

### Transient hypoxia and downregulation of circulating prohepcidin concentrations in healthy young men

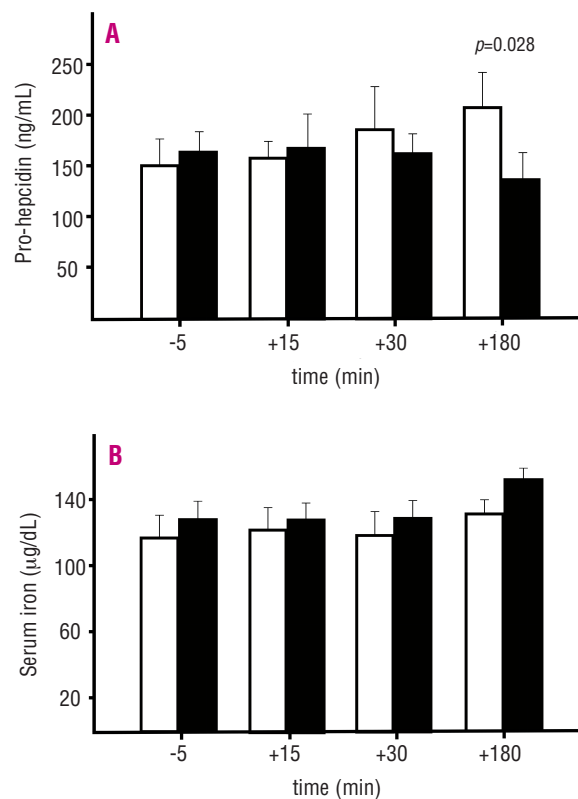
To determine the impact of acute hypoxia on prohepcidin concentrations in humans, we measured concentrations of this peptide in serum collected during and after a transient period (30 min) of hypoxia and during normoxia. Prohepcidin concentrations were significantly lower 150 min after the end of hypoxia than after normoxia ( $p=0.028$ ).

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Systemic oxygen deficiency (i.e. hypoxia) results in elevated serum iron concentrations.<sup>1</sup> This metabolic response to hypoxia appears to be primarily mediated by the downregulation of the hormone hepcidin which has been proposed to reduce circulating iron concentrations.<sup>2</sup> The mechanisms responsible for this downregulation of circulating hepcidin concentrations are unknown but are likely to involve a direct effect of hypoxia on hepatocytes, since it is known that hepcidin gene expression in human HepG2 and Hep3B hepatoma cells is reduced under hypoxic conditions.<sup>3</sup> Hepcidin originates from extrahepatic enzymatic cleavage of its prehormone prohepcidin, which in turn is produced by the liver.<sup>4</sup>

To examine the effects of transient hypoxia on circulating prohepcidin concentrations in humans, we measured prohepcidin levels in serum samples taken during a previous hypoxia study conducted in 14 healthy men of normal weight (age:  $24.3\pm 3.5$  years).<sup>5</sup> Additionally, circulating iron concentrations were measured. The subjects were tested under hypoxic and normoxic conditions while undergoing hyperinsulinemic euglycemic clamp procedures separated by an interval of at least 4 weeks. During the experimental sessions, subjects lay on a bed with the trunk in an almost upright position ( $60^\circ$ ). After 3 hours of clamping, hypoxia (oxygen saturation:  $74.3\pm 2.4\%$ ) was induced for 30 minutes. During the induction of hypoxia and also during the normoxic control period, participants breathed through a tightly fitting face mask connected to a Trajan 808 fresh gas supply (Draeger Medical Technology, Luebeck, Germany). During hypoxia, the oxygen supply was lowered by adjusting the oxygen and nitrogen balance. During normoxia, subjects breathed ambient air with an oxygen saturation averaging  $98.3\pm 1.7\%$ . After 30 min of hypoxia, the oxygen saturation was quickly normalized. Blood samples for determination of circulating iron (Sigma Diagnostics, St. Louis, MO, USA) and prohepcidin (DRG International, Mountainside, NJ, USA) were collected at baseline (-5 min), during hypoxia or normoxia (+15 and +30 min), and 150 min after the hypoxic or normoxic control period (+180 min). Each subject gave written informed consent, and the study was approved by the local ethics committee.

Statistical analyses revealed that circulating prohepcidin concentrations were distinctly lower 150 min after the end of hypoxia (+180 min) after the normoxic session (hypoxic vs. normoxic session:  $136.10\pm 26.69$  vs.  $208.00\pm 34.77$  ng/mL,  $p=0.028$ , after Bonferroni's correction, Figure 1A). However, the undersupply of oxygen from 0 to 30 min did not affect circulating prohepcidin concentrations acutely during this time period (Figure



**Figure 1.** Effects of transient hypoxia on circulating prohepcidin concentrations in humans. Mean values ( $\pm$ SEM) are indicated. Serum prohepcidin and serum iron concentrations (1A and 1B, respectively) before, during and after a 30 min period of hypoxia (black bars) or normoxia (white bars). During the 30-min hypoxic session, the oxygen saturation was lowered to  $\sim 75\%$ . Bonferroni's correction was applied to  $p$  values. The large SEM values are attributable to the inter-individual differences in serum prohepcidin concentrations, not to differences between conditions.

1A). Circulating iron concentrations during and after the hypoxic session did not differ from those during and after the normoxic session ( $p>0.155$ , Figure 1B).

To the best of our knowledge, the present study directly tests for the first time a short exposure to hypoxia (30 min) in humans and its effect on prohepcidin. Our results demonstrate that transient hypoxia causes a reduction in circulating prohepcidin concentrations in healthy young men. Contrary to previous findings of enhanced circulating iron uptake after hypoxic treatment,<sup>6</sup> in our study hypoxia did not cause any changes of this parameter in serum. Considering that previous studies investigated the effects of hypoxia for 3 days to 4 weeks on iron-related measures, the duration of hypoxia in the present study may have been insufficient to cause changes in serum iron measures. However, our results are supported by previous findings that hepcidin but not prohepcidin is significantly related to the iron status,<sup>7-9</sup> suggesting that prohepcidin may decrease after short-term hypoxia, but not hepcidin.

The striking statistical difference between serum prohepcidin levels following hypoxic and normoxic conditions was due partly to the decrease associated with

hypoxia, but also to the unexpected increase observed with normoxia. Considering that all subjects underwent a hyperinsulinemic euglycemic clamp in both conditions, it cannot be ruled out that hyperinsulinemia induced the increase of circulating prohepcidin which occurred during normoxia. Even so, our results clearly demonstrate that hypoxia, which was also performed under hyperinsulinemic euglycemia, is a strong stimulus suppressing the prohepcidin pathway. Although not described in the literature, this increase of serum hepcidin in the normoxic condition might also originate from a circadian oscillation of the peptide. Further studies are needed to clarify this issue.

In summary, our study provides evidence that hypoxia lowers circulating prohepcidin concentrations in humans. The metabolic consequences of this decrease cannot be derived from our study because of the short sampling time and the lack of measurements of serum hepcidin and do, therefore, need to be assessed in further studies.

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## References

1. Toth I, Yuan L, Rogers JT, Boyce H, Bridges KR. Hypoxia alters iron-regulatory protein-1 binding capacity and modulates cellular iron homeostasis in human hepatoma and erythroleukemia cells. *J Biol Chem* 1999;274:4467-73.
2. Ganz T, Nemeth E. Iron imports. IV. Heparin and regulation of body iron metabolism. *Am J Physiol Gastrointest Liver Physiol* 2006;290:G199-G203.
3. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest* 2002;110:1037-44.
4. Kulaksiz H, Theilig F, Bachmann S, Gehrke SG, Rost D, Janetzko A, et al. The iron-regulatory peptide hormone hepcidin: expression and cellular localization in the mammalian kidney. *J Endocrinol*. 2005;184:361-70.
5. Oltmanns KM, Gehring H, Rudolf S, Schultes B, Rook S, Schweiger U, et al. Hypoxia causes glucose intolerance in humans. *Am J Respir Crit Care Med* 2004; 169:1231-7.
6. Leung PS, Srai SK, Mascarenhas M, Churchill LJ, Debnam ES. Increased duodenal iron uptake and transfer in a rat model of chronic hypoxia is accompanied by reduced hepcidin expression. *Gut* 2005;54:1391-5.
7. Taes YE, Wuyts B, Boelaert JR, De Vriese AS, Delanghe JR. Prohepcidin accumulates in renal insufficiency. *Clin Chem Lab Med* 2004;42:387-9.
8. Kemna E, Pickkers P, Nemeth E, van der HH, Swinkels D. Time-course analysis of hepcidin, serum iron, and plasma cytokine levels in humans injected with LPS. *Blood* 2005; 106:1864-6.
9. Hadley KB, Johnson LK, Hunt JR. Iron absorption by healthy women is not associated with either serum or urinary prohepcidin. *Am J Clin Nutr* 2006;84:150-5.