



# Hemostasis in highlanders with excessive erythrocytosis at 5100 m: Preliminary data from the highest city of the world

Ivan Hanco, Benoit Champigneulle, Emeric Stauffer, Aurélien Pichon, Paul Robach, Julien V. Brugniaux, Yann Savina, Stéphane Doutreleau, Philippe Connes, Samuel Vergès

## ► To cite this version:

Ivan Hanco, Benoit Champigneulle, Emeric Stauffer, Aurélien Pichon, Paul Robach, et al.. Hemostasis in highlanders with excessive erythrocytosis at 5100 m: Preliminary data from the highest city of the world. *Respiratory Physiology & Neurobiology*, 2020, 282, pp.103535 -. 10.1016/j.resp.2020.103535 . hal-03492482

**HAL Id: hal-03492482**

**<https://hal.science/hal-03492482>**

Submitted on 8 Sep 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

## **Hemostasis in highlanders with excessive erythrocytosis at 5100 m: preliminary data from the highest city of the world**

Ivan Hanco, MD-MSc<sup>a</sup>; Benoit Champigneulle, MD<sup>b</sup>; Emeric Stauffer, MD-MSc<sup>c</sup>; Aurélien Pichon, PhD<sup>d</sup>; Paul Robach, PhD<sup>e</sup>; Julien V Brugniaux, PhD<sup>f</sup>; Yann Savina, PhD<sup>g</sup>; Stéphane Doutreleau, MD-PhD<sup>h</sup>; Philippe Connes, PhD<sup>i</sup>; Samuel Vergès, PhD<sup>j\*</sup>

a. HP2 Laboratory, INSERM U1042, Grenoble Alpes University, CHU Grenoble Alpes, Grenoble, France. Electronic address: [ihzmd@hotmail.com](mailto:ihzmd@hotmail.com)

b. HP2 Laboratory, INSERM U1042, Grenoble Alpes University, CHU Grenoble Alpes, Grenoble, France. Electronic address: [b.champigneulle@gmail.com](mailto:b.champigneulle@gmail.com)

c. Laboratory LIBM EA7424, Team "Vascular Biology and Red Blood Cell", University of Lyon 1, Lyon, France & Centre de Médecine du Sommeil et des Maladies Respiratoires, Hospices Civils de Lyon, Hôpital de la Croix Rousse, Lyon, France. Electronic address: [emeric.stf@gmail.com](mailto:emeric.stf@gmail.com)

d. Université de Poitiers. Faculté des Sciences du Sport, Laboratoire Move, EA 6314, Poitiers, France. Electronic address: [aurelien.pichon@univ-poitiers.fr](mailto:aurelien.pichon@univ-poitiers.fr)

e. École Nationale des Sports de Montagne, site de l'Ecole Nationale de Ski et d'Alpinisme, Chamonix, France. Electronic address: [paul.robach@ensm.sports.gouv.fr](mailto:paul.robach@ensm.sports.gouv.fr)

f. HP2 Laboratory, INSERM U1042, Grenoble Alpes University, CHU Grenoble Alpes, Grenoble, France. Electronic address: [julien.brugniaux@univ-grenoble-alpes.fr](mailto:julien.brugniaux@univ-grenoble-alpes.fr)

g. HP2 Laboratory, INSERM U1042, Grenoble Alpes University, CHU Grenoble Alpes, Grenoble, France. Electronic address: [yann.savina@univ-grenoble-alpes.fr](mailto:yann.savina@univ-grenoble-alpes.fr)

h. HP2 Laboratory, INSERM U1042, Grenoble Alpes University, CHU Grenoble Alpes, Grenoble, France. Electronic address: [sdoutreleau@chu-grenoble.fr](mailto:sdoutreleau@chu-grenoble.fr)

i. Laboratory LIBM EA7424, Team "Vascular Biology and Red Blood Cell", University of Lyon 1, Lyon, France. Electronic address: philippe.connes@univ-lyon1.fr

j. HP2 Laboratory, INSERM U1042, Grenoble Alpes University, CHU Grenoble Alpes, Grenoble, France. Electronic address: sverges@chu-grenoble.fr

\* **Corresponding author:** Samuel Verges, HP2 Laboratory, INSERM U1042, Grenoble Alpes University, CHU Grenoble Alpes, UM Sports Pathologies, Hôpital Sud, Avenue Kimberley, 38434 Echirolles, France.

Phone: +33 4 76 76 68 60; Fax: +33 4 76 76 89 21; E-mail: SVerges@chu-grenoble.fr

**Word count:** 1447; **References:** 13; **Tables:** 1; **Figures:** 1; **Abstract word count:** 151.

**Author contributions:** IH, BC, ES, AP, PR, JVB, YS, SD, PC and SV designed the study, analyzed and interpreted the data. IH and SV acquired the data. BC and SV performed the statistical analysis. IH and BC wrote the manuscript; SV and PC revisited it for important intellectual content. All authors approved the final version of the manuscript.

## ABSTRACT

Little is known about hemostasis modifications induced by chronic hypoxic exposure in high-altitude residents, especially in those who develop excessive erythrocytosis (EE, i.e. hemoglobin concentration  $\geq 21$  g·dL<sup>-1</sup> in male and  $\geq 19$  g·dL<sup>-1</sup> in female). The aim of this preliminary study was to assess coagulation alterations in highlanders with or without EE using simple hemostatic tests such as bleeding (BT) and clotting (CT) times. Eighty-one male ( $43 \pm 7$  years), permanent residents from La Rinconada (Peru), the highest city in the world (5,100-5,300 m), were evaluated. Thirty-six subjects (44%) presented with EE. EE subjects compared to non-EE subjects had lower BT ( $3.6 \pm 1.2$  vs.  $7.0 \pm 1.9$  minutes,  $p < 0.001$ ) and CT ( $11.7 \pm 1.7$  vs.  $15.1 \pm 2.3$  minutes,  $p < 0.001$ ). These results support the notion that highlanders with EE are in a state of hypercoagulability and call for further hemostasis investigations in this population using more detailed hemostatic methods.

**Keywords:** high-altitude; coagulation; hemostasis; highlanders; excessive erythrocytosis; chronic mountain sickness.

## MAIN TEXT

### 1. Introduction

Chronic hypoxic exposure is a physiological challenge faced by permanent residents at high-altitude (HA) that can lead in some highlanders to excessive erythrocytosis (EE) and chronic mountain sickness (CMS) (Villafuerte and Corante, 2016). If the respiratory or cardio-vascular consequences of acute and chronic HA exposure have been deeply investigated, data regarding the impact of chronic hypoxia on the coagulation cascade are scarce and concern almost exclusively acute HA exposure in lowlanders. In these acute conditions, HA exposure seems to induce a prothrombotic state (Rocke et al., 2018; Zavanone et al., 2017), although this remains debated (Crosby et al., 2003). The consequences of chronic HA exposition on hemostasis in highlanders remains unexplored.

Older yet validated and easy-to-use hemostatic measurements such as bleeding (BT) and clotting (CT) times could represent suitable alternative to more complex albeit extensive investigations that are unfortunately difficult to perform reliably in such extreme conditions and cannot realistically be scaled up in HA low-resource conditions (Bowie and Owen, 1980; Sussman, 1973).

Therefore, this study aims to investigate for the first-time hemostasis in a unique population of highlanders living in the highest city in the world, La Rinconada (5,100-5,300 m), with or without EE. We hypothesized that highlanders with EE would exhibit shorter BT and CT suggesting a state of hypercoagulability.

## **2. Methods**

### **2.1 Participants**

Eighty-one males residing permanently in La Rinconada (a gold mining town located in Southern Peru at an altitude of 5,100-5,300 m and considered the highest city in the world (West, 2002)) were included in the present study after written informed consent. This study was conducted in accordance with the Declaration of Helsinki and was approved by the ethic committee of the National University of San Marcos, Lima, Peru (Protocol number: CIEI-2019-002).

### **2.2. Data collection**

Subjects' baseline characteristics were first collected (age, body mass index (BMI) and duration of residency in La Rinconada) upon inclusion into the study. Pulse oxygen saturation (SpO<sub>2</sub>) and heart rate (HR) were measured at rest, and hematocrit (measured by microhematocrit centrifugation) and hemoglobin concentration ([Hb], Hemocue AB, Angelholm, Sweden) were assessed from an arterialized capillary blood sample. Excessive erythrocytosis (EE) was defined based on the current international consensus as an [Hb]  $\geq$  21 g·dL<sup>-1</sup>; CMS was defined as the association of EE and a Quinzhai score  $\geq$  6 (León-Velarde et al., 2005).

### **2.3 Measurements**

Two coagulation tests were performed by an experimented hematologist (IH) trained in healthcare at HA and low-resource settings.

*Bleeding time.* The BT test classically allows for exploring *in vivo* the primary hemostasis (platelet plug formation). As previously described (Bowie and Owen, 1980), BT was

measured using the Duke method. Briefly, a puncture was performed at the earlobe with a sterile lancet after skin disinfection, and bleeding was cleaned with a filter paper every 30 seconds. Time between the beginning and the end of bleeding was measured with a stopwatch and defined as BT.

*Clotting time.* The CT test explores whole blood coagulation *in vitro*. CT was performed using a Lee and White modified method (Sussman, 1973). A 4-mL venous blood sample was obtained after puncture of a large arm vein and promptly distributed equally in 4 tubes (1 mL of blood in each tube), previously placed on a rack. Room temperature was set and controlled at 15-17°C. After filling the tubes (in less than 5 seconds), the first tube was tilted every 30 seconds, until blood coagulation (*i.e.* until the blood stopped to flow through the wall of the tube). Immediately, the second tube was tilted until clot formation, and then the same manipulation was successively repeated for the last two tubes. CT, measured with a stopwatch, corresponded to the time duration from blood filling of the first tube to blood coagulation of the fourth tube.

## **2.4 Statistical analysis**

Descriptive statistics were reported as mean and standard deviation (SD) for continuous variables and as count (percentage) for categorical variables. Unpaired Student t test was used to compare continuous variables between groups. All tests were 2-sided and a *p* value <0.05 was considered as statistically significant. Statistical analyses were performed using R version 3.6.0 for Mac OS (The R Foundation for Statistical Computing, Vienna, Austria).

## **3. Results**

Demographic characteristics of the subjects are summarized in the table 1. No significant difference in demographic characteristics was observed between participants with or without EE, except BMI. By design, hematocrit and [Hb] levels were different between groups. All highlanders were born at HA (>3500 m) and resided in La Rinconada for  $16 \pm 11$  years. They reported no medication intake, no medical history of chronic respiratory, cardiovascular and hematological diseases and none of them reported history of abnormal bleeding or symptomatic venous or arterial thrombosis.

BT and CT were respectively  $5.5 \pm 2.3$  and  $13.6 \pm 2.7$  minutes for the entire population. EE subjects exhibited significantly lower BT than those without EE ( $p < 0.001$ ; figure 1A). Similarly, CT was significantly lower in subjects with EE compared to participants without EE ( $p < 0.001$ ; figure 1B). When comparing subjects with CMS ( $n=32$ , 40%) vs. subjects without CMS ( $n=49$ , 60%), similar **significant differences** were observed (data not shown).

#### **4. Discussion**

We reported significantly lower BT and CT in highlanders with EE compared to highlanders without EE who lived in the highest city of the world at 5100-5300 m. These data suggest a potential hypercoagulability status in EE highlanders without any past history of symptomatic thrombosis. This preliminary study represents to our knowledge the first report of hemostasis investigation in the setting of permanent HA exposure, since previous studies only focused on transient HA stay in lowlanders (Rocke et al., 2018; Zavanone et al., 2017). **As shown by a recent study from our group reporting an EE prevalence of 47% in a large group of male dwellers from La Rinconada (Hanco et al., 2020), EE is a very frequent condition in highlanders.**



The higher number of red blood cells in the EE group could, at least partly, explain the difference in coagulation activity compared to subjects without EE. Platelet-red blood cell interactions may occur through several adhesion receptors, hence promoting coagulation and reinforcing platelet clot (Litvinov and Weisel, 2017). In addition, the release of several molecules, such as ATP and ADP, by red blood cells has been found to further activate platelets (Litvinov and Weisel, 2017). Moreover, the ability of red blood cells to form aggregates under low flow conditions may, with platelet aggregation, further increase coagulation activity (Litvinov and Weisel, 2017). Although these mechanisms remain hypothetical, the higher hematocrit in EE subjects was likely promoting the prothrombotic status of this population. Moreover, we cannot exclude a potential implication of others prothrombotic factors, such as inflammation, platelet activation, endothelial dysfunction or high serum level of erythropoietin (EPO) that could promote thrombosis by others pathways than increasing hematocrit (Lippi et al., 2010). Indeed, we recently showed that dwellers from La Rinconada exhibited higher EPO serum levels than lowlanders and healthy highlanders living at 3,800 m (Oberholzer et al., 2020); however, the implication of EPO in BT and CT shortening seems unlikely since we observed similar EPO serum levels in highlanders with and without CMS at 5,100 m (Oberholzer et al., 2020). Nevertheless, CMS sufferers may differ from healthy highlanders by presenting lower concentrations of serum EPO receptors and subsequent higher EPO availability (Villafuerte et al., 2014). Implication of EPO as a procoagulant potential factor in EE (and CMS) highlanders remains to be explored. Hence, further investigations are required to elucidate the mechanisms underlying changes in hemostasis associated with chronic hypoxic exposure in highlanders with or without EE.

Our preliminary study has several limitations. Firstly, hemostasis investigations were based on basic clinical tests not allowing to determine the mechanisms underlying the

observed group differences (Bowie and Owen, 1980; Sussman, 1973). However, these tests are accessible and useful in low-resource settings. Secondly, investigators were not blinded according to the EE status of the subjects. Thirdly, we did not include a control group of Andean lowlanders in order to establish whether highlanders from La Rinconada independently of their EE status have different hemostasis status compared to lowlanders; following the same reasoning, comparing our data with values previously measured at sea level does not seem suitable since both tests are known to suffer from a large variability according to the methodology implemented (Bowie and Owen, 1980; Sussman, 1973).

Albeit preliminary, this study is the first report demonstrating hemostasis changes associated with EE in highlanders. Further investigations using more robust and exhaustive methods are required to detangle the effect of chronic hypoxic exposure and EE on hemostasis status. For example, viscoelastic hemostatic testing as thromboelastometry could be a well-validated point-of-care evaluation in an isolated environment with limited medical and biological resources such as La Rinconada (Rocke et al., 2018).

**Acknowledgments:** We thank the voluntary residents of La Rinconada for their personal participation to the study as well as the medical students of the Altiplano University (Puno, Peru) who actively participated to the research program implemented in La Rinconada.

**Funding source:** This study was supported by the Grenoble Alpes University foundation, the “Fonds de dotation AGIR pour les maladies chroniques” and by the French National Research Agency (ANR-12-TECS-0010) in the framework of the “Investissements d’avenir” program (ANR-15-IDEX-02).

## References

- Bowie, E.J.W., Owen, C.A., 1980. Standardization of the bleeding. *Scandinavian Journal of Haematology* 24, 87–94. <https://doi.org/10.1111/j.1600-0609.1980.tb01344.x>
- Crosby, A., Talbot, N.P., Harrison, P., Keeling, D., Robbins, P.A., 2003. Relation between acute hypoxia and activation of coagulation in human beings. *The Lancet* 361, 2207–2208. [https://doi.org/10.1016/S0140-6736\(03\)13777-4](https://doi.org/10.1016/S0140-6736(03)13777-4)
- Hanco, I., Bailly, S., Bailleuil, S., Doutreleau, S., Germain, M., Pépin, J.-L., Vergès, S., 2020. Excessive erythrocytosis and chronic mountain sickness in dwellers of the highest city in the world. *Frontiers in Physiology*. In press. <https://doi.org/doi:10.3389/fphys.2020.00773>
- León-Velarde, F., Maggiorini, M., Reeves, J.T., Aldashev, A., Asmus, I., Bernardi, L., Ge, R.-L., Hackett, P., Kobayashi, T., Moore, L.G., Penaloza, D., Richalet, J.-P., Roach, R., Wu, T., Vargas, E., Zubieta-Castillo, G., Zubieta-Calleja, G., 2005. Consensus Statement on Chronic and Subacute High Altitude Diseases. *High Altitude Medicine & Biology* 6, 147–157. <https://doi.org/10.1089/ham.2005.6.147>
- Lippi, G., Franchini, M., Favaloro, E.J., 2010. Thrombotic complications of erythropoiesis-stimulating agents. *Semin. Thromb. Hemost.* 36, 537–549. <https://doi.org/10.1055/s-0030-1255448>
- Litvinov, R.I., Weisel, J.W., 2017. Role of red blood cells in haemostasis and thrombosis. *VOXS* 12, 176–183. <https://doi.org/10.1111/voxs.12331>
- Oberholzer, L., Lundby, C., Stauffer, E., Ulliel-Roche, M., Hanco, I., Pichon, A., Lundby, A.-K., Villafuerte, F.C., Verges, S., Robach, P., 2020. Re-evaluation of excessive erythrocytosis in diagnosing chronic mountain sickness in men from the world's highest city. *Blood*. <https://doi.org/10.1182/blood.2019004508>
- Rocke, A., Paterson, G., Barber, M., Jackson, A., Main, S., Stannett, C., Schnopp, M., Baillie,

- J., Horne, E., Moores, C., Harrison, P., Nimmo, A., Thompson, A., 2018. Thromboelastometry and Platelet Function during Acclimatization to High Altitude. *Thromb Haemost* 118, 063–071. <https://doi.org/10.1160/TH17-02-0138>
- Sussman, L.N., 1973. The Clotting Time—An Enigma. *Am J Clin Pathol* 60, 651–653. <https://doi.org/10.1093/ajcp/60.5.651>
- Villafuerte, F.C., Corante, N., 2016. Chronic Mountain Sickness: Clinical Aspects, Etiology, Management, and Treatment. *High Altitude Medicine & Biology* 17, 61–69. <https://doi.org/10.1089/ham.2016.0031>
- Villafuerte, F.C., Macarlupú, J.L., Anza-Ramírez, C., Corrales-Melgar, D., Vizcardo-Galindo, G., Corante, N., León-Velarde, F., 2014. Decreased plasma soluble erythropoietin receptor in high-altitude excessive erythrocytosis and Chronic Mountain Sickness. *J. Appl. Physiol.* 117, 1356–1362. <https://doi.org/10.1152/japplphysiol.00619.2014>
- West, J.B., 2002. Highest Permanent Human Habitation. *High Altitude Medicine & Biology* 3, 401–407. <https://doi.org/10.1089/15270290260512882>
- Zavanone, C., Panebianco, M., Yger, M., Borden, A., Restivo, D., Angelini, C., Pavone, A., Grimod, G., Rosso, C., Dupont, S., 2017. Cerebral venous thrombosis at high altitude: A systematic review. *Revue Neurologique* 173, 189–193. <https://doi.org/10.1016/j.neurol.2016.11.004>

## Figure legend

**Figure 1:** Bleeding time (**A**) and clotting time (**B**) according to the excessive erythrocytosis status of the subjects (mean  $\pm$  SD). \*  $p < 0.001$  between groups.

**Table 1:** Characteristics of participants with and without excessive erythrocytosis.

<b>Variable</b>	<b>All participants (n=81)</b>	<b>Participants with EE ([Hb] <math>\geq</math> 21 g·dL<sup>-1</sup>) (n=36)</b>	<b>Participants without EE ([Hb] &lt; 21 g·dL<sup>-1</sup>) (n=45)</b>	<b><i>p</i> value</b>
<b>Age (years)</b>	43 $\pm$ 7	44 $\pm$ 7	43 $\pm$ 8	0.64
<b>Body mass index (kg·m<sup>-2</sup>)</b>	26.47 $\pm$ 3.30	27.38 $\pm$ 3.69	25.74 $\pm$ 2.79	0.03
<b>Hematocrit (%)</b>	61 $\pm$ 10	70 $\pm$ 6	54 $\pm$ 5	<0.001
<b>[Hb] (g·dL<sup>-1</sup>)</b>	20.1 $\pm$ 2.9	22.8 $\pm$ 1.5	17.9 $\pm$ 1.8	<0.001
<b>SpO<sub>2</sub> (%)</b>	81 $\pm$ 5	80 $\pm$ 5	82 $\pm$ 5	0.08
<b>Heart rate (bpm)</b>	85 $\pm$ 11	85 $\pm$ 11	85 $\pm$ 11	0.94
<b>Duration of residency in La Rinconada (years)</b>	16 $\pm$ 11	18 $\pm$ 11	14 $\pm$ 10	0.09
<b>CMS status</b>				
<b>Absent (score=0-5)</b>	49 (61%)	4 (11%)	45 (100%)	
<b>Mild (score=6-10)</b>	22 (27%)	22 (61%)	—	—
<b>Moderate (score=11-14)</b>	9 (11%)	9 (25%)	—	
<b>Severe (score <math>\geq</math> 15)</b>	1 (1%)	1 (3%)	—	

Data are expressed as mean  $\pm$  SD or n (%).  
 EE, excessive erythrocytosis; [Hb], hemoglobin concentration; SpO<sub>2</sub>, pulse oxygen saturation; CMS, chronic mountain sickness. *p* value refers to statistical comparisons between subjects with and without EE.

**Figure 1**

