2017

The role of mitochondria in cardiac development and protection

Pohjoismäki JL

Elsevier BV

info:eu-repo/semantics/article
© Elsevier BV
CC BY-NC-ND https://creativecommons.org/licenses/by-nc-nd/4.0/
http://dx.doi.org/10.1016/j.freeradbiomed.2017.02.032

https://erepo.uef.fi/handle/123456789/1579
Downloaded from University of Eastern Finland's eRepository
The role of mitochondria in cardiac development and protection

Jaakko L. Pohjoismäki & Steffi Goffart

1University of Eastern Finland, Department of Environmental and Biological Sciences, P.O. Box 111, 80101 Joensuu, Finland. §e-mail: Jaakko.Pohjoismaki@uef.fi, tel. +358-505744745

Keywords: mitochondria, mtDNA, metabolism, cardiomyocyte differentiation, postnatal development

Abstract

Mitochondria are essential for the development as well as maintenance of the myocardium, the most energy consuming tissue in the human body. Mitochondria are not only a source of ATP energy but also generators of reactive oxygen species (ROS), that cause oxidative damage, but also regulate physiological processes such as the switch from hyperplastic to hypertrophic growth after birth. As excess ROS production and oxidative damage are associated with cardiac pathology, it is not surprising that much of the research focused on the deleterious aspects of free radicals. However, cardiomyocytes are naturally highly adapted against repeating oxidative insults, with evidence suggesting that moderate and acute ROS exposure has beneficial consequences for mitochondrial maintenance and cardiac health. Antioxidant defenses, mitochondrial quality control, mtDNA maintenance mechanisms as well as mitochondrial fusion and fission improve mitochondrial function and cardiomyocyte survival under stress conditions. As these adaptive processes can be induced, promoting mitohormesis or mitochondrial biogenesis using controlled ROS exposure could provide a promising strategy to increase cardiomyocyte survival and prevent pathological remodeling of the myocardium.
Introduction

The heart is the most energy consuming organ in the human body and derives essentially all of its energy from mitochondrial oxidative phosphorylation (OXPHOS) [1]. The energy demand is not surprising as the heart is a constantly active muscle with enormous oxidative reserve capacity, capable of increasing the cardiac output 7-fold without a need for anaerobic metabolism. In order to provide sufficient amounts of ATP, masses of mitochondria are needed, occupying about one third of the total volume of a cardiomyocytes and corresponding to more than half of the volume of the myofibrils [2].

Due to the heart’s need for efficient oxidative metabolism, mitochondria are extremely important for cardiac development and healthy function. While the common focus has been on the understanding of bioenergetics and metabolic alterations in heart development and disease [3], there is growing evidence that mitochondria can affect cardiomyocyte differentiation and survival also by more subtle means, such as generation of reactive oxygen species (ROS) and regulation of cell death. Besides being essential for cardiomyocyte differentiation and maturation, also mitochondria themselves mature; they increase mass, develop structurally and become functionally specialized in the growing heart [2]. The structural specialization includes the expansion of the mitochondrial network by fission and fusion of mitochondria, which is not restricted to cardiomyocyte development but also later on required for the maintenance of a healthy heart [4].

In this review, we sum up the recent understanding of the role of mitochondria in heart redox regulation, development and cardiomyocyte survival. We also explore options how increased mitochondrial biogenesis could prevent the adverse myocardial remodeling seen in the aging heart and cardiovascular disease.
**Oxidative metabolism and redox regulation**

Mitochondria are cell organelles producing the majority of a cell’s ATP by the means of oxidative phosphorylation. OXPHOS requires the action of four respiratory enzyme complexes (CI-IV, the electron transport chain or ETC) mediating the electron transport from Krebs cycle coupled to the pumping of protons between the mitochondrial intermembrane space, and the release of the generated electrochemical gradient through ATP synthase (FoF1ATPase, complex V). Although the majority of the subunits and all assembly factors of the respiratory complexes are encoded by nuclear genes, 13 central subunits reside in the mitochondrial DNA (mtDNA), making it indispensable for OXPHOS. The proton motive force drives the c-ring rotor in the F\textsubscript{0} subcomplex, generating the torque that powers a sequence of conformational changes in the F\textsubscript{1} subcomplex and catalyzing ATP generation [5]. Complete atomic structures for mammalian respiratory complexes I [6], II [7], III [8], IV [9] as well as yeast complex V [10] have been published, providing insight into the molecular mechanisms of electron transport, proton pumping and ATP generation. The single respiratory complexes are further assembled into different types of supercomplexes or respirasomes [11], containing CI+CIII dimers and varying numbers of CIV, depending on the tissue type and energy demand [12]. Supercomplex architecture has consequences on the efficiency of electron transfer [13, 14], which might be relevant in understanding some of the features of the highly active heart OXPHOS, its control [15] and possible break-down due to pathology [16]. The correlation of supercomplex types and respiration dynamics as well as redox homeostasis in different tissues invites further investigation.

**Mitochondria as a source of free radicals**

Due to their oxidative metabolism mitochondria are also the main source of endogenous reactive oxygen species (ROS) in cells, primarily superoxide radicals (O\textsubscript{2}–) [17]. Although the one electron reduction of O\textsubscript{2} to O\textsubscript{2}– is thermodynamically favored under physiological conditions, only few cellular electron carriers are able to carry out this reaction and for example, the electron transport in mitochondria is tightly coupled to the reduction of O\textsubscript{2} to H\textsubscript{2}O under normal conditions. However, uncontrolled leakage of electrons through complexes I-III can generate superoxide, especially when the rate of ADP phosphorylation is decreased and the membrane proton gradient is high [18, 19]. Besides spontaneous dismutation, superoxide can be rapidly converted to hydrogen peroxidase (H\textsubscript{2}O\textsubscript{2}) by the mitochondrial superoxide dismutase (SOD2) and further to H\textsubscript{2}O by catalase or glutathione peroxidases (GSHs). It should be noted that while
O₂⁻ and H₂O₂ are not especially chemically reactive themselves, their reactions with Fe²⁺ (Fenton reaction) or Fe³⁺ (Haber-Weiss reaction for H₂O₂) generate extremely reactive hydroxyl radicals (OH⁻), responsible for the majority of oxidative damage in cells [20]. The interaction of O₂⁻ and H₂O₂ with iron-sulfur [Fe-S]ₙ cluster containing proteins increases the turnover of these enzymes and the amount of free iron in cells [20]. O₂⁻ and H₂O₂ inactivate key [Fe-S]ₙ hydratases, such as aconitase, and influence iron uptake and storage. Notably, the rapid dismutation of O₂⁻ and the low pH of the mitochondrial intermembrane space does not allow a significant efflux of superoxide from mitochondria. H₂O₂ instead is readily diffusible across membranes through aquaporins [19], thus having a much larger impact on the overall cellular oxidative stress.

**ROS and heart: dearth precedes destruction**

Despite its high oxidative capacity, the basal mitochondrial ROS production in heart is modest compared to skeletal muscle and brain [21]. This counterintuitive observation might be explained by the heart mitochondrial respiratory chain being normally tuned to high OXPHOS output with minimal basal ROS production. Mechanistically this could be achieved through supercomplex organization, which has direct consequences on substrate channeling, electron transport efficiency and ROS production from CI [13, 16, 22]. Consequently, if the ETC is compromised, heart mitochondria become effective ROS generators due to their active OXPHOS [18]. Such a crisis can be caused e.g. by myocardial infarction (MI), resulting in depletion of O₂ in mitochondria and cessation of OXPHOS. Paradoxically, lack of oxygen causes ROS release from mitochondria *in vivo*, although the actual mechanism is unclear [19]. It has been suggested that the increased electron leakage from various parts of the reduced electron transport chain to the residual O₂ might produce O₂⁻ [18], however, hypoxia seems to lower ROS production *in vitro* indicating that the mechanism is more complex *in vivo* [19]. The process is dependent on the ETC as mtDNA-less ρ₀ cells lacking OXPHOS are not able to produce ROS under hypoxic conditions [23] and some evidence suggests the involvement of complex IV [24]. As supercomplex assembly depends on OXPHOS activity and oxygen availability [25], it is likely that supercomplex disassembly due to a sudden change in the substrate availability plays a pivotal role in superoxide production [15, 16, 22]. In addition, the observed low basal ROS levels might also be misleading. Perturbations in OXPHOS causing surges of free radicals could be part of normal cardiac physiology, caused e.g. by the need for higher heart rate during exercise [26]. The fact that reduction of SOD2 enzyme activity has phenotypic
consequences almost solely in heart [27, 28], demonstrates that excess O$_2^-$ production occurs much more frequently in cardiomyocytes than in other tissues.

In hypoxia, ROS release is required as a signal to stabilize HIF-1$\alpha$, resulting in metabolic reprogramming with a generalized hypoxia response, including increased expression of HIF-1$\alpha$ itself [29] (Figure 1). Elevated cytoplasmic ROS levels have been reported to also activate Keap1, the binding partner of nuclear factor erythroid-derived-like 2 (NFE2L2 or Nrf2) [30]. As a result, NFE2L2 is released and translocates into the nucleus where it activates the transcription from antioxidant response element (ARE) containing promoters. ARE promoters control antioxidant genes such as SOD2, heme oxygenases and the nuclear respiratory factor-1 (NRF-1) involved in mitochondrial biogenesis. Interestingly, heterozygous Sod2 knockout (Sod2$^{+/\text{-}}$) mouse hearts having high levels of oxidative stress do not show any activation of NRF-1; instead they show high HIF-1$\alpha$ expression together with a generalized antioxidant response [28]. Due to the compensatory upregulation of antioxidant defenses, Sod2$^{+/\text{-}}$ hearts show only 25% reduction in the SOD2 levels and an over two-fold increase in catalase expression. This is curious, as according to the current paradigm, freely diffusible H$_2$O$_2$ should be the key molecule for cytoplasmic ROS signaling. Less SOD2 should reduce the dismutation of O$_2^-$ to H$_2$O$_2$, whose turnover is facilitated by the increased catalase levels, hence effectively reducing cytosolic H$_2$O$_2$ concentration. Unless the translocation of superoxide from mitochondria has been underestimated, it is likely that at least in this case hydroxyl radicals originating from Fenton reactions within mitochondria could drive the cytosolic ROS signaling.

Besides HIF-1$\alpha$-signaling, hypoxia also causes the activation of anaerobic glycolysis, providing ATP for cell survival by generation of lactate, which in turn causes acidification of the cytoplasm that has repercussions in mitochondria. The sodium-hydrogen antiporters of the plasma membrane will remove the excess H$^+$, but simultaneously increase the cellular Na$^+$, which inhibits the removal of intracellular Ca$^{2+}$ via the Na$^+$/Ca$^{2+}$ exchanger [31]. The high intracellular Ca$^{2+}$ activates the mitochondrial calcium uniporter and in the absence of functional OXPHOS efficiently dissipates the mitochondrial membrane potential, on which the porter is dependent [32]. When sustained, Ca$^{2+}$ stress will trigger necrotic cell death [33]. If O$_2$ levels suddenly increase, as it happens in reperfusion after ischemia, the mitochondrial membrane potential is restored, triggering intake of additional Ca$^{2+}$, opening of the mitochondrial permeability transition pore (MPTP) and resulting in cell death. Additional to calcium dysregulation,
mitochondria produce a burst of $\text{O}_2^-$, causing oxidative damage to mitochondria upon reperfusion [18, 34]. Although it has been suggested that this oxidative damage drives the pathology that develops in the days following reperfusion, there is no evidence that antioxidants could help to curb the tissue damage [35]. Instead, cardiomyocyte death after ischemia-reperfusion and $\text{Ca}^{2+}$ overload is dramatically reduced in mice lacking cyclophilin D, a regulator of MPTP opening [36, 37]. This is interesting as it indicates that much of the oxidative damage is survivable, if cell death due to MPTP activation is prevented. In fact, there are some promising indications that cyclophilin D inhibitors, such as cyclosporine, could reduce infarct size in humans [38, 39]. However, limiting oxidative stress during reperfusion will help to contain the mitochondrial damage and the recovery of the surviving cells [40].

Interestingly, cardiomyocytes are not very sensitive to apoptosis, potentially because adult heart has only a limited capacity for regeneration. For example, the release of cytochrome c, a hallmark of intrinsic apoptotic pathway, is not sufficient to induce apoptosis in isolated cardiomyocytes [41, 42]. Instead, cardiomyocytes seem to focus on damage tolerance and repair [33]. As ROS can activate both survival and death signaling, depending on the intensity of the damage [33], cardiomyocyte damage tolerance should be closely linked to the mitochondrial function. The key mechanism to contain cellular damage and adapt to oxidative stress is to target damaged organelles and cellular macromolecules to degradation in a process called autophagy. Hypoxia and oxidative damage have been shown to induce autophagy in cardiomyocytes [33] and are induced via the mitochondrially regulated AMPK-ULK1 signaling pathway [23]. Interestingly, autophagy seems to have a protective role in mild ischemia but can develop to be detrimental after ischemia-reperfusion [43, 44]. The latter could result from the overload of autophagosome processing as well as delayed clearance of damaged organelles, resulting in increased ROS generation and cell death following MPTP opening. The reported benefits of autophagy are not only mitochondria-related as it is also required for the clearance of stress fibers associated with myocardial injury, preventing pathological remodeling of the heart after ischemia-reperfusion [45]. Chronic exposure to elevated ROS not only increases autophagy [46-48], but also impairs the coupling of excitation-contraction and induces cardiac hypertrophy through apoptosis, necrosis and fibrosis [18]. Again, the distinction between acute and chronic effects is important. Whereas autophagy promotes cell survival after acute stress, continued activation of autophagy can also contribute to the pathology, indicating the existence of an adaptive zone for turnover of cellular components [33].
Mitochondria in heart development

Mitochondrial function is essential for heart development
The heart is the first organ to acquire functionality during the vertebrate embryonic development, in mouse at the embryonic day (E) 8.5, which roughly corresponds to human E20 [3]. Noticeably, in mouse models lacking essential mitochondrial components, such as the ones needed for mtDNA maintenance or gene expression, the embryonic development typically terminates at E8.5 [49-52]. Due to their small size the terminated embryos are not often studied in detail, but where reported, they seem to lack all heart structures [52]. This fits well to the fact that also cardiomyocyte differentiation in vitro requires functional OXPHOS [53]. Therefore it is rather surprising that although the embryonic heart is perfectly capable of oxidative metabolism, it has been thought to rely on anaerobic glycolysis [3]. However, the metabolic signatures of glycolysis might be misleading as fetal cardiomyocytes actually metabolize lactate to pyruvate that is then fed into OXPHOS, a unique feature exploited for selective differentiation of embryonic stem cells into cardiomyocytes [54]. Mitochondria are also otherwise an important sink for lactate as they synthesize a large percentage of their lipids using lactate as a precursor [55].

Metabolic maturation of a growing heart
Another bioenergetically important event in mammalian heart development occurs at the change from pre- to postnatal life (Figure 2). Shortly before birth and during the first weeks of postnatal mouse development, cardiomyocytes switch from hyperplastic to hypertrophic growth [56-58]. The cardiomyocytes become binucleated, increase their myofibril density and mature intercalated discs appear. In humans, this switch is delayed and cardiomyocytes continue to proliferate during the first few months after birth. The increase in contractile components requires a concomitant rise in ATP production during the postnatal hypertrophic growth. As result, mitochondrial biogenesis is induced and the energy metabolism switches from glucose and lactate consuming respiration to the more energy-producing β-oxidation of fatty acids [1]. Due to this increased biogenesis the relative mitochondrial mass of cardiomyocytes is doubled during the early phases of post-natal heart development [2].

Regulation of mitochondrial biogenesis in heart
The regulation of mitochondrial biogenesis is highly complex, as it requires the orchestration of two genomes. As mentioned earlier, only 13 subunits of respiratory chain complexes are encoded by the mitochondrial DNA (mtDNA), whereas the over 1,000 nuclear encoded mitochondrial (NEM) proteins include the components of metabolic pathways, RC complexes, various assembly factors, carrier proteins as well as factors required for maintenance and expression of mtDNA [59].

Several transcriptional activators and regulators are involved in mitochondrial biogenesis. Ubiquitous transcriptional regulators provide a basic expression level of NEM proteins while Nuclear Respiratory Factors 1 and 2 (NRF-1 and NFE2l2 or Nrf2) are required for their dynamic control [1]. Many NEM gene promoters contain cAMP-responsive elements (CRE) enabling regulation via CRE-binding proteins (CREBs) and related factors. The nuclear receptor-type transcription factors called Peroxisome Proliferator-Activated Receptors (PPARs) and their coactivators, such as PGC-1α (known as PPARGC1A in rodents), are important regulators of mitochondrial biogenesis and function, governing for example the metabolic switch from lactate use to β-oxidation of fatty acids by inducing genes involved in fatty acid metabolism and import.

The classical studies on mitochondrial biogenesis have focused on differentiation and adaptation of skeletal muscle where PGC-1α is the most important mitochondrial regulator, sensitive to physiological signals such as fasting, cold and exercise [60]. Therefore, it is interesting that regulation via PGC-1α seems negligible for heart mitochondrial biogenesis [61]. Instead, the physiologically less responsive PPAR coactivator PGC-1β is dramatically upregulated during postnatal hypertrophy, concomitantly with an increase in the NEM gene expression levels. Intriguingly, neither one of the PGC-1s is required later for the maintenance of mitochondrial mass in the adult mouse [62]. While also the CREB family proteins CREM, CREBL2 and CREB3L2 as well as the nuclear transcription factor STAT3 might play a role in the upregulation of mitochondrial components during the postnatal development, none of the NRF genes seems to be involved in the process [61].

The build-up of mitochondrial mass in the postnatal heart begins with an increased expression of mitoribosomal proteins and other components of the mtDNA expression machinery [61, 63].
Once the ribosomal components reach their plateau levels, the genes encoding for the constituents of OXPHOS and β-oxidation pathways are upregulated. This order of events is important as it shows that separate regulatory circuits control the production of translational machinery and mtDNA expression and the nuclear components required for oxidative metabolism. This is rather surprising as according to the basic paradigm the assembly of the respiratory chain complexes happens in an orchestrated manner [64], and the unbalance between nuclear and mitochondrially encoded components of the respiratory complexes can result in suboptimal electron transport and ROS generation, as seen in RC assembly deficient models [65, 66]. Interestingly, during the upregulation of mitochondrial biogenesis during the first days of postnatal life, cardiomyocytes show simultaneous signs of elevated ROS levels capable of eliciting DNA repair responses [61]. This damage response arrests the cell cycle, thus rendering the cardiomyocytes post-mitotic and enabling the switch from hyperplastic to hypertrophic growth [67]. In fact, ROS is known to strongly induce both PGC-1α and PGC-1β [68] and could therefore represent the signal required for the upregulation of OXPHOS that eventually turns the metabolic switch.

As in any cell, mitochondria in cardiomyocytes proliferate by extending the existing organellar network [1]. The increase in mitochondrial mass in the growing heart is accompanied by the structural differentiation to interfibrillar (IFM) and subsarcolemmal mitochondria (SSM) [2]. The two populations are structurally and functionally different: elongated IFM are tightly packed into the space between sarcomere Z-lines and lined with sarcoplasmic reticulum, while the morphologically more variable SSM are located beneath the sarcolemma [69]. Although the two have a nearly identical pattern of substrate utilization, the oxidative capacity of IFM are 1.4 – 1.7 times higher than those of SSM [70]. It has been suggested that IFM primarily supply ATP for the contractile system, whereas SSM provide the energy for the transport of electrolytes and metabolites across the sarcolemma [71]. IFM are also central for mitochondrial Ca2+ cycling due to their proximity to the sarcoplasmic reticulum [72].

Maintenance of heart mitochondrial homeostasis

Adult cardiomyocytes not only contain a large mitochondrial mass, they also maintain a high basal levels of mitochondrial biogenesis, as the intensive oxidative metabolism requires the constant degradation and replacement of damaged mitochondria [46]. Thus the turnover rate, the average lifespan of a mitochondrion, in mature heart is only six days [1]. The mitochondrial
network is under constant remodeling by fusion and fission. Mitochondrial fission, budding off parts of the mitochondrial network, is essential for the identification of dysfunctional and se-
esescent parts of the mitochondrial network and their removal by autophagy [46, 73]. In addi-
tion, mitochondrial fusion is required for mitochondrial maintenance; it is not only essential for cardiomyocyte differentiation [74], but also for the healthy function of an adult heart [4, 75]. However, it is not entirely clear why fragmentation of the mitochondrial network due to impaired fusion has deleterious consequences for the heart, especially as both respiratory chain activity and ATP production are not affected by it [75]. One explanation could be the mainte-
nance of interaction between SSM and IFM at different depths of the cardiomyocyte. Com-
pared to the volume of most cell types, such as 180 μm³ for an endothelial cell, cardiomyocytes are huge, up to 73,453 μm³ in adult rats [2]. This poses a challenge for the efficient distribution of mitochondrial components and metabolites for OXPHOS within the network. While IFM have greater respiratory capacity with smaller ROS production compared to SSM [76], they are enclosed within the myofibrils. Thus, the supply of imported mitochondrial proteins, me-
tabolites and oxygen might be limited by the distance of IFM from the sarcolemma and nucleus, unless network junctions enabled by mitochondrial fusion permit free diffusion. It has been hypothesized that the interaction between IFMs might also facilitate the transmission of mem-
brane potential across mitochondrial subpopulations, enabling steady ATP production under intensive exercise [77, 78]. The impact of these intact IFM network connections on ATP pro-
duction is challenging to assay in vivo, as the effect of the relative position within a host cell on mitochondrial activity is lost when the mitochondria are isolated for oxygraph measure-
ments, possibly explaining the lack of respiratory defects in mitochondrial fusion impaired mouse models [75].

In adult cardiomyocytes contraction work consumes approximately 70% of the produced ATP, most of the remaining ATP is used for regulating ionic homeostasis [79, 80]. Cardiomyocytes maintain high levels of phosphocreatine and ATP, and these levels are constantly monitored by adenosine monophosphate-activated protein kinase (AMPK). A high AMP/ATP ratio activ-
ates AMPK, resulting in suppression of energy using pathways and activation of ATP pro-
duction by increased β-oxidation and glucose catabolism [81]. AMPK is also involved in the mobilization of cellular resources by increasing the degradation of dysfunctional proteins via the ubiquitin proteasome system [82] and autophagy [23]. In skeletal muscle, the contractile
activity-associated rise in intracellular calcium has been shown to induce mitochondrial biogenesis [83, 84]. Skeletal muscle myotubes store calcium in the sarcoplasmic reticulum until a neuronal impulse triggers their influx, thus enabling a logical connection between muscle contractility and regulation of mitochondrial biogenesis. In contrast, calcium release in cardiomyocytes is regulated by external calcium ions binding to voltage-gated calcium channels of the sarcolemma. Also the overall calcium cycling in a constantly contracting system is rather different [85], suggesting that the role of calcium in heart mitochondrial biogenesis is negligible. While endurance training increases the mitochondrial mass of skeletal muscle, its benefits on cardiac mitochondria seem to be most related to ROS tolerance [86], resulting from adaptive increase in antioxidant capacity [26].

ROS tolerance is an interesting consequence of increased mitochondrial activity. In contrast to emergencies, such as sudden ischemia, which cause acutely high levels of ROS, perturbations in the OXPHOS due to suboptimal ETC assembly [13, 14, 16] or substrate availability [19] are expected to generate a more subtle but chronic ROS release, which can be corrected by upregulated mitochondrial biogenesis and quality control [46, 47]. As pointed out earlier, key regulators of mitochondrial biogenesis, such as PGC-1α, are themselves ROS responsive [68]. In fact, heterozygous Sod2 knockout mouse hearts experiencing increased ROS stress have high levels of PGC-1α, PPAR-δ and PPAR-γ expression as tell-tale signs of increased mitochondrial biogenesis [28]. The fact that the expression of PGC-1α is increased under oxidative stress in heart is interesting. As mentioned before, PGC-1β seems to be the key regulator of the developmental mitochondrial biogenesis in the rodent heart, indicating that PGC-1α has a more general-purpose, adaptive role in cells. However, the upregulation of mitochondrial biogenesis does not seem to compensate the mitochondrial dysfunction in these mice. Instead, the heterozygous Sod2 knockout mouse hearts show signs of increased glucose metabolism on the expense of β-oxidation [28], a metabolic change consistent with a hypoxia response regulated by the increased HIF-1α levels [87]. This intriguing fact possibly reveals the hierarchy between hypoxia signaling and the regulation of mitochondrial biogenesis (Figure 1). Further studies are needed to address whether hypoxia signaling is also a response to prolonged, chronic ROS stress, overriding the more adaptive retrograde signals induced by transient ROS, that are aimed to increase mitochondrial biogenesis.

**Mitochondrial DNA maintenance in cardiomyocytes**
Despite recent advances in understanding mammalian mitochondrial DNA replication [88], surprisingly little is known about the regulation of mtDNA maintenance and its tissue specificity [89]. While mitotic cells seem to mainly employ a rather specialized mtDNA replication mechanism, characterized by long persistent RNA intermediates during the lagging strand replication [90], post-mitotic tissues such as heart have more conventional double-stranded DNA (dsDNA) containing replication intermediates [91]. This different replication mechanism could represent an adaptation to the changed redox environment, as oxidative damage can induce a shift towards a synchronous dsDNA replication mode also in cultured cells [92]. A more radical change in the mtDNA maintenance mechanism takes place during the human heart postnatal development, but not in rodents [63, 93]. While fetal human heart mtDNA replicates using a conventional replication mechanism involving replication bubbles, the adult mtDNA maintenance is characterized by abundant recombination intermediates, likely involved in recombination-dependent replication initiation [89, 91]. Although the mtDNA maintenance mode switch, as far as known, is specific for humans, a similar recombination phenotype can be induced in transgenic mouse hearts by overexpression of mitochondrial transcription factor A (TFAM) or the mitochondrial replicative helicase Twinkle [91]. The exact physiological role of the switch is speculative, but circumstantial evidence suggests that mtDNA recombination could be an adaptation to the increased levels of oxidative stress in adult human heart compared to the fetal stage [28, 61, 63, 89, 93].

Although the developmental changes in post-natal hearts of different mammals are rather similar [2], the adaptation strategy against increased ROS levels differs [61]. While human heart changes the mtDNA replication mechanisms, expression of many mtDNA repair enzymes is increased in growing rodent hearts. The adaptation of mtDNA repair to increased ROS is interesting and perhaps relevant in understanding the basic logic of mtDNA maintenance in somatic tissues. In contrast to the billions of base pairs long nuclear genome that has only two copies of most essential genes, mtDNA is only 16.5 kb and exists in hundreds to thousands of copies per cell, making selective degradation of mtDNA more cost efficient for the cell than its repair [94]. Nevertheless, an armory of mtDNA repair mechanisms do exist (reviewed in detail in [95, 96]). As with the nuclear DNA repair mechanisms, the optimal strategy of mtDNA maintenance will depend on the nature of damage.
Due to its proximity to the ETC, mtDNA is prone to frequent oxidative damage, mainly oxidized nucleotides such as 8-oxo-dG [95]. Although this type of modifications do not cause bulky lesions that one would expect to impair transcription and replication, oxidative damage seems to stall mtDNA replication both in vitro [97] and in vivo [92]. In addition, 8-oxo-dG’s are read as dA’s during replication and will result in G>A mutations as observed in the mutation pattern of Sod2+/− mouse hearts [28]. Interestingly, this is not the case in ageing wild-type mice, which seem to accumulate mtDNA mutations caused by replication errors, thus speaking for an efficient repair of oxidative damage in most tissues [98]. Although high levels of mtDNA mutations do clearly impair OXPHOS, they do not increase ROS production [99], undermining the old hypothesis of a mitochondrial vicious circle of ROS and mtDNA damage [100].

The mitochondrial DNA copy number paradox – how much is enough?

As OXPHOS depends on subunits encoded by mtDNA, mtDNA copy number is often expected to correlate with mitochondrial activity and mass in the cell. In fact, mtDNA copy number has been used in a number of studies as an indicator of mitochondrial dysfunction (see for example [101, 102]). However, mtDNA levels are usually measured using the nuclear genome (nDNA) as the reference. In order to compare tissues, they should consist of roughly equally sized cells, which is not the case if cardiomyocyte size varies or if cardiomyocytes have been replaced by other cell types, as in age- or disease related fibrosis [103, 104]. As an example, the mtDNA:nDNA ratio rises sharply during developmental hypertrophy, but due to the parallel increase in cell size the amount of mtDNA per tissue mass is maintained constant [61, 63, 93]. Thus neither mitochondrial mass nor mitochondrial gene expression (both elevated in adult cardiomyocytes) seem to be regulated by mtDNA levels [61]. Instead, the high level of mitochondrial gene expression in the adult heart is achieved by sustained upregulation of mitochondrial transcription factors [61, 63]. Also in skeletal muscle differentiation in vitro [105-107] or insect flight muscles [108] mtDNA copy number increase is not required for increased OXPHOS activity. In fact, the ribonucleotide reductase RRM2B knockout mouse model demonstrates that mtDNA can be severely depleted without obvious effects on OXPHOS [109], further questioning the meaningfulness of mtDNA copy number as a measure of heart mitochondrial biogenesis. It may well be that mtDNA copy number per tissue mass is maintained stable, representing a mere housekeeping role without a bigger developmental or adaptive significance. As mitochondrial transcription factor A (TFAM) and mtDNA levels show good interdependency in cultured cells [110] and during heart development [63], it seems likely that TFAM governs the mtDNA copy number in normal physiological conditions [111].
A role for mtDNA in cardiac protection and ageing

While mtDNA is essential for OXPHOS, its role in the maintenance of heart function exceeds a pure role in energy production. As a rather puzzling observation, the overexpression of TFAM or Twinkle protects mouse cardiomyocytes against ischemia [112, 113] as well as high levels of oxidative stress [28, 114]. While overexpression of these factors seems to have a mild deleterious effect on OXPHOS [115], they also increase mtDNA copy number and induce high levels of recombination [28, 91]. The analysis of a Sod2+/− rescue by Twinkle overexpression indicates that the changes in mtDNA maintenance mechanisms somehow mediate an antiapoptotic effect via p21, but the actual molecular mechanisms remain obscure [28]. The overall picture is confused by the fact that Sod2+/− hearts alone have increased p53 levels. p53 is not only known to respond to ROS but has an emerging role as a regulator of mitochondrial biogenesis [116] and mtDNA maintenance [117] with p21 being one of its key interacting partners [118]. It is known that both mtDNA single-strand breaks [119] as well as complete loss of mtDNA [120] predispose cells to apoptosis, further indicating that mtDNA and its integrity have central roles in cell fate determination.

Paradoxically, despite the importance of functional mitochondria for heart development, the loss of OXPHOS activity is insufficient to trigger the death of an adult cardiomyocyte [121]. Cardiomyocytes that have lost OXPHOS can be detected using histochemistry as cytochrome oxidase negative (COX−) and can have a detrimental effect on the overall heart function. Besides being found in a number of patients with pathogenic mtDNA mutations [122] or deletion syndromes [123], COX− cardiomyocytes are present also in normal aged human heart [124]. Curiously, age related COX− cardiomyocytes have not been detected from mouse, with the exception of aged Twinkle overexpressors (Figure 2) [28]. The species difference could be explained by the different mtDNA maintenance mechanisms in mouse and man. In humans COX− cardiomyocytes accumulate in heart during aging as high levels of mtDNA recombination generate circular deletions, resulting in progressive clonal loss of mitochondrially encoded RC components [125]. COX− cardiomyocytes caused by increased levels of deletions thus represent a trade-off for increasing overall mtDNA integrity and cell survival. Although functionally impaired, COX− cardiomyocytes are a better alternative for overall heart function than the loss of cardiomyocytes and replacement with rigid connective tissue (Figure 2) [28, 113].
This could also explain how Twinkle overexpression protects against cardiac rupture after myocardial infarction [126]. Extensive death of cardiomyocytes will result in a large connective tissue scar, whose subsequent remodeling or inflammation can predispose the affected tissue to rupture under pressure. While COX− cardiomyocytes are less harmful than fibrotic areas, also they are functionally impaired and could have a pathogenic outcome such as conductance block when reaching a certain threshold [121-123].

Mitochondrial medicine for heart diseases

While much is known about the role of mitochondria in heart development and diseases, the application of this published information for any clinical benefit is not trivial. Although e.g. overexpression of TFAM or Twinkle is able to ameliorate the outcome of heart infarction in mice, the effects likely only emulate phenomena already present in human heart. However, these mouse models are extremely useful as humanized models of heart mtDNA maintenance and could provide the means to understand the significance of mtDNA in cardiomyocyte survival.

A central theme worth revisiting is the mitochondrial adaptation to oxidative stress. While a sudden increase in ROS is essential for cardiomyocyte differentiation, oxidative damage is also the central culprit causing tissue damage in ischemia-reperfusion following heart infarction [34]. The adverse effects of ischemia-reperfusion can be partially avoided by ischemic preconditioning, itself a mitochondrial adaptation mechanism [71]. Similarly, it is possible that a controlled permanent increase in oxidative stress in the heart could promote cardiomyocyte survival upon infarction events by increasing the overall stress resistance as a result of mitohormesis, a phenomenon where mild stress considerably increases the tolerance of the cell to further insults [127]. While it might not be possible to prevent cardiomyocyte death caused by the initial insult, improved stress resistance and mitochondrial quality control in the ROS adapted cells could help to contain the damage and have significance to the overall outcome in the patient. Apart from ischemic preconditioning, heart mitohormesis has not yet been addressed experimentally. Oxidative stress is easily induced pharmacologically, thus allowing the development of various treatment strategies. While toxic substances such as rotenone have been used to inhibit OXPHOS in ischemic preconditioning [71], there are therapeutically more plausible agents such as menadione, which divert electrons from the electron transport chain to oxygen, generating superoxide radicals without interfering with ATP production or the redox
status of the NADH pool [128]. Notably, many so-called beneficial bioactive compounds, such as the ellagitannins of blueberries, are in fact effective pro-oxidants [129]. Considering that western diet is generally poor in pro-oxidants, this raises interesting questions regarding the development of diseases connected to reductive stress, such as the Alzheimer’s [130]. As an old remedy, also the benefits of endurance training on cardiac health also seem to involve ROS resistance [86, 131]. Although the benefits of training on cardiac ageing are not linear, it has been suggested that moderate exercise promotes antioxidant signaling via NFE2l2, protecting the aging myocardium from ageing related pathological processes [132].

An important pathological modification of cardiac muscle after infarction or in developing cardiomyopathy is the dedifferentiation of cardiomyocytes, which is accompanied by a metabolic switch to fetal-type carbohydrate metabolism [133]. Although the causality is not well established, it appears that mitochondrial energy metabolism itself could be the key regulator of cardiomyocyte dedifferentiation after ischemic damage and in the failing heart [134]. Potentially the reduced oxidative metabolism together with cardiomyocyte dedifferentiation are adaptive responses required for cell survival and organ regeneration [133], but at the same time contribute to the impairment of cardiac functionality. While it is not proven that the metabolic reprogramming is a cause and not a consequence of cardiac remodeling, the possibility of reversing the mitochondrial energy production back to β-oxidation by forced mitochondrial biogenesis is worth to investigate more. Pharmacologically relevant compounds here would be PPAR agonists, such as bezafibrate, whose benefits in the prevention of cardiovascular events, such as stroke, have been studied intensively [135]. Surprisingly, the effects of PPAR agonists on cardiomyocyte differentiation are virtually unknown. Another promising candidate for the promotion of oxidative metabolism could be the antidiabetic drug metformin, that influences the substrate metabolism for OXPHOS by promoting fatty-acid oxidation [136]. Metformin has been reported to increase myocardial palmitate oxidation in a volume-overload rodent model [137] and although the drug did not influence the overall survival of the animals in the study, it might be applied to influence the pathological reprogramming of cardiomyocytes.

Possible targets for the manipulation of mitochondrial function are not restricted to altering substrate utilization. Both age-related decline in cardiac contractility as well as developing cardiomyopathy result in specific loss of IFM function [71, 138], which might be possible to counteract by promoting mitochondrial biogenesis, resulting not only in increased OXPHOS
but also activating mitochondrial fission \cite{4, 62}. Interestingly, in mitochondrial fusion-deficient mice already a change to high-fat diet was enough to alter cardiac metabolism and prevent the development of cardiomyopathy \cite{75}.

**Conclusions**

Mitochondria are central organelles for heart development and physiology. Besides their fundamental role in ATP production, mitochondria are also the main source of ROS in cardiomyocytes. Free radicals are regarded harmful in the cardiac context, as they contribute to the damage during ischemia as well as pathological remodeling of the myocardium, including maladaptive metabolic modifications due HIF-1α mediated responses. However, also normal physiological stimuli such as endurance exercise can vastly increase ROS, emphasizing the fact that cardiomyocytes are by default highly adapted to ROS exposure. The differentiation of cardiomyocytes both in vitro and in vivo requires functional OXPHOS, and their postnatal maturation, involving the cessation of cell cycle and entry to hypertrophic growth, is dependent on a ROS signal from the boosted electron transport chain. While fetal cardiomyocytes use lactate derived pyruvate to operate OXPHOS, the energetic needs of adult myocardium are met by a switch to β-oxidation with a concomitant increase in mitochondrial mass and RC complexes, resulting in high mitochondrial crista density and differentiation of SSM and IFM. Unlike in skeletal muscle, where PGC-1α is the key controller of mitochondrial biogenesis, its paralogue PGC-1β seems to be the main modulator of the postnatal heart mitochondrial development.

Paradoxically, heart (and perhaps other tissues) seem to have excess of mtDNA copies then they need for the maintenance of OXPHOS. For example, the OXPHOS increase during postnatal development is achieved by upregulation of mtDNA expression without altering its levels per tissue mass. Simultaneously, mtDNA repair and maintenance mechanisms are boosted as a response to increased oxidative stress caused by the highly active OXPHOS. This improved repair is essential as mtDNA integrity influences cardiomyocyte survival. As a tradeoff, the repair processes generate occasional mtDNA deletions, which in time result in a mosaic OXPHOS deficiency in the aging human heart. Although this leads to a less functional, energetically comprised myocardium, it prevents the death of cardiomyocytes, replacement fibrosis and dedifferentiation of surrounding cells that would deteriorate overall performance. A pro-
apoptotic role of mtDNA breaks offer the most parsimonious explanation for the observed cardioprotective role of enhanced mtDNA maintenance in Twinkle and TFAM overexpressor mice.

The fact that mitochondrial adaptations against oxidative damage are physiologically regulated, could enable the controlled preconditioning of cardiomyocytes with ROS. While it might be possible to limit cardiomyocyte death using drugs that inhibit MPTP opening, containing the damage caused by ROS requires the action of existing cellular maintenance mechanisms. Limited ROS exposure, mimicking developmental signals seen in the postnatal stage, could boost the existing mitochondrial repair mechanisms and initiate mitohormesis, an adaptation response preventing the development of pathological changes in the sick or aged heart. Similarly, pharmaceutical compounds such as bezafibrate, capable of inducing mitochondrial biogenesis, might offer means to increase the turnover of damaged mitochondria and to restore mitochondrial network integrity to improve OXPHOS, preventing pathological remodeling of cardiomyocytes.

Acknowledgments

The work supporting this review has been funded by Jane and Aatos Erkko foundation (JP) as well as by the Academy of Finland (SG). Open Access costs have been covered by Jane and Aatos Erkko foundation.

Disclosures

The authors report no conflicts of interest.

References


[M. Floreani, F. Carpenedo, One- and two-electron reduction of menadione in guinea-pig and rat cardiac tissue, General pharmacology 23(4) (1992) 757-62.]


[M. Narasimhan, N.S. Rajasekaran, Exercise, Nrf2 and Antioxidant Signaling in Cardiac Aging, Frontiers in physiology 7 (2016).]


26
Figure legends

Figure 1. ROS and mitochondrial retrograde signaling. In basal conditions, mitochondrial electron transport is tightly coupled to the reduction of O$_2$ to H$_2$O. The homeostasis is maintained mainly by AMPK, which responds to increased OXPHOS demand by monitoring the AMP/ATP ratio and modulating mitochondrial biogenesis and autophagy accordingly. Perturbations in OXPHOS result in electron leakage to O$_2$ past the CIV, perhaps due to partial disassembly of supercomplexes, generating superoxide (O$_2$^{-·}). Hydrogen peroxide (H$_2$O$_2$) is generated from the dismutation of superoxide, facilitated by SOD2. H$_2$O$_2$ diffuses to cytosol, where it can regulate a number of redox sensitive proteins, such as HIF-1α, PGC-1α and NFE2L2. Whereas HIF-1α suppresses mitochondrial function by inducing metabolic switch towards glycolysis and increasing autophagy via AMPK, PGC-1α and NFE2L2 represent more adaptive signals, eliciting antioxidant defenses and DNA repair. The observed impairment of mitochondrial biogenesis due to chronic oxidative stress can be explained by the sustained HIF-1α activation. Outer membrane omitted for clarity.

Figure 2. Intimate connection between the mitochondria and the cardiomyocyte development. The fetal cardiomyocytes convert lactate to pyruvate to run the OXPHOS. Due to the low metabolic needs, fetal heart mitochondria have low crista density but intriguingly have higher levels of mtDNA per tissue mass. During the postnatal development, the oxidative stress caused by the increased mitochondrial biogenesis triggers DNA damage responses, which is needed for the cardiomyocytes to exit cell cycle, enabling hypertrophic growth. During the maturation of the cardiomyocytes, interfibrillar mitochondrial networks form together with increased cristae density and metabolic switch to β-oxidation.

Figure 3. Hypothesis of the tradeoffs in the human heart mitochondrial physiology. During the long adult life, heart mitochondria are exposed to various intrinsic insults caused by the oxidative environment of the metabolically active cardiomyocytes. In most other cells, the turnover of damaged mitochondrial DNA is sufficient to maintain the homeostasis, however mtDNA double-strand breaks can be detrimental to the post-mitotic cardiomyocytes, resulting in apoptotic signaling. Adult human cardiomyocytes have elevated levels of recombination to maintain mtDNA integrity. As a tradeoff, there is a clonal accumulation of mtDNA deletions, resulting
in loss of COX activity (blue cells). However, the loss of cardiomyocytes, resulting in dedifferentiation of surrounding cells together with invasion of connective tissue is much more harmful for the overall performance of the myocardium than the mosaic OXPHOS deficiency seen in the aged human heart [124, 125]. The COX/SDH histochemistry example is from 20-month old Twinkle overexpressor heart, representing a humanized model of cardiac ageing. COX deficient cells stained blue, see the supplementary data in Pohjoismäki et al. [28] for details.