

suitably equipped laboratory. Future work will focus on immune profile guided therapeutic trials for immuno-dysfunction as well as an exploration of whether the immune defects identified are simply additive or if there is synergism between them.

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Figures for this abstract can be accessed at www.ics.ac.uk/Meetings_Seminars/main_meetings/gold_medal_ab

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Research Gold Medal Presentations

Iron is an important factor in pulmonary physiology

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Scientific background

The interaction between hypoxia and pulmonary physiology is central to intensive care medicine. Physiological responses to hypoxia include an increase in pulmonary arterial pressure caused by hypoxic pulmonary vasoconstriction. This phenomenon is important in intensive care medicine, such as in patients with acute respiratory distress syndrome (ARDS),¹ as well as in other areas of anaesthesia and medicine.² However it can become pathological – pulmonary hypertension frequently complicates hypoxic lung disease and worsens patient survival.^{3,5} The mechanisms underlying pulmonary responses to hypoxia are poorly understood. This body of work has investigated these mechanisms and their therapeutic implications. Our original hypothesis was that the hypoxia-inducible factor

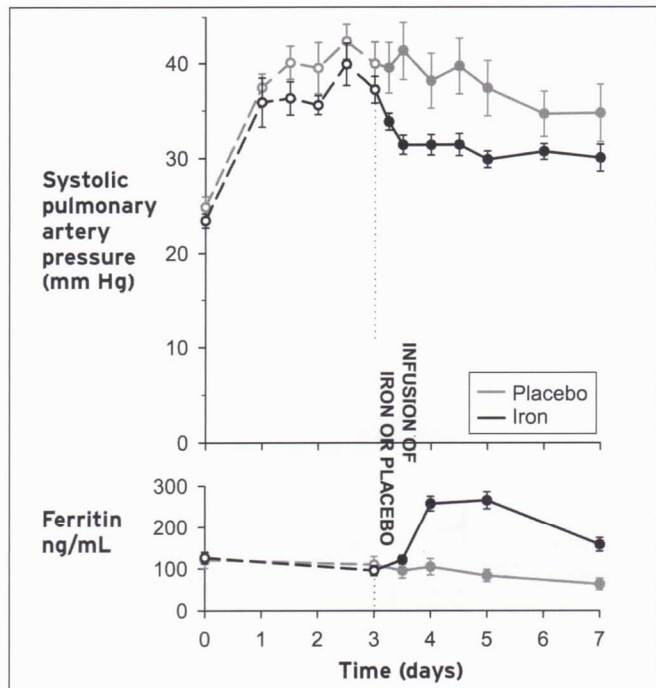


Figure 1. Effect of intravenous iron on systolic pulmonary artery pressure and serum ferritin in healthy sea-level residents during altitude hypoxia at 4,340 metres. Baseline measurements (Day 0) were made at sea level prior to ascent to high altitude. Dashed lines and open symbols indicate pre-infusion measurements, while solid lines and closed symbols indicate post-infusion measurements. Infusions were given immediately after measurements were made on the morning of Day 3 (indicated by the dotted vertical line). Black lines and symbols indicate the group that received an infusion of iron sucrose 200 mg (n=11), while grey lines and symbols indicate the group that received a placebo infusion (n=11). Infusion with iron reversed much of the elevation in pulmonary artery systolic pressure caused by hypoxia ($p<0.02$).¹¹ Values shown are mean – SEM.

(HIF) family of transcription factors, which are known to control intracellular responses to hypoxia,⁶ also control systemic cardiopulmonary responses to hypoxia to some extent. In each of the human studies, the primary outcome measure was pulmonary artery systolic pressure assessed by Doppler echocardiography.

Study of patients with a rare mutation

Although HIF is best known as the transcriptional activator of erythropoietin, in fact it controls cellular responses to hypoxia throughout the body.⁶ In order to examine involvement of HIF in systemic physiology, we studied the cardiopulmonary phenotype associated with Chuvash polycythaemia, an extremely rare autosomal recessive disease in which HIF-mediated gene expression is pathologically activated. Twelve participants were exposed to 10-minute periods of hypoxia on a mouthpiece system, using the technique of dynamic end-tidal forcing to control end-tidal partial pressures of oxygen (PETO₂) and carbon dioxide. Mild hypoxia (PETO₂ 70 mm Hg [9.3 kPa]) and moderate hypoxia (PETO₂ 50 mm Hg [6.7 kPa]) were tested. Patients with Chuvash polycythaemia were found to have pulmonary hypertensive, ventilatory and heart rate responses to hypoxia.^{7,8} Remarkably, the increase in pulmonary arterial pressure caused by hypoxia was 5-10 times greater in patients than control participants ($p<0.01$). This was the first study to elucidate a specific genetic determinant of human cardiopulmonary hypoxia physiology.

Study of mice with an engineered mutation

In a related animal study we used whole body plethysmography to measure the ventilatory response to acute hypoxia (12% oxygen) in various genetically engineered knockout mice. Compared with littermate controls, the ventilatory response was ~60% greater in mice deficient for prolyl hydroxylase domain-2, an enzyme that regulates HIF activity ($p<0.01$).⁹

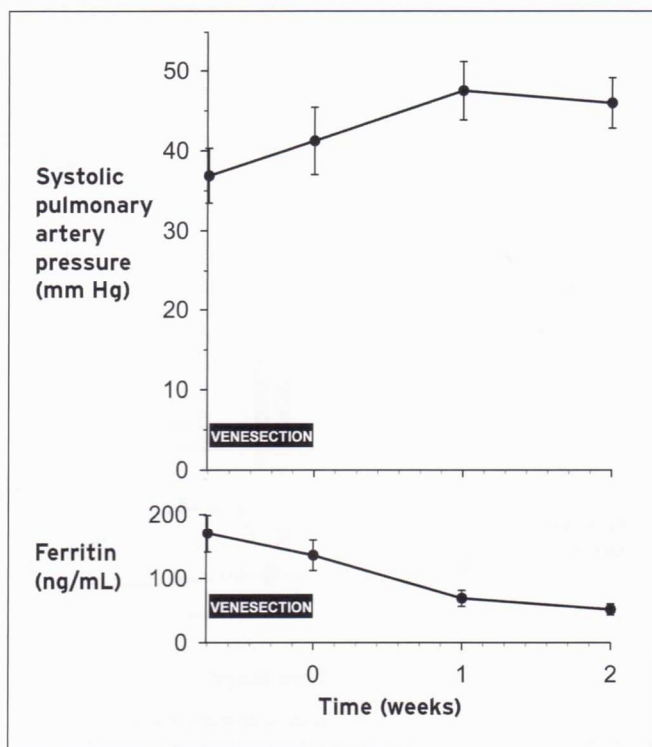


Figure 2. Effect of venesection on systolic pulmonary artery pressure and serum ferritin in chronic mountain sickness patients during altitude hypoxia at 4,340 metres. Eleven patients with chronic mountain sickness were studied. Measurements shown in this figure are those made prior to any iron or placebo infusions. The black bar indicates the period over which staged isovolaemic venesection of two litres of blood occurred. Over the following two weeks, a progressive reduction in ferritin ($p < 0.001$) was accompanied by a substantial increase in pulmonary artery systolic pressure compared with pre- and post-venesection values ($p < 0.03$). Haematocrit and cardiac output remained stable over this period and did not account for these changes (complete results reported in *JAMA* publication).¹¹ Values shown are mean \pm SEM.

Laboratory study of iron and pulmonary physiology

Together these human and animal studies implicated HIF in regulating cardiopulmonary responses to hypoxia. Our next study sought to manipulate these responses by manipulating HIF pharmacologically, exploiting the observation that intracellular HIF activity varies with iron availability.⁶ Eight healthy volunteers took part in a placebo-controlled crossover study.¹⁰ Each volunteer undertook two experimental days, one beginning with an intravenous infusion of saline placebo and one beginning with an intravenous infusion of iron sucrose 200 mg. Following the infusions, volunteers were exposed to acute hypoxia (as above) and to eight hours of sustained hypoxia in our laboratory's purpose-built hypoxia chamber (PETO₂ 55 mm Hg [7.3 kPa]). The normal increase in pulmonary arterial pressure caused by sustained hypoxia (a 23% increase in this study) was prevented by prior infusion of iron ($p < 0.001$).¹⁰

Field study of iron and pulmonary physiology using altitude hypoxia

Before this research programme, iron had not been considered as a factor in the aetiology or clinical management of any form of pulmonary hypertension. To explore whether this novel effect of iron might be clinically important, we conducted a major field study in the remote Andes of Peru.¹¹ Two randomised, double-blinded, placebo-controlled protocols were conducted in Cerro de Pasco (altitude 4,340 m) where barometric pressure is ~450 mm Hg (equivalent to breathing ~12% oxygen at sea level). In the first protocol, 22 healthy sea-level residents were studied over one week of hypoxia, and received intravenous iron sucrose (200 mg) or saline placebo on the third day. Infusion of iron reversed ~40% of the pulmonary hypertensive response to hypoxia ($p < 0.02$; see **Figure 1**). In the

second protocol, 11 high-altitude residents diagnosed with chronic mountain sickness (haemoglobin > 21 g/L) were studied over one month of hypoxia. Patients underwent staged isovolaemic venesection of two litres of blood, and two weeks later received intravenous iron sucrose (400 mg) or placebo, which were subsequently crossed over. Progressive iron deficiency induced by venesection was associated with a ~25% increase in pulmonary artery systolic pressure ($p < 0.03$; see **Figure 2**). This study demonstrated that hypoxic pulmonary hypertension is profoundly influenced by iron availability in a manner that is consistent with the biochemistry of HIF. These findings were recently published in the *Journal of the American Medical Association*.¹¹

Conclusions and ongoing work

This research programme has provided evidence that HIF, which coordinates the cellular response to hypoxia, also plays a major role in regulating the actual organ systems upon which cellular oxygen delivery ultimately depends. It has further established that hypoxic pulmonary hypertension is attenuated by iron supplementation and exacerbated by iron depletion. These findings have immediate clinical implications for the patients studied and, more generally, suggest there may be a place for careful adjustment of iron balance in the broader management of hypoxic lung disease. It is unusual for an agent as familiar, safe and inexpensive as iron to offer promise for a novel indication, and even a small beneficial effect in critically ill patients such as those with ARDS or end-stage pulmonary hypertension would be significant. A recent editorial commented that our work 'has vast clinical implications for the sickest patients seen in the hospital' and asked the question 'Should hypoxic patients in the intensive care unit receive supplemental iron?'.¹² At the least, until further evidence is forthcoming it may be prudent to avoid iron deficiency in critically ill patients with pulmonary hypertension.¹¹ It is interesting that although the reported prevalence of true iron deficiency in intensive care is ~10%,^{13,14} in practice this is rarely treated or even measured. We are preparing to extend our work with studies in intensive care and studies of ascorbate (vitamin C), which has the same intracellular effect on HIF as iron and may present a better therapeutic alternative.^{15,16}

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