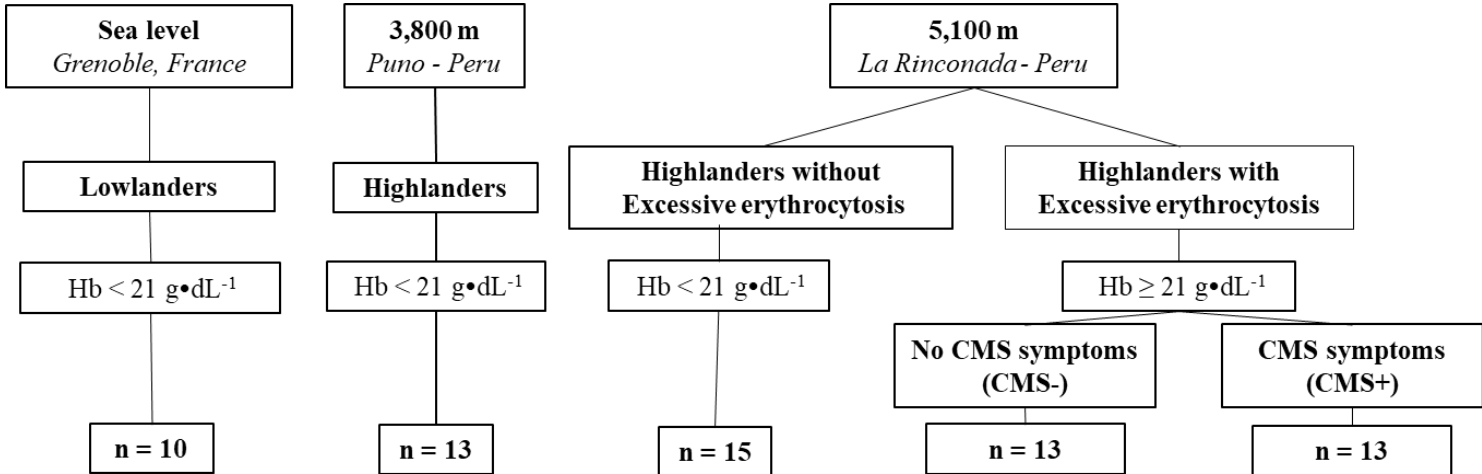
**Table 4. Correlation between hematological characteristics, viscosity and post-occlusion hyperemia in highlanders at 5,100 m**

	SpO <sub>2</sub> (%)	[Hb] (g·dL <sup>-1</sup> )	Ht (%)	Viscosity at 11.25 s <sup>-1</sup> (mPa.s)	Viscosity at 22.5 s <sup>-1</sup> (mPa.s)	Viscosity at 45 s <sup>-1</sup> (mPa.s)	Viscosity at 90 s <sup>-1</sup> (mPa.s)
<b>SpO<sub>2</sub> (%)</b>	-						
<b>[Hb] (g·dL<sup>-1</sup>)</b>	<b>-0.67</b>	-	-				
<b>Ht (%)</b>	<b>-0.67</b>	-	-				
<b>Viscosity at 11.25 s<sup>-1</sup> (mPa.s)</b>	0.10	-0.14	0.10	-	-	-	-
<b>Viscosity at 22.5 s<sup>-1</sup> (mPa.s)</b>	-0.12	0.11	0.20	-	-	-	-
<b>Viscosity at 45 s<sup>-1</sup> (mPa.s)</b>	-0,23	0.34	0.37	-	-	-	-
<b>Viscosity at 90 s<sup>-1</sup> (mPa.s)</b>	0.33	0.48	0.48	-	-	-	-
<b>AUC [Hbtot] (μmol·s)</b>	-0.36	0.37	0.16	0.10	0.18	0.35	0.48
<b>Δmin/max [Hbtot] (μmol<sup>-1</sup>)</b>	<b>-0.59</b>	0.51	0.35	0.19	0.41	<b>0.62</b>	<b>0.68</b>

Results are Spearman correlation coefficient (rho). [Hb], hemoglobin concentration; Ht, Hematocrit; [Hbtot], total hemoglobin concentration. Bold values are statistically significant correlation (p < 0.05)

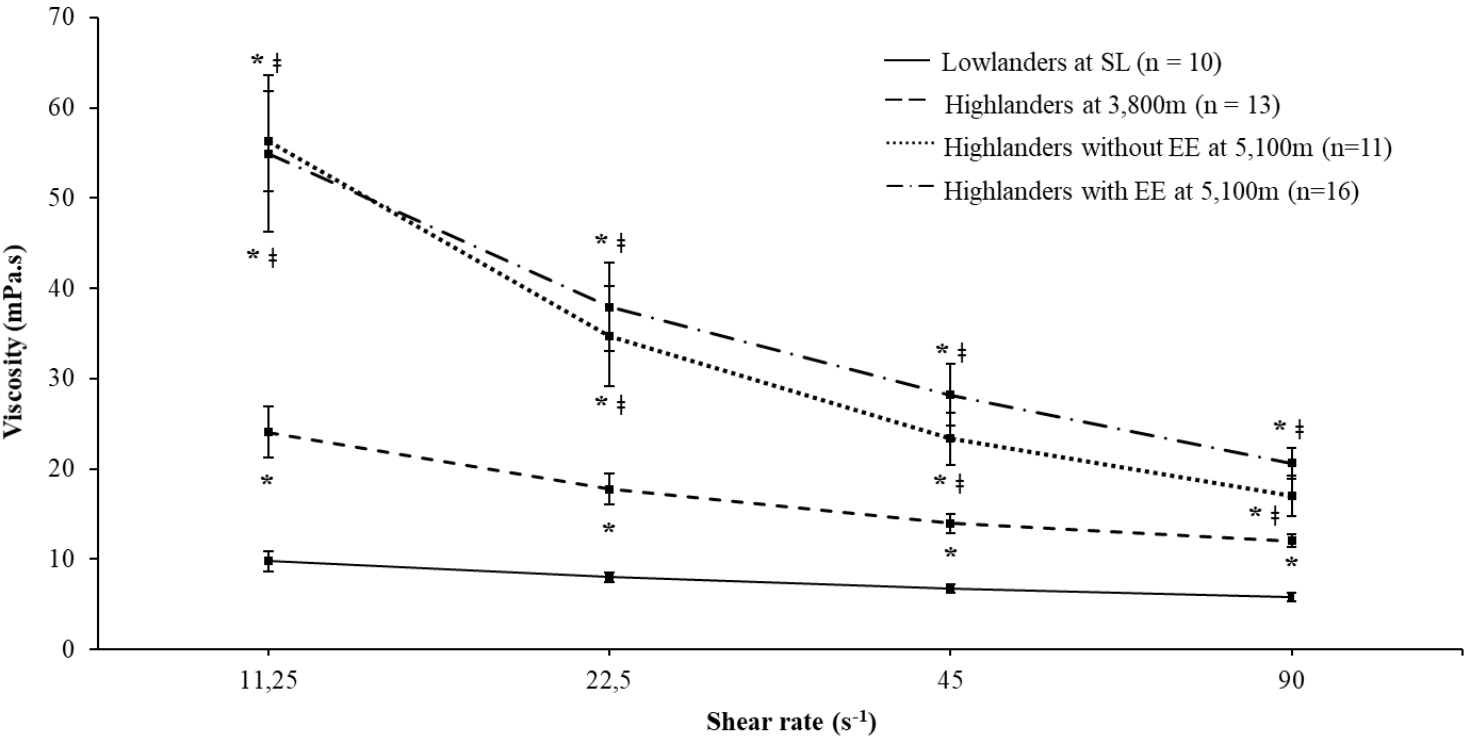
**Figure 1. Flow chart.**

CMS, Chronic Mountain Sickness; Hb, Hemoglobin concentration.



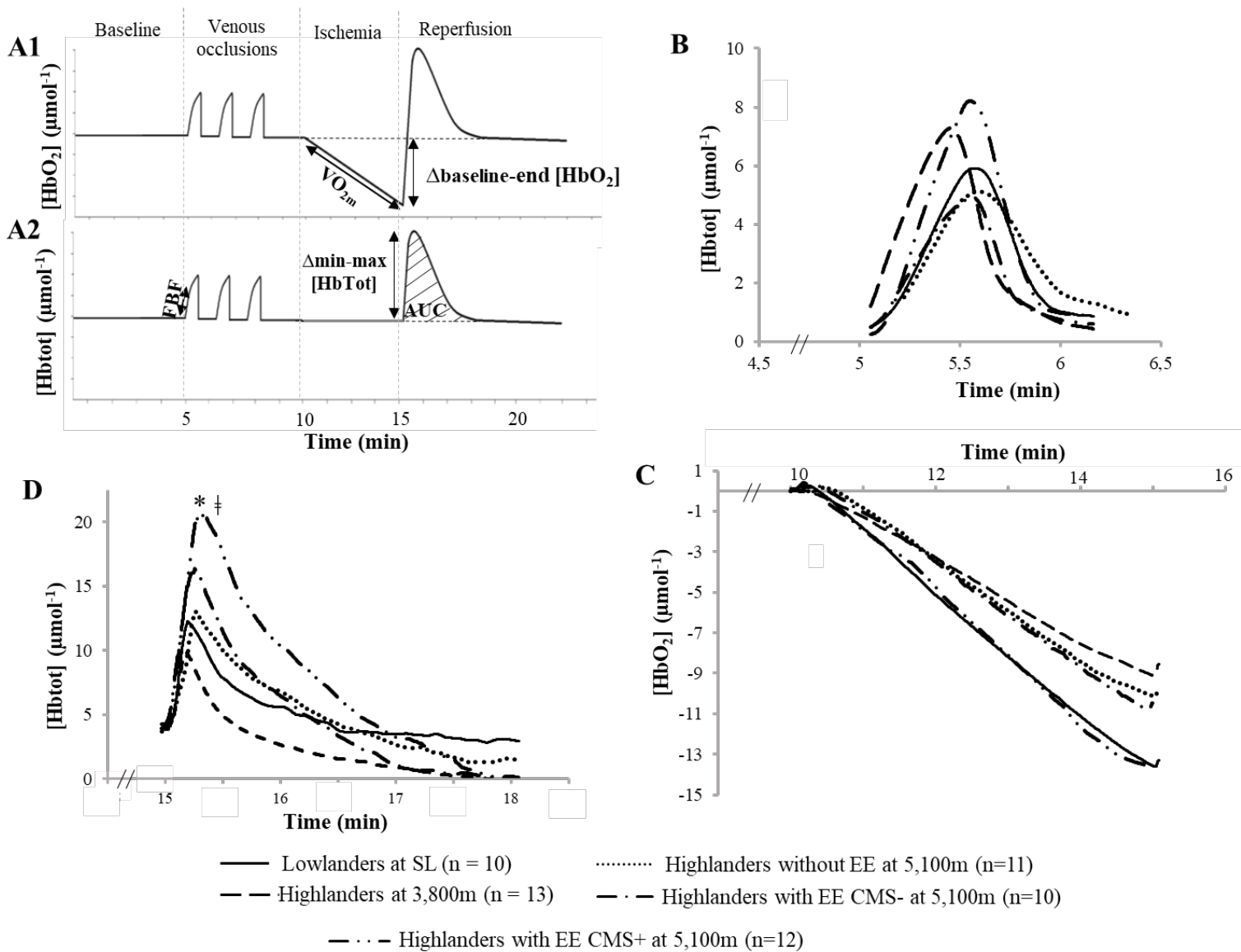
**Figure 2. Blood viscosity measured at different shear stress levels.**

EE, excessive erythrocytosis; SL, sea level. \* significantly different compared to lowlanders, † significantly different compared to highlanders at 3,800 m.



### Figure 3. NIRS recordings during the ischemia-reperfusion test.

Typical changes in oxyhemoglobin ( $[HbO_2]$ ) (Panel A1) and total haemoglobin concentration ( $[Hbtot]$ ) (Panel A2). Changes in  $[Hbtot]$  during the venous occlusions phase (Panel B) and during the reperfusion phase following complete ischemia (Panel D). Changes in  $[HbO_2]$  during the complete ischemia phase (Panel C). \* significant difference between Lowlanders at SL and Highlanders with EE CMS + at 5,100m for  $\Delta$ min-max  $[Hbtot]$  and AUC  $[Hbtot]$ , † significant difference between Highlanders at 3,800m and Highlanders with EE CMS + at 5,100m for  $\Delta$ max/min  $[Hbtot]$ .



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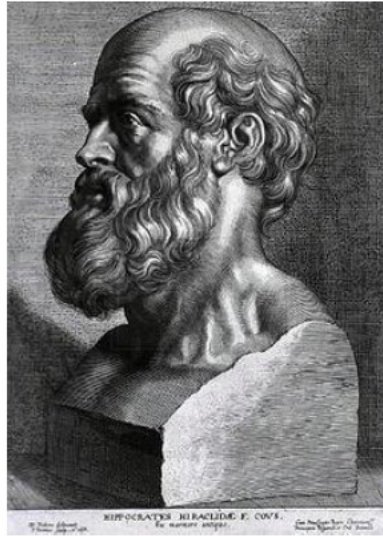
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*Je ne permettrai pas que des considérations de religion, de nation, de race, de parti ou de classe sociale viennent s'interposer entre mon devoir et mon patient.*

*Je garderai le respect absolu de la vie humaine.*

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