The Iron Paradigm of Pulmonary Arterial Hypertension
Popeye Knows Best

Gopinath Sutendra, Sébastien Bonnet

Iron is a metal not only important in maintaining the structural integrity of the Earth’s core but also the normal physiological function of metazoans. In fact, the requirement of iron as a cofactor for iron–sulfur and iron-dependent proteins, many of which are involved in a host of metabolic and regulatory pathways, helped form the foundation for the iron–sulfur world theory on the origin of life by the German chemist Günter Wächtershäuser.1 It is therefore not surprising that a shift in the delicate balance of iron levels is implicated in a variety of pathological conditions that seem to have an underlying metabolic abnormality. For example, iron overload can increase the risk of metabolic syndrome,2 whereas iron deficiency is associated with cancer.3

A newly emerging metabolic disease is pulmonary arterial hypertension (PAH), a complex vascular remodeling disease resulting in pulmonary artery (PA) occlusion with an accompanying right ventricular (RV) hypertrophy and failure.4,5 PAH PA vascular cells, including endothelial cells, smooth muscle cells, and fibroblasts, seem to have a metabolic switch from mitochondrial-derived glucose oxidation to cytoplasmic-derived glycolysis,6–8 similar to cancer.9 The complexity of this disease is due, in part, to a diverse range of activated signaling pathways in the proliferative and apoptosis-resistant PA smooth muscle cells (PASMCs). These pathways range from activation of pro-proliferative transcription factors, such as hypoxia-inducible factor 1α, signal transducer and activator of transcription 3 and nuclear factor of activated T cells to induced epigenetic modifications, including microRNA aberrant expression,10 methylation resulting in decreased expression of mitochondrial enzymes, such as manganese superoxide dismutase11 or single-nucleotide polymorphisms to sirtuin-3.15 Although a primary mitochondrial suppression in PASMCs has provided strong evidence for how many of these seemingly unrelated pathways may converge into 1 central metabolic pathway,6,7 it still remains unclear which primary signals result in inhibition of mitochondrial or metabolic proteins in PAH. Intriguingly, many of the complexes, dehydrogenases, reductases, hydroxylases, and demethylases important for mitochondrial or metabolic function are iron-dependent. For example, both complex I and II of the mitochondrial electron transport chain have multiple iron–sulfur clusters,13 whereas iron is a required cofactor for the hydroxylases that regulate hypoxia-inducible factor 1α stability and transcriptional activity,14 suggesting that iron deficiency could result to mitochondrial suppression. This is in keeping with a previous report that shows iron deficiency can suppress mitochondrial function and induce a metabolic switch from glucose oxidation to glycolysis.15 Taken together, these observations suggest that iron deficiency could explain the mitochondrial suppression and metabolic changes, along with the proliferative and apoptosis-resistant signaling pathways identified in PAH (Figure). Furthermore, iron deficiency has also been shown to be associated with clinical PAH16,17; however, evidence supporting iron deficiency as a direct trigger in the development of PAH is lacking.

In this issue of Circulation Research, Cotroneo et al,18 provide evidence that iron deficiency in Sprague-Dawley rats can induce mitochondrial suppression resulting in PA vascular proliferation and apoptosis resistance, PA occlusion and PAH. Many of the molecular features observed in PAH were also present in the iron-deficient lungs, including activated hypoxia-inducible factor 1α, nuclear factor of activated T cells, and signal transducer and activator of transcription 3. In vitro, treatment of human PASMCs with the iron chelator deferoxamine resulted in suppressed mitochondrial function as indicated by increased mitochondrial membrane potential, decreased reactive oxygen species production, and decreased mitochondrial complex I activity. Furthermore, a metabolic switch from glucose oxidation to glycolysis was detected in the lungs of iron-deficient animals. This study suggests that iron deficiency in the lungs may be central to the mitochondrial suppression and metabolic remodeling observed in PAH. Intriguingly, therapeutic intervention with the metabolic modulating compound dichloroacetate, which is known to increase mitochondrial glucose oxidation, or the tyrosine kinase inhibitor imatinib, reversed the iron-deficiency–mediated PAH. Although increasing mitochondrial function with dichloroacetate has previously been shown to reverse many preclinical animal models of PAH,6,19 along with other proliferative diseases, including cancer,20 these results suggest that dichloroacetate could increase mitochondrial function in an iron-independent manner, or alternatively, regulate iron homeostasis.
An intriguing finding was that systemic vessels were completely unaffected in iron-deficient animals, similar to what is observed in patients with PAH. Michelakis et al. had previously shown the existence of mitochondrial diversity between the systemic and PASMCs, with differences in the expression profile of mitochondrial electron transport chain complexes and dismutases. In that study, the authors concluded that these differences in systemic and pulmonary SMC mitochondria could explain why the PAs constrict in response to hypoxia, whereas the systemic arteries dilate, as mitochondria are important oxygen sensors in the vasculature. Taken together with this study, it is feasible that similar to oxygen sensing, the PAs may also be more sensitive to iron depletion than the systemic arteries; however, more work is still required to explain this interesting finding.

A major limitation for the current preclinical models of PAH is that they do not completely recapitulate the human disease. These include the more commonly used inducible forms of PAH, with the use of a toxin, such as monocrotaline in rats or with chronic hypoxia, either alone or in combination with the vascular endothelial growth factor inhibitor (Sugen, Inc, Redwood City, CA) in mice or rats. This study suggests that iron deficiency in rats could be a new preclinical model for PAH. Although this model does recapitulate many of the features observed in patients with PAH, including a profound pulmonary vascular remodeling, prominent infiltration of the PA vasculature with inflammatory cells, increased (18F)-fluorodeoxyglucose positron emission tomography signal in the lungs, RV hypertrophy, increased mean PA pressure, and pulmonary vascular resistance, further studies are required to address if these animals have increased mortality and succumb to RV failure. It will also be important to determine if an iron-deficient diet can also induce PAH in mice, a more relevant species for transgenic models.

An interesting observation of this study was an increase in cardiac output in the iron-deficient animals, which is in contrast to what is normally observed in patients with PAH. It is feasible that the increase in cardiac output may have been a consequence of a modest anemia (as there was a ≈25% decrease in hemoglobin levels compared with control animals), resulting to increased PA blood flow. This increase in cardiac output, commonly observed in anemic patients, is a compensatory mechanism to sustain oxygen delivery to the tissues. Therefore, in this scenario, the original insult resulting in PAH may not have been a primary metabolic dysfunction in the PA vascular cells, but rather a result of flow-induced PA endothelial damage, initiating a cascade of events resulting in PASMC proliferation and vascular remodeling. Further work needs to address if the increase in cardiac output was a consequence or direct trigger in this model of PAH.

One caveat of this work is that it is in contrast to other studies that claim iron deficiency may be beneficial for PAH. For example, in the monocrotaline model of PAH in Sprague-Dawley rats, dietary iron restriction has been shown to attenuate PA vascular remodeling and RV failure. In that study by Naito et al., an iron-deficient diet was initiated 1 day after monocrotaline injection and resulted in decreased PA wall thickness and a modest decrease in RV systolic pressures. A limitation of that study was that neither mean PA pressures nor cardiac output was measured. Potential explanations for the contradictory effects of an iron-deficient diet between these 2 studies may include differences in tissue iron levels or hemoglobin levels. In both studies an iron-deficient diet resulted in depleted iron levels in the serum. However, it also depleted iron stores in the lung, liver, and myocardium and reduced hemoglobin levels and erythropoiesis in this study by Cotroneo et al., whereas an iron-deficient diet did not result to any significant differences in hemoglobin levels or erythropoietin concentration in the Naito et al. study. An intriguing concept may be that a modest decrease in iron levels may prove beneficial in

**Figure.** Suppressed iron-dependent signaling may explain the metabolic remodeling in pulmonary arterial hypertension (PAH) pathogenesis. Iron is a required cofactor for all iron–sulfur and iron-dependent proteins, many of which are required for mitochondrial function. These include mitochondrial Krebs cycle enzymes, complexes I and II of the electron transport chain, proteins or microRNAs implicated in mitochondrial biogenesis or function and transcription factors known to suppress mitochondrial function, such as hypoxia-inducible factor (HIF) 1α via regulation of hydroxylases. Iron-deficiency-mediated mitochondrial suppression can result in (1) decreased glucose oxidation via inhibition of the pyruvate dehydrogenase complex (PDC) by HIF1α-mediated expression of pyruvate dehydrogenase kinase (PDK), (2) increased mitochondrial membrane potential, (3) suppressed mitochondrial-mediated apoptosis, (4) an enhanced inflammatory response, (5) decreased generation of mitochondria-derived reactive oxygen species (mROS) or α-ketoglutarate (αKG), and (6) activation of HIF1α nuclear factor of activated T cells (NFAT) and signal transducer and activator of transcription 3 (STAT3), all of which promote cellular proliferation and PAH. In addition, activation of HIF1α, NFAT, and STAT3 can be both a cause and a result of mitochondrial suppression, reinforcing a positive feedback loop. The traffic light below depicts the proteins and pathways implicated either in PAH (red) or by iron regulation (green).
PAH, by selectivity inhibiting specific iron-dependent proteins known to be implicated in PAH signaling, such as the nuclear factor of activated T cells–activating phosphatase calcineurin\(^\text{25}\) without compromising mitochondrial function, whereas a more significant decrease in iron levels is required for global mitochondrial suppression and PAH pathogenesis. Although an attractive explanation, more detailed work is required to elucidate the precise mechanism for how iron deficiency may be both beneficial and detrimental in PAH.

An important finding from this work was that iron supplementation resulted in partial reversal of iron-deficient–mediated PAH. Although iron supplementation (via intravenous injection of ferric carboxymaltose twice a week) resulted in \(\approx 4\)-fold increase in lung iron levels, a \(\approx 2\)-fold increase was also detected in the myocardium and blood ferritin levels were increased \(\approx 22\)-fold compared with control animals. As there are currently 2 clinical trials (NCT01446848 and NCT01288651 according to clinicaltrials.gov) on the use of iron supplementation as a therapeutic intervention in patients with PAH, caution needs to be practiced as increased iron levels could result in a tissue-specific iron overload, resulting in detrimental effects. For example, high levels of iron in the myocardium could result in iron-overload cardiomyopathy.\(^\text{26}\)

As iron metabolism varies among tissues, the precise mode of delivery, ensuring repletion in the lung while avoiding over-load in the heart or other organs, may be a clinical challenge.

The work by Cotroneo et al.\(^\text{18}\) not only suggests that PAH may need to be added to the long list of iron-related diseases, but also adds merit to Popeye's concept on the health benefits of iron-rich foods, potentially providing clues for new and exciting therapeutic options for this deadly disease.

**Sources of Funding**

This work was supported, in part, by grants from the Heart and Stroke Foundation of Canada, the Canadian Institute for Health Research, and a Canadian Research Chair of Canada to S. Bonnet.

**Disclosures**

None.

**References**


**Key Words:** Editors | iron-deficiency | metabolism | mitochondria | pulmonary hypertension