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Blood viscosity and its determinants in the highest city in the world

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Key points

- Highlanders develop unique adaptative mechanisms to chronic hypoxic exposure, including substantial haemoglobin and haematocrit increases.
- However, a significant proportion of populations living permanently at high altitude develop maladaptive features known as chronic mountain sickness (CMS).
- This study aimed to assess the effects of permanent life at high altitude on clinical and haemorheological parameters (blood viscosity and red blood cell aggregation) and to compare clinical and haemorheological parameters of dwellers from the highest city in the world according to CMS severity.
- Blood viscosity increased with altitude, together with haemoglobin concentration and haematocrit. At 5100 m, highlanders with moderate-to-severe CMS had higher blood viscosity mainly at high shear rate and even at corrected haematocrit (40%), with a lower red blood cell aggregation.
- Blood viscosity may contribute to CMS symptomatology but the increased blood viscosity in CMS patients cannot solely be explained by the rise in haematocrit.

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Abstract Chronic mountain sickness (CMS) is a condition characterised by excessive erythrocytosis (EE). While EE is thought to increase blood viscosity and subsequently to trigger CMS symptoms, the exact relationship between blood viscosity and CMS symptoms remains incompletely understood. We assessed the effect of living at high altitude on haemoglobin, haematocrit and haemorheological parameters (blood viscosity and red blood cell aggregation), and investigated their relationship with CMS in highlanders living in the highest city in the world (La Rinconada, Peru, 5100 m). Ninety-three men participated in this study: 10 Caucasian lowlanders, 13 Andean highlanders living at 3800 m and 70 Andean highlanders living at 5100 m (35 asymptomatic, CMS score $\leq$5; 15 with mild CMS, CMS score between 6 and 10; 20 with moderate-to-severe CMS, CMS score >10). Blood viscosity was measured at native and corrected haematocrit (40%). Haemoglobin concentration and haematocrit increased with the altitude of residency. Blood viscosity also increased with altitude (at 45 s^{-1}: 6.7 $\pm$ 0.9 mPa s at sea level, 14.0 $\pm$ 2.0 mPa s at 3800 m and 27.1 $\pm$ 8.8 mPa s at 5100 m; $P < 0.001$). At 5100 m, blood viscosity at corrected haematocrit was higher in highlanders with moderate-to-severe CMS (at 45 s^{-1}: 18.9 $\pm$ 10.7 mPa s) than in highlanders without CMS (10.2 $\pm$ 5.9 mPa s) or with mild CMS (12.1 $\pm$ 6.1 mPa s) ($P < 0.05$). In conclusion, blood viscosity may contribute to CMS symptomatology but the increased blood viscosity in CMS patients cannot solely be explained by the rise in haematocrit.

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Introduction

Chronic hypoxic exposure is a physiological challenge for about 140 million individuals permanently living at high altitude (>2500 m) worldwide (Moore, 2017). The adaptive responses to chronic hypobaric hypoxia aim to preserve oxygen delivery to the tissues (West, 2017). The mechanisms typically involved are an increase in red blood cells and haemoglobin concentration ([Hb]) in order to increase arterial oxygen content (Beall, 2007).

However, a significant proportion of highlanders (5–33%) develops signs of maladaptive features to chronic hypoxia generally known as chronic mountain sickness (CMS) (Villafuerte & Corante, 2016). Based on the current consensus, CMS is a syndrome defined by excessive erythrocytosis (EE) ([Hb] $\geq$21 g dl^{-1} and $\geq$19 g dl^{-1} for males and females, respectively), associated with various signs and symptoms such as breathlessness, palpitations, sleep disturbance, cyanosis, dilatation of veins, paraesthesia, headache and tinnitus (León-Velarde *et al.* 2005). Relative hypoventilation as well as specific hormonal, cellular and genetic factors may promote CMS (León-Velarde & Richalet, 2006; Zhou *et al.* 2013; Villafuerte & Corante, 2016; Bermudez *et al.* 2020) but the exact pathophysiological mechanisms underlying EE and CMS symptoms still remain debated.

Prolonged exposure to hypoxia is known to increase haematocrit and induce haematological changes (Beall, 2007). Furthermore, both continuous and intermittent hypoxia increase whole blood and plasma viscosity in rat models (Yelmen *et al.* 2011; Pichon *et al.* 2012; Kang *et al.* 2016). Tremblay and colleagues (Tremblay, 2019; Tremblay *et al.* 2019) recently reported that Andeans living in Cerro de Pasco (Peru, 4330 m) with EE had higher blood viscosity than those without EE, and that blood hyperviscosity partly contributed to the decreased flow-mediated dilatation observed in highlanders with EE. In addition, the authors reported that decreasing blood viscosity by isovolaemic haemodilution improved vascular function in individuals with EE. However, they only focused on a population with mild CMS (mean Qinghai CMS score of 7 $\pm$ 3) and it remains to be clarified whether blood hyperviscosity underlies the severity of CMS. Pichon *et al.* (2012) previously demonstrated that the use of acetazolamide, a drug used for the treatment of CMS in high altitude residents (Richalet *et al.* 2005), lowered blood viscosity and subsequently attenuated pulmonary and systemic vascular resistance in rats chronically exposed to hypoxia (>5500 m). These findings suggest that blood hyperviscosity could contribute, at least in part, to the maladaptive features found in some high-altitude residents. However, although blood viscosity is affected by haematocrit/haemoglobin level, there is no clear direct relationship between these two parameters since an increase in haematocrit/haemoglobin level does not always result in a rise in blood viscosity (Lemonne *et al.* 2015, 2017; Connes *et al.* 2018).

The first aim of this study was to assess the effects of permanent life at high altitude on clinical and haemorheological parameters (blood viscosity and red blood cell aggregation). Since this study is the first to investigate blood viscosity in a unique population living

above 5000 m in the highest city in the world (La Rinconada, Peru), healthy highlanders at 3800 m and lowlanders at sea level were included to highlight the specific mechanisms associated with permanent life at such an extreme altitude. We hypothesized that blood viscosity would progressively increase with the altitude of residency in highlanders when compared with lowlanders. The second aim of this study was to compare clinical and haemorheological parameters of dwellers from La Rinconada according to CMS severity, in order to clarify the mechanisms underlying the maladaptive features associated with chronic hypoxic exposure at high altitude. We hypothesized that blood viscosity would increase with the severity of CMS.

Methods

Ethical approval

The study was approved by the ethics committee of Universidad Nacional Mayor de San Marcos (CIEI-2019-002) and performed according to the *Declaration of Helsinki*. This study is part of a larger research programme (Expedition 5300) investigating the pathophysiological consequences of living permanently at high altitude (Enserink, 2019).

Population

Ninety-three men between 18 and 60 years of age were included in this study: 10 Caucasian lowlanders living at sea level (Grenoble, France, 204 m), 13 Andean highlanders permanently living at 3800 m (Puno, Peru), and 70 Andean highlanders permanently living at 5100 m (La Rinconada, Peru), among which 35 were asymptomatic (CMS score ≤5; CMS−), 15 reported mild CMS (CMS score 6–10) and 20 reported moderate-to-severe CMS (CMS score >10) symptoms. All highlanders at 3800 m had a CMS score ≤5. CMS scores were determined using the Qinghai CMS score (León-Velarde *et al.* 2005). Based on clinical interviews and examinations, subjects with a medical history of respiratory and cardiovascular diseases and diabetes were excluded in order to avoid cases of secondary CMS (León-Velarde *et al.* 2005). None of the lowlanders at sea level were acclimatized to high altitude at the time of the tests (no sojourn above 2000 m over the past 3 months) and none of the highlanders reported a prolonged stay at low altitude (more than 5 days below 3500 m) over the past 3 months. The highlanders were all Andean natives from high-altitude (≥3800 m). At 5100 m, all highlanders were working in gold mine facilities. All participants were informed about the procedure and gave their written informed consent prior to participation in this study.

Study design

Each participant was assessed during a single experimental session. The Qinghai CMS score includes the following criteria: symptoms of breathlessness and/or palpitations, sleep disturbance, cyanosis, paraesthesia, headache, tinnitus, dilatation of veins and haemoglobin concentration and the presence of EE (León-Velarde *et al.* 2005). Pulse oxygen saturation (S_{pO_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 assessed in the seated position after 5 min at rest, according to standard recommendations (O'Brien *et al.* 2003; Pretto *et al.* 2014). Haemoglobin concentration, haematocrit and blood rheological evaluations were performed on a venous blood sample collected from the antecubital vein in EDTA tubes according to the current recommendations (Baskurt *et al.* 2009).

Measurement and data analysis

Haemoglobin concentration, haematocrit, blood viscosity and red blood cell aggregation. Haemoglobin concentration ([Hb]) was measured *in situ* with a HemoCue system (HemoCue Hb201+, HemoCue AB, Ängelholm, Sweden) and haematocrit (Ht) by microcentrifugation (Hemata STAT – II, Separation Technology Inc., Sandford, USA). We compared [Hb] measurements provided by the HemoCue system and by another blood analyser (ABL80, Radiometer, Copenhagen, Denmark) in a subsample of highlanders at 5100 m ($n = 57$). Bland Altman analysis showed good agreement, with a mean difference between the two measurements of 0.46 ± 0.89 g dl^{-1}, which was constant throughout the range of [Hb] (19–26 g dl^{-1}). Also, [Hb] and Ht were well correlated ($R^2 = 0.89$; $P < 0.001$). Blood viscosity was measured at native Ht and at several shear rates (11.25 s^{-1}, 22.5 s^{-1}, 45 s^{-1}) using a cone-plate viscometer (Brookfield DVII with CPE40 spindle, Ametek Brookfield, Middleborough, USA), according to the guidelines for haemorheological laboratory techniques (Baskurt *et al.* 2009). In a subgroup of highlanders living at 5100 m, blood viscosity was also measured at a corrected Ht (40%) using autologous plasma for dilution. Red blood cell (RBC) aggregation was measured using the Myrenne aggregometer (Roentgen, Germany) at stasis. As recommended, RBC aggregation was measured at a corrected Ht (40%) to specifically assess RBC aggregation properties (which depend on plasma factors and RBC aggregability), without any influence of the amount of RBC available in the blood samples (Baskurt *et al.* 2009). Briefly, pre-existing RBC aggregates were dissociated at a shear rate of 600 s^{-1} before stopping the shear rate. Then, the intensity of the light of an infra-red laser passing

Table 1. Clinical and biological characteristics of healthy individuals according to the altitude of residency

	Lowlanders at SL ($n = 10$)	Highlanders at 3800 m ($n = 13$)	Highlanders at 5100 m ($n = 35$)
Age (years)	32.8 ± 7.2	28.8 ± 11.4	38.1 ± 9.7$^{\#}$
Duration of residency (years)	-	-	10.3 ± 8
BMI (kg m^{-2})	22.8 ± 1.7	26.5 ± 3.2*	26 ± 2.9*
[Hb] (g dl^{-1})	14.3 ± 0.9	18.3 ± 1.3*	21.1 ± 2.3$^{*\#}$
Haematocrit (%)	41.9 ± 2.5	57.0 ± 4.7*	64.6 ± 7.9$^{*\#}$
S_{pO_2} (%)	98.1 ± 1.0	91.6 ± 1.4*	83.7 ± 4.3$^{*\#}$
CMS score	0.4 ± 0.7	0.5 ± 0.8	2.2 ± 1.8$^{\#}$
Systolic BP (mmHg)	121.7 ± 9.2	110.8 ± 7.7*	118.2 ± 10.3$^{\#}$
Diastolic BP (mmHg)	70.4 ± 8.2	72.3 ± 8.7	78.7 ± 9.4$^{*\#}$

Results are mean ± SD. BP, blood pressure; BMI, body mass index; CMS, chronic mountain sickness; [Hb], haemoglobin concentration; SL, sea level. *Significantly different from lowlanders, $^{\#}$significantly different from highlanders at 3800 m ($P < 0.05$).

Table 2. Clinical and biological characteristics of highlanders at 5100 m according to the CMS score

	Highlanders without CMS at 5100 m ($n = 35$)	Highlanders with mild CMS at 5100 m ($n = 15$)	Highlanders with moderate-to-severe CMS at 5100 m ($n = 20$)
Age (years)	38.1 ± 9.7	46.1 ± 10.6$^{\$}$	43.9 ± 7.4$^{\$}$
Duration of residency (years)	10.3 ± 8	14.6 ± 10.3	18.5 ± 7.3$^{\$}$
BMI (kg m^{-2})	25.9 ± 2.9	27.3 ± 3.5	25.6 ± 2.2
[Hb] (g dl^{-1})	21.1 ± 2.3	23.1 ± 1.2$^{\$}$	24.1 ± 1.5$^{\$}$
Haematocrit (%)	64.6 ± 8	71.4 ± 4.2$^{\$}$	75.6 ± 4.4$^{\$}$
S_{pO_2} (%)	83.7 ± 4.3	78.9 ± 5$^{\$}$	77.9 ± 7.2$^{\$}$
CMS score	2.23 ± 1.8	7.9 ± 1.1$^{\$}$	12.7 ± 2.1$^{\$\S}$
Systolic BP (mmHg)	118.2 ± 10.3	118.4 ± 11.8	113.1 ± 14.9
Diastolic BP (mmHg)	78.7 ± 9.4	79.1 ± 10.1	74.5 ± 9.1

Results are mean ± SD. BP, blood pressure; BMI, body mass index; CMS, chronic mountain sickness; [Hb], haemoglobin concentration. $^{\$}$Significantly different from highlanders at 5100 m without CMS, §significantly different from highlanders at 5100 m with mild CMS ($P < 0.05$).

through the suspension was measured for 10 s to derive an index of RBC aggregation (Baskurt *et al.* 2009).

Statistical analysis

Comparisons between groups were performed using a one-way ANOVA test. When the analysis of variance revealed a significant difference ($P < 0.05$), *post hoc* Fisher's LSD tests were applied for between-group comparisons. To test our hypotheses, a first analysis was performed to investigate the effects of altitude in healthy individuals (comparison of lowlanders *vs.* highlanders at 3800 m *vs.* highlanders at 5100 m without CMS). A second analysis was performed to assess the effect of CMS in highlanders living at 5100 m by comparing highlanders without CMS to those with mild or moderate-to-severe CMS. To explore the factors influencing the effect of altitude and CMS severity, multivariate linear regression models were performed. Statistical analyses were performed using IBM SPSS version 22. All data are expressed as mean ± SD.

Results

Clinical and biological characteristics (Tables 1 and 2)

Comparison in healthy individuals according to altitude of residency. Highlanders at 5100 m without CMS were older than highlanders at 3800 m ($F = 8.5$, $P < 0.001$). Lowlanders had a lower BMI than highlanders at 3800 m and 5100 m ($F = 4.1$, $p = 0.005$). S_{pO_2} decreased ($F = 41.6$, $p < 0.001$) while [Hb] ($F = 64.0$, $p < 0.001$) and Ht ($F = 64.6$, $p < 0.001$) increased with altitude of residency. Highlanders at 3800 m had lower systolic blood pressure ($F = 2.0$, $P < 0.05$) and highlanders at 5100 m had higher diastolic blood pressure compared to the two other groups ($F = 2.7$, $p = 0.04$).

Comparison in highlanders at 5100 m according to CMS severity. Highlanders at 5100 m without CMS were younger than those with mild or moderate-to-severe CMS ($F = 4.9$, $p = 0.011$). The duration of residency in highlanders at 5100 m with moderate-to-severe CMS was higher than those without CMS ($F = 6.0$, $p = 0.04$). Highlanders at 5100 m with CMS had lower S_{pO_2} ($F = 8.67$, $P < 0.001$) and higher [Hb] ($F = 17.2$, $P < 0.001$) and Ht ($F = 19.9$, $P < 0.001$) than those without CMS, while no significant difference was observed between the two CMS groups. There was no significant difference between groups regarding BMI, systolic and diastolic blood pressure.

Haemorheology

Comparison in healthy individuals according to altitude of residency. Red blood cell aggregation did not differ according to the altitude of residency ($F = 0.2$, $p = 0.8$; Fig. 1*A*).

At all shear rates blood viscosity increased (11.25 s^{-1}, $F = 33.9$, $P < 0.001$; 22.5 s^{-1}, $F = 33.5$, $P < 0.001$; 45 s^{-1}, $F = 40.3$, $P < 0.001$) with altitude of residency (Fig. 2*A–C*).

Comparison in highlanders at 5100 m according to CMS severity. Highlanders at 5100 m with moderate-to-severe CMS had lower RBC aggregation than highlanders at 5100 m without CMS ($F = 1.8$, $p = 0.044$; Fig. 1*B*).

At native Ht, blood viscosity was not different between the three groups at 11.25 s^{-1} ($F = 0.5$, $p = 0.6$; Fig. 2*D*) and at 22.5 s^{-1} ($F = 1.9$, $p = 0.16$; Fig. 2*E*). However, at higher shear rate (i.e. 45 s^{-1}, $F = 3.41$, $p = 0.04$; Fig. 2*F*), highlanders without CMS had lower blood viscosity than those with moderate-to-severe CMS. Blood viscosity at 45 s^{-1} also tended to be higher in highlanders with mild CMS compared to highlanders without ($p = 0.068$). When measured at corrected Ht in a subgroup of 37 highlanders at 5100 m (no CMS $n = 7$, mild CMS $n = 12$, moderate-severe CMS $n = 18$), highlanders with moderate-to-severe CMS had higher blood viscosity than those with mild CMS or no CMS at all shear rates (11.25 s^{-1}: $F = 3.4$, $p = 0.05$; 22.5 s^{-1}: $F = 3.5$, $p = 0.04$, at 45 s^{-1}: $F = 3.6$, $p = 0.04$; Fig. 3*A-C*).

Multivariate analyses

To explore the effects of age and BMI (i.e. variables showing significant differences between groups, Table 1) on the differences in blood viscosity between lowlanders, highlanders at 3800 m and highlanders at 5100 m, we performed a multivariate analysis, using the shear rate at 45 s^{-1} (which shows the most pronounced differences between groups; Fig. 2). The model was significant ($R^2 = 0.59$; $P < 0.001$) with blood viscosity and group categories being independently associated ($\beta = 0.81$; $P < 0.001$). Neither age ($\beta = 0.035$; $p = 0.73$), nor BMI ($\beta = -0.19$; $p = 0.09$) had a significant and independent influence on blood viscosity.

To explore the role of factors potentially underlying CMS severity but not involved in the definition of CMS *per se* (i.e. excessive erythrocytosis and severe hypoxaemia; León-Velarde *et al.* 2005), we performed a multivariate linear regression model including age,

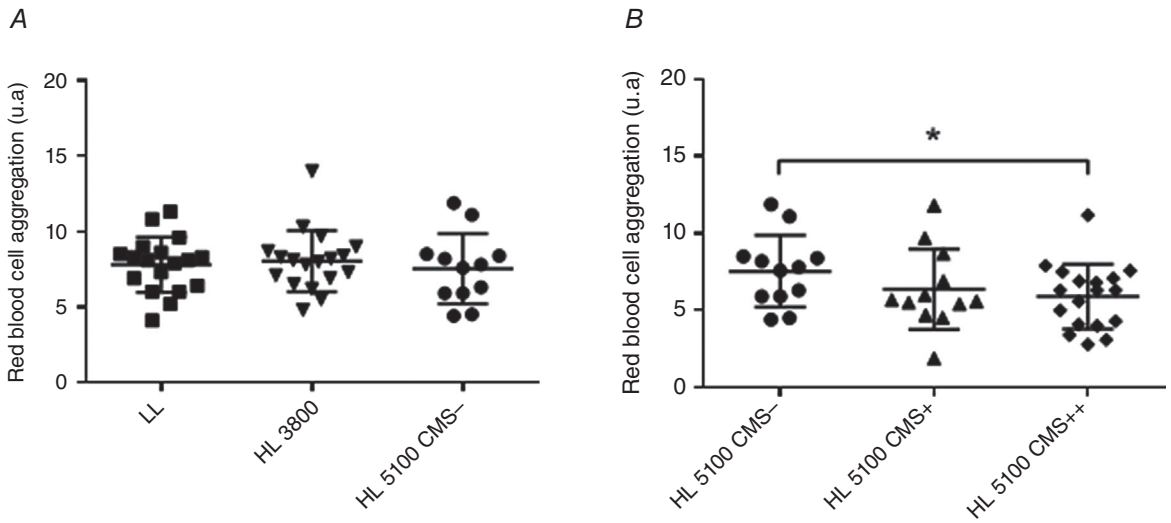

Figure 1. Red blood cell aggregation
A, effect of altitude of residency in healthy subjects ($n = 48$). *B*, effect of CMS score in highlanders at 5100 m ($n = 42$). LL, lowlanders; HL 3800, highlanders at 3800 m; HL 5100 CMS−, highlanders at 5100 m without CMS; HL 5100 CMS +, highlanders at 5100 m with mild CMS; HL 5100 CMS++, highlanders at 5100 m with moderate-to-severe CMS. *$P < 0.05$. One-way ANOVA test and *post hoc* Fisher's LSD tests for between-group comparisons.

the duration of residency in La Rinconada and blood viscosity at a shear rate of 45 s^{-1} (which shows the most pronounced differences between groups; Fig. 2). The model was significant ($R^2 = 0.28$; $P < 0.001$) and only blood viscosity was independently associated with the CMS score ($\beta = 0.28$; $P < 0.05$). The duration of residency in La Rinconada was marginally associated with CMS score ($\beta = 0.26$; $p = 0.055$) and no independent association between age and CMS score was observed ($\beta = 0.19$; $p = 0.16$).

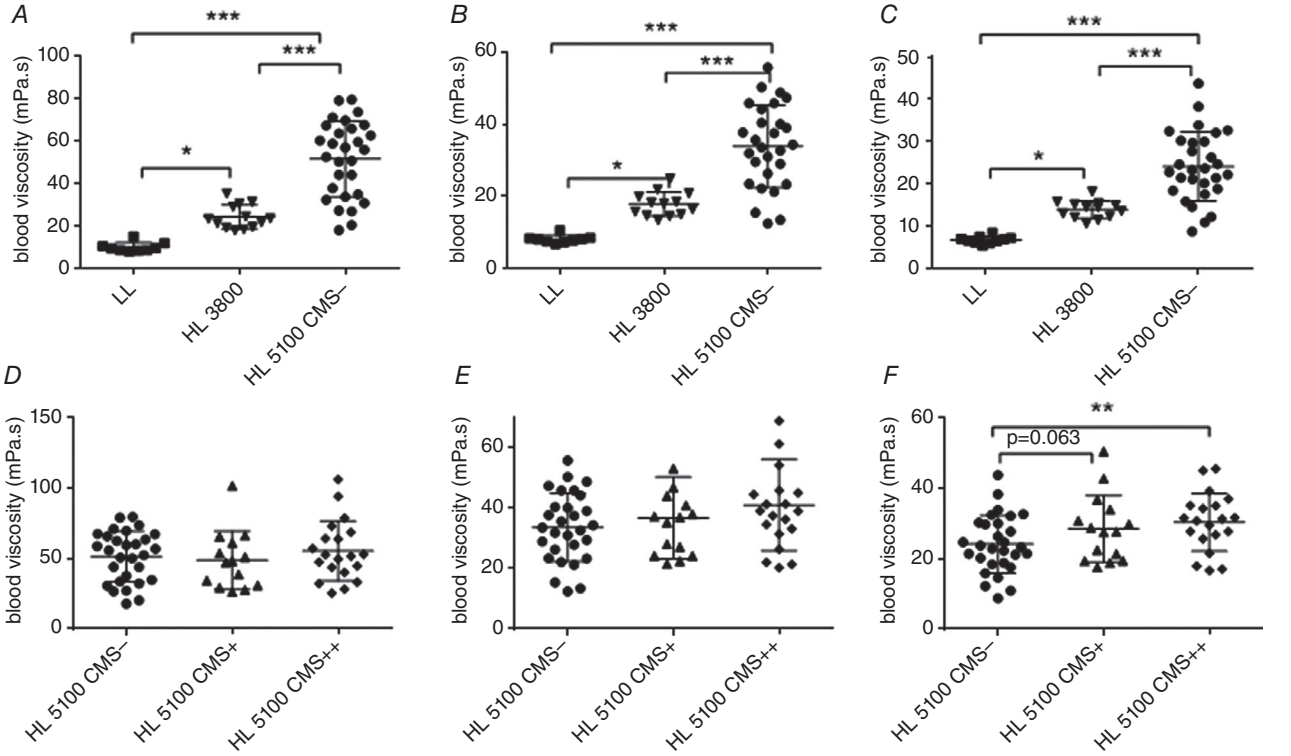

Figure 2. Blood viscosity
A–C, effect of altitude of residency in healthy subjects ($n = 58$) at different shear rates: 11.25 s^{-1} (*A*), 22.5 s^{-1} (*B*), 45 s^{-1} (*C*). *D–F*, effect of the CMS score in highlanders at 5100 m ($n = 70$) at different shear rates: 11.25 s^{-1} (*D*), 22.5 s^{-1} (*E*), 45 s^{-1} (*F*). LL, lowlanders; HL 3800, highlanders at 3800 m; HL 5100 CMS–, highlanders at 5100 m without CMS; HL5100 CMS+, highlanders at 5100 m with mild CMS; HL 5100 CMS++, highlanders at 5100 m with moderate-to-severe CMS. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. One-way ANOVA test and *post hoc* Fisher's LSD tests for between-group comparisons.

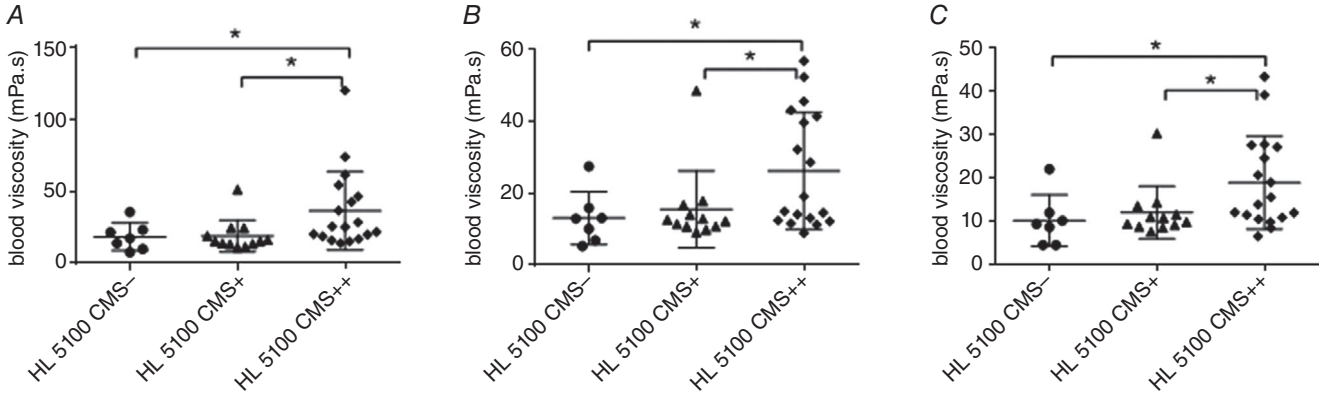

Figure 3. Blood viscosity at corrected haematocrit (40%)
Effect of CMS score in highlanders at 5100 m ($n = 37$) at different shear rates: 11.25 s^{-1} (*A*), 22.5 s^{-1} (*B*), 45 s^{-1} (*C*). HL 5100 CMS–, highlanders at 5100 m without CMS; HL 5100 CMS+, highlanders at 5100 m with mild CMS; HL 5100 CMS++, highlanders at 5100 m with moderate-to-severe CMS. $^*P < 0.05$. One-way ANOVA test and *post hoc* Fisher's LSD tests for between-group comparisons.

Discussion

The main objectives of the present study were to independently evaluate the effect of permanent residency at altitude and of CMS on haemorheological parameters. The main results are that (1) haemoglobin concentration, haematocrit and blood viscosity progressively increased with altitude of residency in healthy individuals, and (2) at 5100 m, blood viscosity was increased in patients suffering from moderate-to-severe CMS, suggesting that blood viscosity may play a role in CMS symptom aetiology.

Clinical characteristics

Highlanders at 5100 m without CMS symptoms were younger than those with mild or moderate-to-severe symptoms. Older age is considered a risk factor for CMS (Monge *et al.* 1989), possibly due to an age-dependent reduction in ventilation leading to an accentuation of hypoxaemia (Sime *et al.* 1975). It is further admitted that haematocrit increases with age among highlanders (León-Velarde *et al.* 2000). Moreover, the duration of residency at high altitude (and the concomitant duration of exposure to low oxygen pressure) is considered as a risk factor for CMS (Penazola *et al.* 2017). The multivariate analysis showed that while age was not independently associated with the CMS score, the duration of residency in La Rinconada could play a small role in CMS severity within the present population.

The loss of ventilatory acclimatization to altitude manifesting in hypoxaemia is thought to be one of the mechanisms leading to CMS (León-Velarde & Richalet, 2006). Highlanders suffering from CMS have a blunted ventilatory response to hypoxia leading to a relative hypoventilation. This lower level of alveolar ventilation induces more severe hypoxaemia than in healthy highlanders. As a consequence, the erythropoietin (EPO) response would be enhanced leading to exaggerated polycythaemia (Winslow *et al.* 1989). However, EPO availability rather than EPO concentration *per se* may be critical, lower EPO soluble receptor levels leading in some highlanders to increased EPO availability and subsequently to EE and CMS (Villafuerte *et al.* 2014).

Haemorheology

Paradoxically, although high altitude residents are known to experience profound haematological changes such as hypoxia-induced polycythaemia (Beall, 2007), very few data are available regarding their blood rheological characteristics. Kametas *et al.* (2004) previously reported lower blood viscosity in Andean women at 4800 m than those found in our study. However, the authors made an estimation of blood viscosity and did not measure it directly, making the comparison with our study difficult. Moreover, at sea level, women usually have lower blood viscosity than men (Gudmundsson & Bjelle, 1993; Kameneva *et al.* 1999) and one could speculate that this difference persists at high altitude. More recently, Tremblay *et al.* (2018) measured blood viscosity in Sherpa on ascent to 5050 m and also found lower values compared to the values observed in the present study but this finding may be attributable to their rather low haematocrit values and also to the lack of change during the ascension ($44 \pm 3\%$ at 1400 m and $44 \pm 2\%$ at 5050 m). Moreover, the authors measured blood viscosity only at 225 s^{-1} while measurements were performed at 11.25, 22.5 and 45 s^{-1} in the present study using the same viscometer as Tremblay *et al.* (2018). Because blood is a shear thinning fluid, as well as because of important phenotypic differences between Andeans and Sherpas (Beall, 2007), comparisons of values from Tremblay *et al.* and the present study are rather difficult. However, it is likely that blood viscosity was significantly higher in our population since measurements were not possible at 225 s^{-1} because the percentage of torque obtained on the viscometer was higher than 100% at shear rates higher than 90 s^{-1}. In the present study, as expected, Andean highlanders chronically exposed to hypoxia exhibited higher blood viscosity compared to individuals living at sea level, and these effects were not related to BMI or age differences (parameters known to potentially affect blood viscosity; Brun *et al.* 2011; Simmonds *et al.* 2013). In support of this, chronic hypoxic exposure in rat models was shown to increase haemoglobin concentration, haematocrit and blood viscosity (Yelmen *et al.* 2011; Pichon *et al.* 2012).

At 5100 m, blood viscosity measured at native haematocrit and at the two highest shear rates was higher in highlanders with moderate-to-severe CMS than in highlanders without CMS, suggesting that blood viscosity could also play a role in the development of CMS. A rise in blood viscosity has previously been observed in Andeans with EE (Tremblay *et al.* 2019) but the relationship between CMS symptoms and blood viscosity has not yet been studied. Surprisingly, blood viscosity measured at the lower shear rate (i.e. 11.5 s^{-1}) was not different between the three groups living in La Rinconada, despite the higher haematocrit and haemoglobin observed in both groups with CMS compared to highlanders without CMS. Blood viscosity depends on RBC aggregation, plasma viscosity and haematocrit at low shear rate, while haematocrit exerts less influence at high shear rate. A loss of RBC deformability may also profoundly affect blood viscosity (Cho & Cho, 2011; Nader *et al.* 2019). RBC aggregation was lower in highlanders with moderate-to-severe CMS compared to individuals without CMS, which could have partly offset the effects of the increased haematocrit and haemoglobin levels on blood viscosity measured at the lowest shear rate. The reasons for the lower RBC aggregation found in individuals with moderate-to-severe

CMS are unknown and need to be explored further but several mechanisms such as lower plasma fibrinogen, thrombospondin, von Willebrand factor concentrations (plasma factors) or difference in RBC membrane sialic contents (cellular factors) (Baskurt & Meiselman, 2013; Nader *et al.* 2017; Gondelaud *et al.* 2020) could be involved. At higher shear rates, blood viscosity was higher in the most severe group compared to highlanders without CMS but no significant difference in blood viscosity was noted between the mild CMS group and healthy highlanders despite the fact that haematocrit and haemoglobin concentration were higher in the two groups with CMS. Nevertheless, the multivariate analysis showed that blood viscosity was independently associated with the CMS score, emphasizing that blood viscosity may play a role in CMS severity. Altogether, these results suggest that RBC deformability could be severely impaired in the most severe CMS group, which, in association with the high haematocrit/haemoglobin levels, could lead to a further rise in blood viscosity. When measured at corrected haematocrits, highlanders with moderate-to-severe CMS maintained a very high blood viscosity in comparison to individuals with no CMS (+100% at 22.5 s^{-1}) and also compared to highlanders with mild CMS (+70% at 22.5 s^{-1}). This further supports the idea that RBC deformability was different between highlanders with moderate-to-severe CMS and the two other groups. In contrast, the mild group had only a slightly higher blood viscosity than the group with no CMS (+20%), which suggests that RBC deformability, and perhaps plasma viscosity, was not dramatically different between these two groups. Unfortunately, it was not possible to measure these two parameters in the present study, nevertheless it has been shown that RBCs stimulated by altitude hypoxia in highlanders from the Tibetan plateau have an increased osmotic fragility and higher haemolytic rate, suggesting a loss of deformability (Zhong *et al.* 2015). Whether these data from Tibetans apply to Andean populations remains hypothetical. Therefore, further studies are required to better understand the relationship between polycythaemia and blood viscosity at high altitude. Other specific hemorheological parameters (plasma viscosity and red blood cell deformability, as well as its determinants, i.e. RBC internal viscosity, RBC membrane deformability and RBC surface-to-volume ratio) should be investigated to elucidate the mechanisms involved in CMS.

Limitations

The potential impact of toxic exposure in mining facilities remains unclear and difficult to evaluate. Previous studies suggested an effect of increased blood concentrations of zinc, lead and cobalt on red blood cell production and haematocrit (Hutchison & Stark, 1961; Jefferson *et al.* 2002; Gonzales *et al.* 2011). In La Rinconada, mercury is used to extract gold and represents the main risk of contamination, but its potential impact on blood viscosity remains unknown. Mining at altitude has also been reported to accelerate pulmonary disease, like silicosis and other pneumoconiosis, known to worsen hypoxaemia and to affect cardiopulmonary function (Vearrier & Greenberg, 2011). The pathogenic role of environmental factors on blood viscosity and CMS onset probably is insufficiently considered and should therefore be investigated in future studies.

Conclusions

The present study investigated for the first time blood viscosity and some of its determinants (RBC aggregation and haematocrit) in the highest city in the world (5100 m), in highlanders chronically exposed to severe hypobaric hypoxia with or without CMS. Living at high altitude resulted in an increase in haemoglobin concentration, haematocrit and blood viscosity. At 5100 m, highlanders with CMS had higher blood viscosity mainly at high shear rate and even at corrected haematocrit, with a lower red blood cell aggregation, suggesting that lower red blood cell deformability might promote increased blood viscosity and contribute to the occurrence of CMS symptoms. Further studies are required to clarify the relationship between chronic hypoxic exposure, red blood cell deformability and other determinants of blood viscosity.

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Additional information

Competing interests

None declared.

Author contributions

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 and final approval of the version to be published. All authors 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. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Keywords

chronic mountain sickness, haemorheology, high-altitude native, hypoxia

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document