Interplay of Mitochondrial Biogenesis and Oxidative Stress in Heart Failure

Running title: Cooper; Role of mitochondrial biogenesis in heart failure

Marcus P. Cooper, MD

Dept of Medicine, Division of Cardiovascular Medicine, University of Massachusetts Medical School, Worcester, MA

Address for Correspondence:
Marcus P. Cooper, MD
University of Massachusetts Medical School
368 Plantation Street
Albert Sherman Center, 7th Floor West, AS7-1053
Worcester, MA 06105
Tel: 508-856-6945
Fax: 508-856-6933
E-mail: marcus.cooper@umassmed.edu


Key words: glycolysis, fatty acid oxidation, Editorial, mitochondrial DNA mutation, mitochondrial DNA deletion, cardiomyopathy, heart failure, mitochondria, oxidative stress, metabolism
The dawn of mitochondrially powered cells began 1-2 billion years ago when an amitochondriate host subsumed α-Proteobacterium, a hydrogen-producing symbiont\(^1\). Equipped to oxidize nutrients, eukaryotic cells acquired a ‘boost’ to cellular energy, enabling the emergence of multi-cellular mammals, sustained by a nearly inexhaustible muscular pump— the heart.

In this issue of *Circulation*, MacLellan and colleagues\(^2\) examine interplay of mitochondria biogenesis and oxidative stress in human cardiomyopathy. They reveal that disparate changes in mitochondrial biogenesis and mitochondrial oxidative stress distinguish between ischemic cardiomyopathy and dilated cardiomyopathy.

Using a complement of ultrastructural, biochemical and genetic analyses, MacLellan and colleagues convincingly show that mitochondrial content is increased in dilated cardiomyopathic (DCM) hearts (n= 8) but not ischemic cardiomyopathic (ICM) hearts (n= 8). Irrespective of heart failure etiology, oxidative phosphorylation (OXPHOS) was severely impaired, a finding universally reported by others. Given that mitochondrial content was increased about 2-fold in DCM hearts, these data may imply better maintenance of total cellular OXPHOS in DCM hearts compared with ICM hearts. Because mitochondrial mass is influenced by proliferation (mitochondrial biogenesis) and clearance (mitophagy), the authors investigated the mechanistic basis for increased mitochondrial content in DCM hearts. Notably, *PGC-1α (PPARGC1a)*, a potent regulator of mitochondrial biogenesis\(^3\), as well as several target genes of PGC-1α were induced at the level of mRNA and protein in DCM hearts but not in ICM hearts. These genetic data replicate a previously reported study\(^4\). Even so, this topic is not immune to controversy or the Proteus phenomenon, as findings by others oppugn the conclusions of MacLellan and colleagues. Using a mixed cohort, one study showed that PGC-1α, which promotes mitochondrial biogenesis, was reduced in both ICM and DCM hearts\(^5\). Induction of adenine
nucleotide translocator (SLC25A4), a gene whose induction signifies mitochondrial biogenesis, was reported by Wallace and colleagues in ICM hearts (but not DCM hearts)\(^6\), implying mitochondrial biogenesis is specific to ICM. Similar disparities are found throughout the literature, some of which are mentioned in the work by MacLellan and colleagues. What then accounts for these differences? The obvious answer is that differences in patient demographics, classification, heart failure staging or medical treatment could have influenced conclusions. Alternatively, the effect of cardiomyopathy on mitochondrial proliferation might depend on a variable independent of heart failure classification. To this end, MacLellan and colleagues evaluated additional mitochondrial variables in cardiomyopathic hearts. Most notably, in DCM hearts, mutation of mitochondrial DNA, a sensor of oxidative stress within mitochondria, was increased about 4-fold compared with non-failing control hearts, while no significant difference was found between non-failing hearts and ICM hearts.

Nearly a half century ago, DNA was identified inside mitochondria\(^7,8\). Using electron microscopy, fine fibrous (rod-like) structures were initially observed in the matrix of mitochondria from several organisms\(^7\), including amoeba\(^9\) and mouse oocyte mitochondria\(^10\). Using an assortment of fixatives, fibers within mitochondria exhibited staining patterns consistent with DNA, and further studies, using metabolic labeling, confirmed initial observations. Two decades after the discovery of mitochondrial DNA, Young and colleagues\(^11\) sequenced the entire human mitochondrial DNA, revealing a circular genome of 16, 569 base pairs that encodes 37 genes: 2 rRNA, 22 tRNAs and 13 polypeptides. Unlike mitochondrial DNA of yeast or most prokaryotes, the genome of mammalian mitochondria proved highly compact with very little non-coding sequence, and its genetic code and tRNAs differed from the nucleus. Considering that there are 50 to several hundred mitochondria per cell and each mitochondrion
contains 5-10 genomes, a single cell contains several hundred to a few thousand mitochondrial genomes. In cooperation with nuclear encoded subunits, proteins encoded by mitochondrial DNA comprise the respiratory chain and ATP synthase, a multi-enzyme system critical for OXPHOS. A by-product of respiration, reactive oxygen species are liberated by mitochondria, exposing mitochondrial DNA to constant oxidative stress. Due to its location within the mitochondrial matrix and lack of histones, mitochondrial DNA is highly susceptible to oxidative damage. To maintain genomic integrity, mitochondria utilize an armamentarium of detoxifying enzymes and rely on a machinery of base excision repair enzymes that mend oxidative damage.

When oxidative stress exceeds defense mechanisms, de novo mutations (and deletions) accrue in the mitochondrial genome, giving rise to a variegated population of mitochondrial DNA genomes, a mixed population of mitochondrial genomes referred to as heteroplasmy. The extent of heteroplasmy varies across tissues and is particularly increased in heart and skeletal muscle. Assuming a low fixed error rate for the mitochondrial DNA polymerase, mitochondrial DNA is an incidental sensor of oxidative stress that reflects the equilibrium between DNA damage and repair.

In this issue of Circulation, MacLellan and colleagues exploited high-throughput sequencing to quantify mitochondrial DNA mutations (0.04% in DCM versus 0.01% in non-failing hearts) and deletions (0.05% in DCM versus 0.003% in non-failing hearts). They identify a higher frequency of mitochondrial DNA mutations and deletions in patients with dilated cardiomyopathy but not in those with ischemic cardiomyopathy. Given that heteroplasmic mutations approaching 60% were required to observe biochemical defects in OXPHOS, a mutation frequency of 1 out of 1000-2500 mitochondrial genomes was unlikely to directly influence OXPHOS. Nonetheless, the increase in mutations and deletions is indicative of
increased oxidative stress in DCM hearts. Interestingly, irrespective of the heart failure etiology, MnSOD activity in whole cell lysate was similar, despite DCM hearts having twice as many mitochondria. Because MnSOD is localized to mitochondria, this implies reduced detoxification activity per mitochondrion in DCM hearts and may explain the increased frequency of mitochondrial DNA mutations and deletions. Similar to MacLellan and colleagues, others have reported an association between oxidative stress and mitochondrial biogenesis in cardiomyopathic hearts. Wallace and colleagues found increased oxidative stress and likely induction of mitochondrial content in ICM hearts. This group also reported a 7-220 fold increase in mitochondrial DNA deletions in ICM heart, which showed the greatest oxidative stress. In maternally inherited cardiomyopathy, one group found an association between oxidative stress and mitochondrial biogenesis, including an induction of PGC-1α. The central theme of all these reports, including the study by MacLellan and colleagues, is that cardiomyopathy coupled with marked increases in oxidative stress is associated with induction of PGC-1α and mitochondrial biogenesis; while, cardiomyopathy with a lesser degree of oxidative stress is associated with reduced (or normal) levels of PGC-1α. MacLellan and colleagues also show a decline in a single base excision repair protein for ICM and DCM hearts. These data, however, require cautious interpretation, since repair activity was not measured, and ICM hearts do not have increased mitochondrial DNA mutations.

Regarding cardiomyopathy and mitochondrial biogenesis, several interesting questions remain. Is mitochondrial biogenesis adaptive or maladaptive? While the salutary effect of increasing OXPHOS via PGC-1α-mediated mitochondrial biogenesis is intuitive, heart muscle has a limited capacity for mitochondrial biogenesis due to its dense myofibrillar network. In mice, Kelly and colleagues showed that unchecked mitochondrial biogenesis crowds the
contractile apparatus, impairing myocardial performance and culminating in dilated cardiomyopathy\textsuperscript{18}. In conditional transgenic mice, controlled induction of PGC-1\textalpha{} still promoted dilated cardiomyopathy\textsuperscript{19}, accompanied by dysmorphic mitochondria and myofibrillar degeneration. Analogous to mitochondrial biogenesis reported by MacLellan and colleagues in DCM hearts, mitochondrial content was increased about 2-fold in the inducible mouse model\textsuperscript{19}. Shutting off the transgene in these mice reversed cardiomyopathy as well as aberrant changes in mitochondria\textsuperscript{19}. Interestingly, in the study by MacLellan and colleagues, implantation of a left ventricular assist device reduced mitochondrial DNA mutations in DCM hearts. Based on the accumulated data and recent data by MacLellan and colleagues, a simple (but unifying) model can be hypothesized (Figure 1). Irrespective of heart failure etiology, a disproportionate increase in oxidative stress triggers the induction of PGC-1\textalpha{}, culminating in mitochondrial proliferation. This in turn would have implications for fuel utilization, as induction of PGC-1\textalpha{} was associated with cardiac induction of genes involved in fatty acid oxidation\textsuperscript{4,18}, a process which further exacerbates oxidative stress. Under this scenario, oxidative stress, which is injurious to contractile function\textsuperscript{20}, and mitochondrial proliferation, which crowds the contractile apparatus\textsuperscript{18,19}, would collectively impair myocardial performance. While increased PGC-1\textalpha{} and mitochondrial biogenesis might initially preserve OXPHOS, over time increased mitochondrial content (and/or repression of contractile genes) would lead to myofibrillar disarray and contractile dysfunction as reported by Kelly and colleagues\textsuperscript{19}. Presumably, differences in loading of the heart and host factors determine the degree of oxidative stress, as unloading of DCM hearts with left ventricular assist devices reduced the frequency of mitochondrial DNA mutations, indicating reduced oxidative stress. In contrast, if oxidative stress is not sufficiently elevated in the cardiomyopathic heart, there is a decline in PGC-1\textalpha{}, mitochondrial content and
OXPHOS, culminating in impaired contractile function. A decline in PGC-1α and mitochondrial content would likely necessitate a reliance on glycolysis, a reversal of the so called “fetal switch”. Further work is necessary to determine which signals down regulate mitochondrial biogenesis in certain heart failure patients. Based on this discussed model, induction of mitochondrial biogenesis is initially adaptive but over time proves maladaptive due to ill effects on metabolism and the contractile apparatus, whereas insufficient mitochondrial content limits myocardial performance due to reduced OXPHOS. In heart failure, this implies there is delicate balance between myocardial content and myocardial performance (Figure 1).

Although controversy continues to bedevil this topic, the work by MacLellan and colleagues in this issue of Circulation does move us closer to a consensus. Cardiomyopathies differ not only in their clinical etiology, but also at the level of mitochondrial biogenesis and oxidative stress. Apart from advancing our basic understanding of mitochondrial biogenesis in human heart failure, their work may inform the search for diagnostic and therapeutic interventions. Classifying cardiomyopathies as “glycolytic” heart failure or “oxidative” heart failure may lead us closer to more tailored therapies.

Conflict of Interest Disclosures: None.

References:


**Figure Legend:**

**Figure 1.** Model showing the interplay of mitochondrial biogenesis and oxidative stress in human cardiomyopathy (CM). As illustrated by the leftward pointing “Glycolytic” arrow, if oxidative stress is not markedly increased, there is a decline in PGC-1α and mitochondrial biogenesis, culminating in impaired oxidative phosphorylation (OXPHOS) and myocardial performance. Cardiomyopathic hearts traversing the “glycolytic pathway” show a greater preference for glucose utilization. Because oxidative stress is not sufficiently elevated, mitochondrial DNA does not show an increase in mitochondrial DNA mutations. On the other hand, if there is a marked elevation in oxidative stress (perhaps dependent on loading or host factors), there is induction of PGC-1α, mitochondrial biogenesis and enzymes involved in fatty acid oxidation. Despite increased mitochondrial content, OXPHOS per mitochondrion is
impaired (due to increased oxidative stress). Cardiomyopathic hearts traversing the “oxidative pathway” show a greater preference for fatty acids, the oxidation of which further exacerbates oxidative stress, which in turn promotes mitochondrial DNA mutations and worsens myocardial performance. Depending on medical or mechanical therapies, loading conditions and host factors, it is hypothesized that a cardiomyopathic heart can transition between states, that is, between “glycolytic” heart failure and “oxidative” heart failure.
Interplay of Mitochondrial Biogenesis and Oxidative Stress in Heart Failure
Marcus P. Cooper

Circulation, published online April 15, 2013;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/early/2013/04/15/CIRCULATIONAHA.113.003177

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office.
Once the online version of the published article for which permission is being requested is located, click Request
Permissions in the middle column of the Web page under Services. Further information about this process is
available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/