

## **Letter to Blood**

### **Suppression of plasma hepcidin by venesection during steady-state hypoxia**

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Hepcidin inhibits iron uptake from the gut and release of iron from macrophages in the reticuloendothelial system. Suppression of plasma hepcidin occurs in lowlanders after ascent to high altitude,<sup>1,2</sup> reflecting the increased iron demand for erythropoiesis, but the mechanism and primary stimulus for hepcidin suppression in this setting remains unclear.

It has been suggested that hypoxia *per se*, possibly via the hypoxia-inducible factor (HIF) family of transcription factors, provides a stimulus for transcriptional suppression of hepcidin.<sup>3</sup> This is supported by the hepcidin suppression seen in patients with congenital upregulation of HIF proteins, even after correction for hemoglobin and iron status.<sup>4</sup> However, others have argued that hepcidin suppression does not result from hypoxia directly,<sup>5,6</sup> but rather from the hypoxia-induced increase in erythropoietic drive. Hepcidin levels are suppressed in patients with excessive ineffective erythropoiesis at sea level,<sup>7</sup> although they remain normal in healthy high-altitude residents exposed to long term hypoxia and in patients with polycythemia vera at sea level, in whom erythropoietic drive is likely to be relatively stable.<sup>8,9</sup> In recent years, numerous mediators have been proposed as the link between erythropoiesis and hepcidin suppression (the so-called ‘erythroid regulator’). Candidates include growth-differentiation factor 15 (GDF15), soluble transferrin receptor (sTfR), erythropoietin (EPO) and the novel hormone erythroferrone, recently described in mice and reported to be the product of the gene *Fam132b*.<sup>10</sup> The protein product of the homologous human gene (*FAM132B*) is known as FAM132B, CTRP15 or myonectin.

We studied patients with chronic mountain sickness (CMS), a condition characterised by excessive erythrocytosis and elevated blood viscosity in high altitude residents.<sup>11</sup> Venesection provides symptomatic relief in such patients, and therefore presents an opportunity to examine the effects of acutely enhanced erythropoietic drive on a background of chronic, steady-state hypoxia.

Ten male patients (age  $44 \pm 10$  years, mean  $\pm$  SD) were recruited in Cerro de Pasco, Peru (4340 m above sea level), as part of a wider study into iron supplementation at high

altitude.<sup>12</sup> All were high-altitude natives with CMS, as defined by excessive erythrocytosis (hemoglobin  $\geq 21$  g/dL) and hypoxemia with no other medical explanation.<sup>11</sup> At baseline (day 0), arterial oxygen saturation was measured and venous blood was sampled for assessment of haematocrit, ferritin, transferrin saturation, hepcidin, EPO, sTfR, GDF-15 and FAM132B. Patients then underwent isovolemic venesection of 500 ml on each of days 1-4 (total volume 2000 mL), with repeat pulse oximetry and blood sampling on days 5, 12 and 19.

The results are shown in Figure 1. Despite significant hypoxemia (arterial SpO<sub>2</sub>  $83 \pm 1$  %, mean  $\pm$  SEM), and exaggerated erythropoiesis, baseline hepcidin levels in CMS patients ( $8.8 \pm 3.0$  ng/ml) were similar to those reported both for healthy Peruvian lowlanders at sea level<sup>2</sup> and healthy high-altitude populations in Ethiopia.<sup>8</sup> This seems likely to reflect the relatively stable, albeit elevated, erythropoietic activity in these groups, and is in keeping with classical concept of an erythroid regulator that signals imbalance between erythropoietic drive and iron supply, rather than absolute marrow activity.<sup>13</sup>

Venesection produced a 20% fall in hematocrit by day 5 ( $P < .01$ , paired Student's  $t$  test), accompanied by a rise in plasma EPO ( $P < .05$ ) and a marked fall in plasma hepcidin ( $P < .05$ ). This fall occurred without any significant rise in GDF15, sTfR or FAM132B, and before the subsequent reduction in serum ferritin and transferrin saturation. Hepcidin suppression persisted for the duration of the study period, with undetectable plasma levels in most patients on days 12 and 19.

These findings support erythroid activity, rather than hypoxia *per se*, as the major stimulus for hepcidin suppression at high altitude. Our results show a temporal association between changes EPO and hepcidin following venesection, but do not identify a clear role for systemic GDF15, sTfR, FAM132B or iron availability in linking erythropoietic drive to hepcidin suppression in this setting.

**Acknowledgements:** We are grateful to the participants in this study, which was approved by the Oxford Tropical Research Ethics Committee (Oxford, UK) and the Universidad Peruana Cayetano Heredia Research Ethics Committee (Lima, Peru). All volunteers gave written, informed consent. The research was funded by the Wellcome Trust.

**Contributions:** N.P.T. and T.G.S. planned and performed research, analysed and interpreted data and wrote the manuscript. S.L.L., C.G. and M.R.C. planned and performed research. K.L.D., D.R.M. and P.A.R. planned the research and interpreted the data.

**Conflict-of-interest disclosure:** The authors declare no competing financial interests.

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## References

1. Piperno A, Galimberti S, Mariani R, et al. Modulation of hepcidin production during hypoxia-induced erythropoiesis in humans in vivo: data from the HIGHCARE project. *Blood*. 2011;117(10):2953-2959.
2. Talbot NP, Lakhali S, Smith TG, et al. Regulation of hepcidin expression at high altitude. *Blood*. 2012;119(3):857-860.
3. Peyssonnaud C, Zinkernagel AS, Schuepbach RA, et al. Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). *J Clin Invest*. 2007;117(7):1926-1932.
4. Gorduek VR, Miasnikova GY, Sergueeva AI, et al. Chuvash polycythemia *VHL*<sup>R200W</sup> mutation is associated with down-regulation of hepcidin expression. *Blood*. 2011;118(19):5278-5282.
5. Volke M, Gale DP, Maegdefrau U, et al. Evidence for a lack of a direct transcriptional suppression of the iron regulatory peptide hepcidin by hypoxia-inducible factors. *PLoS One*. 2009;4(11):e7875.
6. Liu Q, Davidoff O, Niss K, Haase VH. Hypoxia-inducible factor regulates hepcidin via erythropoietin-induced erythropoiesis. *J Clin Invest*. 2012;122(12):4635-4644.
7. Tanno T, Bhanu NV, Oneal PA, et al. High levels of GDF15 in thalassemia suppress expression of the iron regulatory protein hepcidin. *Nat Med*. 2007;13(9):1096-1101.
8. Lundgrin EL, Janocha AJ, Koch CD, et al. Plasma hepcidin of Ethiopian highlanders with steady-state hypoxia. *Blood*. 2013;122(11):1989-1991.
9. Tarkun P, Mehtap O, Atesoğlu EB, Geduk A, Musul MM, Hacıhanefioglu A. Serum hepcidin and growth differentiation factor-15 (GDF-15) levels in polycythemia vera and essential thrombocythemia. *Eur J Haematol* 2013;91(3):228-235.

10. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet.* 2014;46(7):678-684.
11. León-Velarde F, Maggiorini M, Reeves JT, et al. Consensus statement on chronic and subacute high altitude diseases. *High Alt Med Biol.* 2005;6(2):147-157.
12. Smith TG, Talbot NP, Privat C, et al. Effects of iron supplementation and depletion on hypoxic pulmonary hypertension: two randomized controlled trials. *JAMA* 2009;302(13):1444-1450.
13. Finch C. Regulators of iron balance in humans. *Blood.* 1994;84(6):1697-1702.

## Figure legend

**Figure 1. Effect of venesection in high altitude residents with chronic mountain sickness.** Open bar represents 500 mL isovolemic venesection on each of four consecutive days (days 1-4; total volume 2000 mL). Hematocrit was estimated by microcentrifugation (mean of two measurements). Arterial oxygen saturation was measured by pulse oximetry (Nonin Onyx, Nonin Medical, USA). Serum for analysis of ferritin, iron and total iron binding capacity (TIBC) was stored at 4°C and analysed within 72 h at sea level (Medlab, Lima, Peru). Transferrin saturation was calculated as  $100(\text{serum iron}/\text{TIBC})$ . Serum was stored at -20°C for EPO ELISA (Medlab, Lima, Peru). Plasma was stored at -20°C for hepcidin (Bachem, UK), GDF15 and sTfR (R&D Systems, UK) and FAM132B ELISA. In the case of FAM132B, samples were assayed using two independent ELISA kits (Cusabio Biotech, China and Avisaera Bioscience, USA). Results are provided for the Cusabio Biotech assay (manufacturer's product code CSB-EL008059HU) because the detection range most closely matched the range of FAM132B values in our samples, but neither assay revealed any evidence of an effect of venesection on FAM132B. Individual time points (mean  $\pm$  SEM) were compared with baseline (day 0) using paired Student's *t* tests. \* indicates statistically significant difference ( $P < .05$ ).

Figure 1

