Effects of Iron Supplementation and Depletion on Hypoxic Pulmonary Hypertension
Two Randomized Controlled Trials

Thomas G. Smith, MBBS, DPhil
Nick P. Talbot, BMBCh, DPhil
Catherine Privat, BSc
Maria Rivera-Ch, PhD
Annabel H. Nickol, MBBS, PhD
Peter J. Ratcliffe, BMBCh, MD
Keith L. Dorrington, DM, DPhil
Fabiola León-Velarde, PhD
Peter A. Robbins, BMBCh, DPhil

Hypoxia-induced pulmonary hypertensive disorders are a major cause of morbidity and mortality in respiratory and cardiac diseases and at high altitude. Hypoxia causes pulmonary hypertension through hypoxic pulmonary vasoconstriction and vascular remodeling. Convergent discoveries in the biochemistry of oxygen sensing and in cardiopulmonary physiology have recently established the importance of the hypoxia-inducible factor (HIF) family of transcription factors in regulating these processes.

Hypoxia-inducible factor controls an extensive range of transcriptional responses to hypoxia throughout the body. Emerging evidence supports a role for HIF in regulating systemic responses to hypoxia across the principal organ systems responsible for oxygen delivery to cells, encompassing erythropoiesis as well as pulmonary vascular tone.

Context Hypoxia is a major cause of pulmonary hypertension in respiratory disease and at high altitude. Recent work has established that the effect of hypoxia on pulmonary arterial pressure may depend on iron status, possibly acting through the transcription factor hypoxia-inducible factor, but the pathophysiological and clinical importance of this interaction is unknown.

Objective To determine whether increasing or decreasing iron availability modifies altitude-induced hypoxic pulmonary hypertension.

Design, Setting, and Participants Two randomized, double-blind, placebo-controlled protocols conducted in October-November 2008. In the first protocol, 22 healthy sea-level resident men (aged 19-60 years) were studied over 1 week of hypoxia at Cerro de Pasco, Peru (altitude 4340 m). In the second protocol, 11 high-altitude resident men (aged 30-59 years) diagnosed with chronic mountain sickness were studied over 1 month of hypoxia at Cerro de Pasco, Peru.

Intervention In the first protocol, participants received intravenous infusions of Fe(III)-hydroxide sucrose (200 mg) or placebo on the third day of hypoxia. In the second protocol, patients underwent staged isovolemic venesection of 2 L of blood. Two weeks later, patients received intravenous infusions of Fe(III)-hydroxide sucrose (400 mg) or placebo, which were subsequently crossed over.

Main Outcome Measure Effect of varying iron availability on pulmonary artery systolic pressure (PASP) assessed by Doppler echocardiography.

Results In the sea-level resident protocol, approximately 40% of the pulmonary hypertensive response to hypoxia was reversed by infusion of iron, which reduced PASP by 6 mm Hg (95% confidence interval [CI], 4-8 mm Hg), from 37 mm Hg (95% CI, 34-40 mm Hg) to 31 mm Hg (95% CI, 29-33 mm Hg; P = .01). In the chronic mountain sickness protocol, progressive iron deficiency induced by venesection was associated with an approximately 25% increase in PASP of 9 mm Hg (95% CI, 4-14 mm Hg), from 37 mm Hg (95% CI, 30-44 mm Hg) to 46 mm Hg (95% CI, 40-52 mm Hg; P = .003). During the subsequent crossover period, no acute effect of iron replacement on PASP was detected.

Conclusion Hypoxic pulmonary hypertension may be attenuated by iron supplementation and exacerbated by iron depletion.

Trial Registration clinicaltrials.gov Identifier: NCT00952302

JAMA. 2009;302(13):1444-1450 www.jama.com

©2009 American Medical Association. All rights reserved.
nary, cardiac, and vascular function.13,14 These findings are reflected in the clinical realm, in which rare mutations that have in common the potential to activate the HIF system have now been linked to the development of pulmonary hypertension10-12 and erythrocytosis.15

Hypoxia-inducible factor is primarily regulated through oxygen-dependent proteosomal degradation, which confers its ability to sense and respond to variations in oxygen availability.16 However, proteosomal degradation of HIF is also iron-dependent, requiring Fe(II) as an obligate cofactor.17-19 In cultured cells, HIF degradation is respectively potentiated and inhibited by increasing and decreasing iron availability.20,21 At the systemic level, our laboratory recently established that iron availability interacts with hypoxia in regulating the pulmonary circulation, in a manner that is consistent with involvement of HIF.22 In healthy iron-replete volunteers, intravenous infusions of iron blunted the pulmonary vasoconstrictive response to hypoxia, while reduction of iron availability with the iron chelator desferrioxamine enhanced hypoxic pulmonary vasoconstriction. These experiments were limited to studying responses in healthy volunteers over a period of 8 hours, and their importance for patients with hypoxic pulmonary hypertensive disease is unknown. Our aim was to investigate the time course and extent of the interaction between iron and hypoxia on pulmonary hypertension over days and weeks of hypoxia at high altitude.

METHODS

This study was conducted in October-November 2008. All protocols were randomized, double-blind, and placebo-controlled. In the sea-level resident (SLR) protocol, healthy volunteers (aged 19-60 years), recruited in Lima, Peru (at sea level), were studied during 1 week in the town of Cerro de Pasco, Peru (altitude 4340 m), where mean barometric pressure is approximately 450 mm Hg (equivalent to breathing approximately 12% oxygen at sea level). The primary outcome measure was the effect of iron infusion on pulmonary artery systolic pressure (PASP) assessed by Doppler echocardiography.

In the chronic mountain sickness (CMS) protocol, native high-altitude residents (aged 30-59 years) of Cerro de Pasco diagnosed with CMS were studied over 1 month in Cerro de Pasco. Chronic mountain sickness is characterized by an excessive erythropoietic response to the hypoxia of high altitude and is frequently complicated by pulmonary hypertension.4 Chronic mountain sickness is treated with venesection, which induces iron deficiency, and in these patients it is therefore possible to study the effects of both decreasing (through venesection) and increasing (through intravenous iron infusion) iron availability. The primary outcome measure was the effect of venesection-induced iron depletion on PASP.

In both protocols, randomization was performed at a single time point by permuted blocking using the random allocation rule, with numbered identical paper slips drawn randomly from a hat. In the CMS protocol, randomization was stratified by baseline PASP. All participants were men, who were taking no medications or supplements and who provided written informed consent. The Oxford Tropical Research Ethics Committee (Oxford, England) and the Universidad Peruana Cayetano Heredia Research Ethics Committee (Lima, Peru) approved the study, which was conducted in accordance with the Declaration of Helsinki. Remuneration of participants was intended to be neutral, providing appropriate compensation for loss of earnings incurred through taking part in the study without any undue incentive to participate. This was carefully determined in discussion with the research ethics committees and with consideration for local wages and the socioeconomic status of participants.

SLR Protocol

There were 22 participants in the SLR protocol (Figure 1). Volunteers were in good health, of lowland ancestry (at least second-generation born and raised at sea level), and had not traveled to high altitude within the preceding year. Baseline echocardiographic measurements and venous blood analyses of hematocrit, iron status, and erythropoietin concentration were conducted in Lima, Peru (day 0). Participants then ascended by road to Cerro de Pasco over approximately 8 hours, where repeated measurements were made throughout the following week. On the morning of the third day at high altitude, participants received an intravenous infusion of Fe(III)-hydroxide sucrose (doses of 200 mg in 100 mL administered over 30 minutes; Vifor Inc, St Gallen, Switzerland) or saline placebo (100 mL).

CMS Protocol

Eleven patients participated in the CMS protocol (Figure 1). Each patient had been previously diagnosed with CMS according to standard diagnostic criteria: excessive erythrocytosis (hemoglobin ≥21 g/dL) occurring in natives or long-term residents living more than 2500 m above sea level, hypoxemia, and absence of chronic pulmonary disease or concomitant medical conditions that worsen hypoxemia.23 At the commencement of the study, each patient had an increased hematocrit equivalent to a calculated hemoglobin concentration of more than 21 g/dL, with no history of venesection within the preceding year. Following baseline echocardiographic measurements and venous blood analyses (day 0), patients underwent isovolemic venesection of 500 mL on each of 4 consecutive days to remove a total of 2 L of blood (days 1-4). Measurements were repeated following the completion of venesection (day 5) and at weekly intervals (days 12 and 19). Patients then received intravenous infusions of Fe(III)-hydroxide sucrose (total dose 400 mg, administered in 2 infusions separated by 2 days, each consisting of a dose of 200 mg in 100 mL administered over 30 minutes) or saline placebo.
Table 1. Participant Characteristics in the Sea-Level Resident and Chronic Mountain Sickness Protocols

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo Group (n = 11)</th>
<th>Iron Group (n = 11)</th>
<th>Placebo-First Group (n = 5)</th>
<th>Iron-First Group (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>35 (11)</td>
<td>38 (12)</td>
<td>45 (9)</td>
<td>46 (12)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72 (14)</td>
<td>66 (22)</td>
<td>66 (6)</td>
<td>57 (6)</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.68 (0.03)</td>
<td>1.69 (0.10)</td>
<td>1.58 (0.05)</td>
<td>1.61 (0.07)</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>45 (1)</td>
<td>45 (1)</td>
<td>72 (7)</td>
<td>73 (7)</td>
</tr>
<tr>
<td>PASP, mm Hg</td>
<td>25 (4)</td>
<td>23 (2)</td>
<td>37 (10)</td>
<td>36 (13)</td>
</tr>
</tbody>
</table>

Abbreviation: PASP, pulmonary artery systolic pressure.

Table 2. PASP Changes in Individual Participants in the Sea-Level Resident Protocol

<table>
<thead>
<tr>
<th>Participant</th>
<th>Increment in PASP on Ascent to High Altitude, mm Hg</th>
<th>Increment in PASP Following Infusion, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant</td>
<td>Placebo Group</td>
<td>Iron Group</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>9</td>
</tr>
</tbody>
</table>

Abbreviation: PASP, pulmonary artery systolic pressure.

Echocardiographic Measurements

Pulmonary artery systolic pressure was determined by using a standard Doppler technique. A Vivid-i echocardiography machine (GE Medical Systems, Chalfont St Giles, Buckinghamshire, England) was used to determine the maximum systolic pressure gradient across the tricuspid valve and PASP was calculated using the modified Bernoulli equation and an estimated right atrial pressure of 5 mm Hg. Cardiac output was also determined echocardiographically.

Statistical Analyses

The study was powered to detect a difference of 6 mm Hg or more in PASP between groups or before and after venesection, based on a power of 80%, a 2-sided significance of .05, and the changes and variance recorded in our previous studies using Doppler echocardiography to measure PASP during hypoxia. Differences between paired observations in each protocol were assessed statistically by using t test and differences between experimental groups in each protocol were assessed by using analysis of variance (ANOVA) (SPSS version 16.0, SPSS Inc, Chicago, Illinois). Differences were considered significant at the P < .05 level. For the SLR protocol, a repeated measures ANOVA was employed using the four 12-hourly observations immediately before the infusion and the four 12-hourly observations immediately after the infusion. The within-participant factor was whether the measurement was made before or after infusion. The between-participant factor was whether the participant belonged to the placebo group or iron group.
To examine the effects of venesection in the CMS protocol, a paired t test was used to compare PASP before and after venesection, and before and after induction of iron deficiency. To examine the effects of iron infusion in the CMS protocol, a standard ANOVA was used with participant as a random factor and with time (days 19, 25, and 31 measurements), and whether the measurement was post–iron infusion as fixed factors. In all cases, actual changes rather than percentage changes were analyzed and there were no missing values in the analyses. All values are reported as mean (95% confidence interval [CI]) unless otherwise stated.

RESULTS
Baseline characteristics of participants are shown in Table 1. All infusions were well tolerated. Infusion of iron or placebo did not significantly affect cardiac output in either protocol.

SLR Protocol
Minor self-limiting symptoms of acute mountain sickness (headache, fatigue, nausea, difficulty sleeping) were common on ascent to high altitude, but there were no serious adverse effects. Baseline echocardiographic and hematological variables did not differ significantly between the iron and placebo groups. Incremental changes in PASP associated with exposure to high altitude and with iron or placebo infusions are shown for the individual participants in Table 2. Mean (SD) values for PASP are shown in Figure 2. Across all participants, there was a significant increase in PASP with exposure to high altitude of 14 mm Hg (95% CI, 12-17 mm Hg), from 24 mm Hg (95% CI, 23-25 mm Hg) to 39 mm Hg (95% CI, 36-41 mm Hg; P < .001) at day 3 of altitude. Infusion of iron reversed much of the increase in PASP caused by hypoxia, with PASP decreasing by 6 mm Hg (95% CI, 4-8 mm Hg), from 37 mm Hg (95% CI, 34-40 mm Hg) to 31 mm Hg (95% CI, 29-33 mm Hg) in the iron group compared with a decrease in the placebo group of 2 mm Hg (95% CI, –5 to 1 mm Hg), from 40 mm Hg (95% CI, 35-45 mm Hg) to 38 mm Hg (95% CI, 32-44 mm Hg). This difference between groups was significant (P = .01), and the effect of iron was evident within 4 hours of infusion.

Mean values for hematocrit, serum erythropoietin, transferrin saturation, and ferritin are shown in Figure 2. Across all participants, by day 3 at altitude, hematocrit had increased by 2% (95% CI, 1%-3%) (P < .001), erythropoietin had increased by 60 mU/mL (95% CI, 36-83 mU/mL) (P < .001), transferrin saturation had decreased by 10% (95% CI, 6%-13%) (P < .001), and ferritin had decreased by 21 ng/mL (95% CI, 7-35 ng/mL; to convert to pmol/L, multiply by 2.47) (P = .008). Serum iron decreased during this period by 24 µg/dL (95% CI, 13-35 µg/dL; to convert to µmol/L, multiply by 0.179), from 96 µg/dL (95% CI, 86-107 µg/dL) to 72 µg/dL (95% CI, 63-81 µg/dL) (P < .001). In the first 24 hours postinfusions, the reduction in erythropoietin in the iron group (decrease of 25 mU/mL;
Baseline hematocrit was 73% (95% CI, 69%-76%), equivalent to a calculated hemoglobin concentration of approximately 24 g/dL. Baseline PASP was elevated at 37 mm Hg (95% CI, 30-44 mm Hg) consistent with the high incidence of pulmonary hypertension in CMS. Venesection reduced hematocrit by 19% (actual change, 14%; 95% CI, 12%-16%) and caused a 10% increase in cardiac output (0.4 L/min; 95% CI, 0.1-0.6 L/min). Associated with these changes was an increase in PASP of 4 mm Hg (95% CI, 0-8 mm Hg) to 41 mm Hg (95% CI, 33-49 mm Hg). During the following 2 weeks, hematocrit and cardiac output remained stable and the progressive development of iron deficiency (indicated by a 66% reduction in ferritin of 118 ng/ml; 95% CI, 102-134 ng/ml; P < .001) was accompanied by a further significant increase in PASP that peaked at 48 mm Hg (95% CI, 40-55 mm Hg) and plateaued at 46 mm Hg (95% CI, 40-52 mm Hg) before the crossover period (P = .03). Overall, PASP increased by approximately 25% (9 mm Hg; 95% CI, 4-14 mm Hg) from baseline to the initial infusions (P = .003). During the crossover period, the group that received iron first (iron-first group) was compared with the group that received placebo first (placebo-first group) using ANOVA. In this between-group analysis, there was no significant effect of iron on PASP (P = .64), indicating that replacement of iron did not acutely oppose the increase in pulmonary artery pressure associated with iron depletion.

**COMMENT**

In contrast with other vascular beds, hypoxia constricts rather than dilates the pulmonary vasculature. An immediate increase in pulmonary artery pressure is followed by a slower-onset, progressive elevation that may arise from HIF-regulated alterations in gene expression and may thereby depend on iron availability. The results from the SLR protocol demonstrate that acute administration of iron results in substantial and sustained reversal of the hypoxia-induced elevation in pulmonary arterial pressure observed at high altitude. These findings have implications for hypoxic disease states involving pulmonary hypertension, including chronic obstructive pulmonary disease, congenital heart disease, and high-altitude diseases such as CMS and high-altitude pulmonary edema.

High-altitude pulmonary edema is a potentially fatal condition that occurs in lowlanders ascending rapidly to high alt...
titude and evidence suggests that it arises from an excessive pulmonary vasoconstrictive response to hypoxia. Our findings suggest that iron might attenuate this response in high-altitude pulmonary edema, as it did in the SLR protocol. The opposite might also be true—iron deficiency may be harmful in hypoxic pulmonary hypertensive disease. Iron deficiency can develop rapidly during the acute erythropoietic response provoked by ascent to altitude, and in future studies it will be of interest to understand whether there is any interaction between negative iron balance and adverse responses to altitude such as high-altitude pulmonary edema. Our findings also raise a concern that iatrogenic iron deficiency created by venesection could exacerbate certain types of chronic pulmonary hypertension.

This possibility is supported by the results from the CMS protocol in which reduction of iron availability was associated with a substantial increase in pulmonary arterial pressure. This effect, which was evident over several weeks, is consistent with the pulmonary sequelae of acute iron chelation and implies that clinical iron deficiency could exacerbate pulmonary hypertension in chronic pulmonary and cardiac disease. We are not aware of any published studies investigating such a link. However, intercurrent iron deficiency may provide an explanation for the puzzling lack of sustained pulmonary antihypertensive benefits from venesecting secondary erythrocytosis in cor pulmonale. These findings may also be relevant in complications of congenital heart disease such as Eisenmenger syndrome, where pulmonary hypertension, hypoxemia (although not alveolar hypoxia), and secondary erythrocytosis coexist. Venesection is now discouraged in these patients as the ensuing iron deficiency is thought to be detrimental, although there is no evidence that it affects the underlying cardiopulmonary pathology.

Our findings also have implications for the clinical management of CMS, where the imperative to limit red blood cell mass must now be balanced against the potential for worsening pulmonary hypertension through iron deficiency. Our findings further suggest that careful adjustment of iron balance may have a place in the broader management of hypoxic pulmonary hypertensive disease. In patients in the CMS group, replacement of iron did not acutely oppose the pulmonary hypertensive effects of iron deficiency. However, in the presence of long-standing pulmonary vascular remodelling, it is possible that effects of iron replenishment may be delayed (our protocol did not test this possibility), and it is further possible that the dose of 400 mg was inadequate to reverse rapidly the effects of venesection approximately 1 g of iron. Overall, this preliminary study justifies further exploration of the therapeutic potential of manipulating iron balance in hypoxic pulmonary hypertensive disease.

Although these human studies were predicated on the known biochemistry of HIF and genetic studies implicating HIF in regulating cardiopulmonary function, it is difficult to prove with certainty that HIF underlies the observed pulmonary effects of iron. Surrogate markers such as peripheral concentrations of HIF-regulated gene products (eg, erythropoietin) do not necessarily reflect HIF activity in the pulmonary circulation. Alternative theoretical explanations for the effects of iron reported in our study include possible involvement of some unknown iron-dependent oxygen sensor or the possibility that iron availability modified pulmonary arterial pressure through the formation of reactive oxygen species. However, our findings are entirely consistent with the respective pulmonary vascular manifestations of up- and down-regulation of HIF observed in humans and animals.

Irrespective of the precise mechanism, our study establishes an important role for iron in high-altitude physiology and associated hypoxic pulmonary disease. Until further evidence is forthcoming, we suggest that it may be prudent to avoid iron deficiency in hypoxic patients with pulmonary hypertension and in high-altitude pulmonary edema—susceptible individuals returning to high altitude.

**Table 3. PASP Changes in Individual Participants in the Chronic Mountain Sickness Protocol**

<table>
<thead>
<tr>
<th>Initial Infusion</th>
<th>Increment in PASP Following Venesection and Iron Depletion, mm Hg</th>
<th>Increment in PASP Following Initial Infusion, mm Hg</th>
<th>Increment in PASP Following Crossover Infusion, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo first</td>
<td>19</td>
<td>4</td>
<td>−7</td>
</tr>
<tr>
<td>Placebo first</td>
<td>1</td>
<td>9</td>
<td>−16</td>
</tr>
<tr>
<td>Placebo first</td>
<td>14</td>
<td>−7</td>
<td>−4</td>
</tr>
<tr>
<td>Placebo first</td>
<td>8</td>
<td>−5</td>
<td>4</td>
</tr>
<tr>
<td>Placebo first</td>
<td>−7</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>2 (−2 to 7)</td>
<td>−4 (−9 to 0)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence interval; PASP, pulmonary artery systolic pressure.

**Increment in PASP following venesection and iron depletion compares measurements made at baseline with those made on day 19 immediately before initial infusions.**

©2009 American Medical Association. All rights reserved.

(Reprinted) *JAMA*, October 7, 2009—Vol 302, No. 13 1449
REFERENCES


