Journal of the American College of Cardiology
© 2013 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 61, No. 18, 2013
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2013.02.012

Heme Levels Are Increased in Human Failing Hearts

Arineh Khechaduri, MS, Marina Bayeva, PhD, Hsiang-Chun Chang, BA, Hossein Ardehali, MD, PhD
Chicago, Illinois

Objectives The goal of this study was to characterize the regulation of heme and non-heme iron in human failing hearts.

Background Iron is an essential molecule for cellular physiology, but in excess it facilitates oxidative stress. Mitochondria are the key regulators of iron homeostasis through heme and iron-sulfur cluster synthesis. Because mitochondrial function is depressed in failing hearts and iron accumulation can lead to oxidative stress, we hypothesized that iron regulation may also be impaired in heart failure (HF).

Methods We measured mitochondrial and cytosolic heme and non-heme iron levels in failing human hearts retrieved during cardiac transplantation surgery. In addition, we examined the expression of genes regulating cellular iron homeostasis, the heme biosynthetic pathway, and micro-RNAs that may potentially target iron regulatory networks.

Results Although cytosolic non-heme iron levels were reduced in HF, mitochondrial iron content was maintained. Moreover, we observed a significant increase in heme levels in failing hearts, with corresponding feedback inhibition of the heme synthetic enzymes and no change in heme degradation. The rate-limiting enzyme in heme synthesis, delta-aminolevulinic acid synthase 2 (ALAS2), was significantly upregulated in HF. Overexpression of ALAS2 in H9c2 cardiac myoblasts resulted in increased heme levels, and hypoxia and erythropoietin treatment increased heme production through upregulation of ALAS2. Finally, increased heme levels in cardiac myoblasts were associated with excess production of reactive oxygen species and cell death, suggesting a maladaptive role for increased heme in HF.

Conclusions Despite global mitochondrial dysfunction, heme levels are maintained above baseline in human failing hearts. (J Am Coll Cardiol 2013;61:1884–93) © 2013 by the American College of Cardiology Foundation

Heart failure (HF) rates have soared over the last decade, with almost 6 million Americans affected; the prevalence of the disease is rising steadily (1). Molecular mechanisms of HF are complex (2), but mitochondrial dysfunction is an early and common finding in hypertrophied and failing hearts (3). Virtually all aspects of mitochondrial physiology are deranged in HF (4), including mitochondrial number and biogenesis (5), energetic capacity (6), and production of reactive oxygen species (ROS) (7). In addition to generating adenosine triphosphate (ATP), mitochondria play a major role in the regulation of iron balance and synthesis of heme and iron-sulfur (Fe/S) clusters (8,9); however, these processes have not been systematically examined in failing hearts.

Indirect evidence points to a link between cellular and mitochondrial iron regulation and HF. Iron is an essential molecule that functions as a cofactor in mitochondrial cytochromes, antioxidant enzymes, and more, and its deficiency is associated with cardiomyopathy (10). In contrast, the redox properties of iron make it an ideal catalyst for the production of a toxic hydroxyl radical (OH ·) by the Fenton reaction (11), and cardiac dysfunction is a prominent feature of iron overload diseases, such as hemochromatosis (12). Elevated iron levels were noted in the hearts of mice overexpressing the alpha subunit of the Gq protein, a model of cardiomyopathy (13); however, cellular distribution of iron has not been assessed. Recently, we have shown that accumulation of iron, specifically in the mitochondria through a reduction in the levels of ATP-binding cassette transporter B8 (a protein involved in mitochondrial iron export), leads to the development of cardiomyopathy in mice (14). Similarly, aggregation of iron inside the mitochondria has been observed in the hearts of Friedreich's ataxia patients (15), who develop progressive and lethal cardiac dysfunction (16). Thus, maintenance of iron balance inside the heart appears to be critical for its function, but it remains unknown how iron regulation is altered in failing human hearts.

From the Feinberg Cardiovascular Research Institute, Northwestern University School of Medicine, Chicago, Illinois. Dr. Bayeva is supported by the American Heart Association (AHA) Midwest Affiliate Predoctoral Fellowship (10PRE4430021). Dr. Ardehali is supported by the National Institutes of Health Grants (K02 HL107448, R01 HL104181, and 1P01 HL108795). Dr. Ardehali has been a member of the Speaker's Bureau for Merck and has been a consultant for Takeda, Cubist Pharma, and the Gerson Lehrman Group. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received November 21, 2012; revised manuscript received January 15, 2013, accepted February 5, 2013.

Iron enters the mitochondria through an inner membrane transporter mitoferrin 2 (Mfrn2) (17,18), and can either be stored in a complex with mitoferritin (FtMt) or used in heme and Fe/S cluster biosynthetic pathways (19). Heme production begins in the mitochondria with condensation of glycine and succinyl coenzyme A to form delta-aminolevulinic acid (ALA) by the rate-limiting enzyme ALA synthase (ALAS) (9). The next 5 conversions are carried out in the cytosol. Finally, the synthesis is completed in the mitochondria with insertion of an iron atom into protoporphyrin IX (PPIX) to form heme by ferrochelatase (9) (Fig. 1). In the heart, heme functions as a catalytic or structural subunit of mitochondrial electron transport chain (ETC) complexes, myoglobin, antioxidant enzymes, and components of the cytochrome p450 (20). Moreover, heme can be broken down by heme oxygenases (HMOX) into elemental iron, carbon monoxide, and cardioprotective antioxidant biliverdin (21). Despite the essential role heme plays in the heart, this molecule remains understudied outside of the erythropoietic system.

Fe/S cluster assembly requires over 20 enzymes and scaffolding proteins, and takes place primarily in the mitochondria (8). Fe/S clusters are incorporated into mitochondrial, cytoplasmic, and nuclear proteins involved in oxidative phosphorylation, DNA repair, purine metabolism, and heme production (22,23). Disruption of Fe/S cluster synthesis may also lead to cardiomyopathy, as noted in patients with Friedreich's ataxia (24) and mice with deletion of ABCB8 (14), although these conditions are also associated with mitochondrial iron accumulation and ROS.

Given the critical role mitochondria play both in iron homeostasis and in HF, we aimed to characterize the changes in cytosolic and mitochondrial heme and non-heme iron regulation in human failing hearts. Our results demonstrate that both cytosolic and mitochondrial heme levels are increased in failing hearts, with feedback inhibition of the heme synthetic enzymes, except for ALAS2, which is increased in HF, and whose expression was previously reported to be restricted to the hematopoietic system. We also show that in H9c2 cardiac myoblasts, ALAS2 expression and heme levels are regulated by hypoxia and erythropoietin (EPO), the 2 pathways that are often altered in failing hearts. Furthermore, we demonstrate that increased heme levels are associated with elevated oxidative stress and loss of viability in cultured cardiomyoblasts.

Methods

Human samples. Tissue samples were obtained from the tissue bank at Feinberg Cardiovascular Research Institute (Northwestern University, Chicago, Illinois) and consisted of samples from nonfailing (n = 10) and failing ischemic (n = 10) human hearts. Failing ischemic tissues were obtained from the explanted hearts of cardiac transplantation recipients. Nonfailing heart tissue samples were obtained from unmatched organ donors whose hearts were unsuitable for transplantation, but who had no known cardiac disease. Explanted hearts were immediately placed in cold cardioplegic solution and subsequently frozen in liquid nitrogen. Protocols for tissue procurement were approved by the Institutional Review Board of Northwestern University. Informed consent was obtained from all transplantation patients and from the families of organ donors before tissue collection.

Cell culture. H9c2 cardiac myoblasts were purchased from ATCC and kept in complete Dulbecco's Modified Eagle Medium (ATCC, Manassas, Virginia) supplemented with 10% fetal bovine serum (Invitrogen Grand Island, New York) and 1% penicillin-streptomycin. For hypoxic experiments, cells were maintained in a hypoxic chamber at 37°C and 5% carbon dioxide in the presence of 1% oxygen for up to 8 days. Medium was replaced every 2 days, and cells were collected under hypoxia before analysis. For pharmacological treatments, cells were grown to 80% to 90% confluence and incubated with 10-μM hemin (Sigma-Aldrich, St. Louis, Missouri) and 0.6 mg/ml EPO (Sigma-Aldrich) for 48 h in complete medium.

Abbreviations and Acronyms

ALA = delta-aminolevulinic acid
ALAS2 = ALA synthase 2
ATP = adenosine triphosphate
ETC = electron transport chain
EPO = erythropoietin
Fe/S = iron-sulfur
HF = heart failure
HMOX1 = heme oxygenase 1
Mfrn2 = mitoferrin 2
FtMt = mitochondrial ferritin
PCR = polymerase chain reaction
PPIX = protoporphyrin IX
qRT = quantitative reverse-transcriptase
ROS = reactive oxygen species
TfR1 = transferrin receptor 1

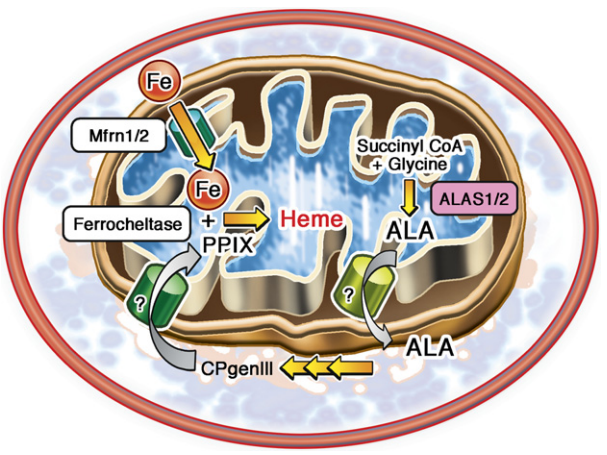

Figure 1 Schematic Representation of Cellular Heme Synthesis Pathway

ALA = delta-aminolevulinic acid; ALAS2 = ALA synthase 2; CoA = coenzyme A; CPgenIII = coproporphyrinogen III ; Fe = iron; Mfrn2 = mitoferrin 2; PPIX = protoporphyrin IX.