Mitochondria in heart failure

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This review focuses on the evidence accumulated in humans and animal models to the effect that mitochondria are key players in the progression of heart failure (HF). Mitochondria are the primary source of energy in the form of adenosine triphosphate that fuels the contractile apparatus, and are thus essential for the pumping activity of the heart. We evaluate changes in mitochondrial morphology and alterations in the main components of mitochondrial energetics, such as substrate utilization and oxidative phosphorylation coupled with the level of respirasomes, in the context of their contribution to the chronic energy deficit and mechanical dysfunction in HF.

Keywords   Heart failure • Mitochondria • Respirasomes • Oxidative phsophorylation • Reversible phosphorylation

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1. Introduction

Heart failure (HF) is an enormous medical and societal burden. More than 2% of the US population is affected, with an annual mortality rate for mild-to-moderate failure of 10%[1,2] and for severe failure over 50%, and a cost in the USA of $28 billion annually for treatment.[3] HF is a complex multifactorial syndrome characterized by mechanical dysfunction of the myocardium and the inability of the heart to supply adequate amounts of blood to meet the perfusion and metabolic needs of the body. Defects in bioenergetics, increased preload and afterload, altered signal transduction pathways, abnormalities of calcium homeostasis, as well as neurohormonal dysregulation are major pathogenic factors for myocardial dysfunction in HF. Despite significant advances in therapy, particularly inhibition of the renin–angiotensin and sympathetic nervous systems, HF remains a progressive syndrome.

In order to support both electrical and mechanical activities (contractility and diastolic relaxation), the heart needs a continuous energy supply. This requirement is met by the daily synthesis of approximately 30 kg of the high-energy molecule adenosine triphosphate (ATP),[4] produced mainly by mitochondrial oxidative phosphorylation. Cardiac energy deficits have been reported in HF[5–7] due to alterations in all components of cardiac energetics.[2,8] Therefore, the improvement of myocardial energetics becomes a promising approach to the treatment of HF.

Mitochondrial oxidative phosphorylation relies not only on the activities of individual complexes, but also on the coordinated action of supramolecular assemblies (respirasomes) of the electron transport chain (ETC) complexes in both normal[9] and failing

heart.[10] In human mtDNA mutant cybrids with impaired mitochondrial respiration, the recovery of mitochondrial function correlates with the formation of respirasomes[11] suggesting that respirasomes represent regulatory units of mitochondrial oxidative phosphorylation[12] by facilitating the electron transfer between the catalytic sites of the ETC. We recently reported a decrease in mitochondrial respirasomes in HF[10] that fits in the category of a new mitochondrial cytopathy.

This review provides an overview of the regulation of the cardiac mitochondrial function in health and disease, and the current state of knowledge concerning the contribution of mitochondrial energy deficit to the progression of HF.

2. Cardiac mitochondrial populations in heart and disease

2.1 Morphology

Conventional transmission electron microscopy of mammalian cardiac tissue reveals mitochondria to be elliptical individual organelles situated either in clusters beneath the sarcolemma (subsarcolemmal mitochondria, SSM) or in parallel, longitudinal rows ensconced within the contractile apparatus (interfibrillar mitochondria, IFM) (Figure 1). The two mitochondrial populations differ in their cristae morphology,[13] with a lamelliform orientation in SSM, whereas the cristae orientation in IFM is tubular.[14]

The morphology of mitochondria is responsive to changes in cardiomyocytes. For example, following endurance exercise in mice,

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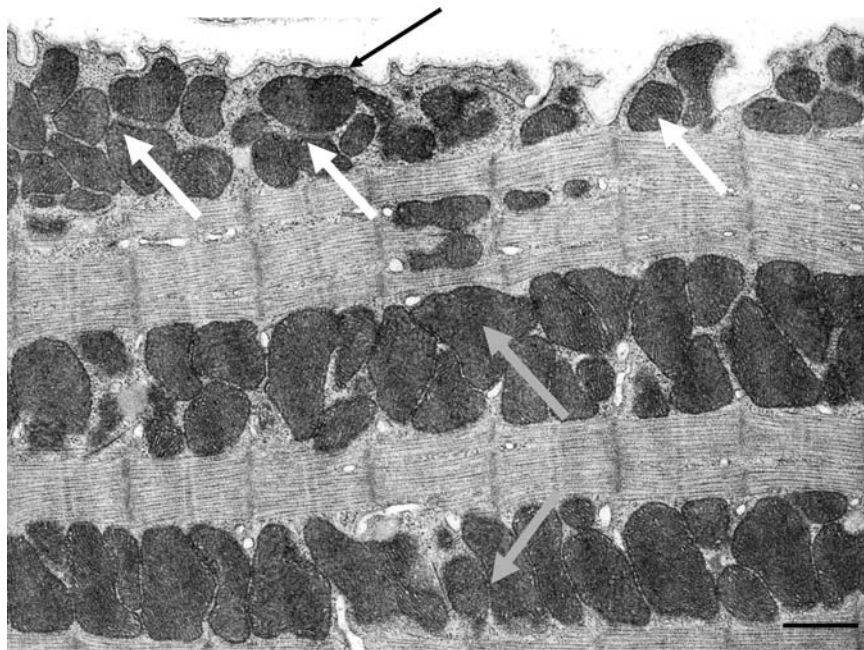
Figure 1 Cardiac mitochondria. Transmission electron micrograph of mouse myocardium. Clusters of subsarcolemmal mitochondria (SSM, white arrows) are situated immediately beneath the sarcolemma (black arrow); longitudinal rows of mitochondria that are located within the contractile apparatus (myofibrils) are interfibrilar mitochondria (IFM, grey arrows).

cardiac hypertrophy is accompanied by giant mitochondria with disorganized cristae.[15] Cardiac hypertrophy secondary to aortic constriction in rats[16] is associated with increased mitochondrial size, as well as increased replication of mitochondrial DNA. Alterations of other non-mitochondrial cardiomyocyte compartments may be associated with changes in mitochondria. For example, transgenic mice harbouring a missense allele of cardiac troponin T to mimic the human familial hypertrophic cardiomyopathy experience cardiac myofibrillar degeneration associated with an increased number of small mitochondria with a loss of membranes and cristae.[17]

By examining endocardial biopsies from humans with hypertrophic and dilated cardiomyopathy, Baandrup et al.[18] found giant mitochondria with decreased matrix density in some cardiomyocytes, associated with increased mitochondrial number. Also, in a canine model of chronic HF of moderate severity, an increased number of smaller mitochondria with the loss of matrix density and intramitochondrial myelin figures[19] was reported. These alterations in mitochondrial morphology are associated with changes in mitochondrial bioenergetics, such as decreased oxidative phosphorylation measured in both permeabilized cardiac fibres[19] and isolated mitochondria.[10]

2.2 Mitochondrial biogenesis

Modifications in mitochondrial morphology are usually associated with changes in mitochondrial number. The dividing of pre-existent organelles is dependent on mitochondrial fission, requires neosynthesis of phospholipids and proteins, and is controlled at the level of transcription of both mitochondrial and nuclear DNA. Mitochondrial transcription is promoted by the binding of the mitochondrial transcription factor A (mtTFA) on an upstream enhancer of the two mitochondrial DNA strands. The peroxisome proliferator-activated receptor (PPAR) gamma co-activator (PGC-1α) is considered the master synchronizer of the two genomes, by increasing the transcriptional activity of the nuclear respiratory factors (NFRs) on mtTFA promoter and of the PPARα. NFRs increase the expression of mtTFA and the vast majority of nuclear-encoded subunits of the tricarboxylic acid cycle and ETC complexes. The transcription factor PPARα increases the expression of genes encoding for the mitochondrial fatty acid (FA) transport and oxidation enzymes. The PGC-1α controls the optimal mitochondrial content that is critical for cardiomyocyte function.[20,21]

2.2.1 Mitochondrial biogenesis in physiological hypertrophy

Mitochondrial content needs to parallel cardiomyocyte growth in order to meet cellular energy requirement. However, whether cardiomyocyte enlargement induced by physiological stimuli (i.e. sustained exercise training) is accompanied by an increase in mitochondrial biogenesis is not clear, and was reported either increased[22] or unchanged.[23,24] Also, it is still debated if physiological stimuli drive an increase in mitochondrial content independent of cardiomyocyte hypertrophy. Notable, FA oxidation was reported unchanged[25] suggesting the absence of a metabolic remodelling in physiological hypertrophy.

2.2.2 Mitochondrial biogenesis in pathological hypertrophy

The activities of the mitochondrial marker enzyme citrate synthase and of cytochrome c oxidase, as well as the expression of oxidative phosphorylation genes[26,27] were reported unchanged when measured in the homogenates of cardiac tissue originating from hypertrophied hearts. These data suggest that mitochondrial biogenesis matches

the energy demand of the hypertrophic cardiomyocyte during compensated cardiac hypertrophy.

2.2.3 Mitochondrial biogenesis in HF

A downregulation of the entire pathway of mitochondrial biogenesis was reported in HF. First, a decrease in cardiac expression of the transcription factor PGC-1α was found in different experimental models of HF.[28–30] Second, downregulation of NFRs and mtTFA was also reported,[28] as well as of tricarboxylic acid cycle and ETC enzymes.[28,31,32]

In contrast, primary mitochondrial defects in both humans and animal models are associated with increased mitochondrial proliferation and cardiomyopathy. For example, Sebastiani et al.[32] found an increase in mtDNA and mitochondrial proliferation with myofibril displacement, associated with increased expression of genes involved in mitochondrial biogenesis in humans with cardiomyopathies induced by mitochondrial defects. Increased mitochondrial proliferation was also found in murine models of cardiomyopathies associated with ablation of the adenine nucleotide translocase 1,[33] frataxin,[34] Mn-SOD,[35] and mtTFA.[36] The increased mitochondrial mass in the myocardium of mtTFA knockout mice is accompanied by a decrease in mtDNA and transcripts for cytochrome c oxidase subunit I (encoded by mtDNA), as well as reduced expression of PPARα transcripts, PPARα-dependent genes, and impaired ETC. The severe ATP depletion indicates that the mitochondrial defect cannot be compensated by the increase in mitochondrial number. Of notice, this model of cardiomyopathy is characterized by a discrepancy between increased mitochondrial proliferation and decreased mitochondrial maturation (decreased mtDNA and mtDNA transcripts, with defects in all ETC complexes containing mtDNA-encoded subunits). Single polymorphisms in the PGC-1α gene have been identified which correlate with an increased risk of hypertrophic cardiomyopathy.[37] Taken together, these observations suggest that primary alterations in mitochondrial biogenesis may contribute to cardiac pathologies.

2.3 Biochemical properties

Cardiac SSM and IFM differ in both their inherited properties and response to disease. Rat,[38] rabbit,[39] hamster,[40] and human[41] heart IFM oxidize substrates at higher rates compared with heart SSM, with no differences in their coupling abilities and cytochrome content. The greater permeability of SSM to exogenous NADH[42] suggests a decrease in their inner membrane intactness. SSM also are more susceptible to calcium overload-mediated cytochrome c release and mitochondrial damage compared with IFM.[43]

An increased susceptibility of cardiac SSM to ischaemic damage has been observed in rat[44] and rabbit.[45] During ischaemia of the isolated perfused rabbit heart, cardiac SSM sustain a loss of the phospholipid cardiolipin (CL), with decrease in oxidative phosphorylation through cytochrome oxidase and loss of cytochrome c.[45] SSM also produce reactive oxygen species (ROS) following ischaemic damage.[46] In contrast, cardiac IFM experience an age-related increased susceptibility towards mitochondrial permeability transmission pore opening[47] associated with decreased yield and oxidative phosphorylation[39] due to a defect in the cytochrome c binding site in complex III. Ageing-related mitochondrial defects predispose to a greater susceptibility to ischaemia compared with adult hearts due to a defect in the iron−sulfur protein in complex III.[48] Despite an increase in the

antioxidant defense mechanisms, a significant increase in oxidative stress markers was described in cardiac IFM compared with SSM with age.[49]

Cardiac IFM are also more sensitive to diabetic damage. Experimental-induced diabetes in rats causes a decrease in CL content, oxidative phosphorylation, and complex III activity, associated with an increase in superoxide production and oxidative-induced modifications of proteins and lipids in cardiac IFM.[50] Also, increased apoptotic susceptibility of cardiac IFM was reported in experimental diabetes, with lower mitochondrial membrane potential and antiapoptotic proteins, and increased permeability transition pore opening.[51]

These data emphasize the importance of studying both mitochondrial populations when attempting to elucidate the contribution of mitochondrial dysfunction to cardiac failure. In contrast, other studies report that both cardiac mitochondrial populations are equally affected in HF. For example, a decrease in the content of CL and activity of complex III occur acutely after myocardial infarction in both cardiac SSM and IFM, which lead to decreased mitochondrial respiration and increased ROS production.[52] A loss of CL also was reported in dilated cardiomyopathy in humans and spontaneously hypertensive HF in rats, and correlated with reduced complex IV activity in both populations of cardiac mitochondria.[53] In a model of microembolisms-induced HF of moderate severity in dogs, we found that both populations of heart mitochondria experience a decrease in oxidative phosphorylation and amount of supercomplexes,[10] without changes in CL content and molecular species.

3. Myocardial substrate metabolism in the normal and failing heart

3.1 Substrate utilization in the normal heart

ATP utilized by the heart is synthesized mainly by means of oxidative phosphorylation in the inner mitochondrial membrane, a process that involves the coupling of electron transfer and oxygen consumption with phosphorylation of ADP to ATP. The catabolism of exogenous substrates (FAs, glucose, pyruvate, lactate, and ketone bodies) provides the reduced intermediates, NADH (nicotinamide adenine dinucleotide, reduced) and $FADH_2$ (flavin adenine dinucleotide, reduced), as donors for mitochondrial electron transport.

The myocardium has the remarkable ability to switch between carbohydrate and fat as fuel sources (metabolic flexibility) so that ATP production is maintained at a constant rate in the face of diverse physiological and dietary conditions. The catabolism of FA provide up to 90% of the ATP in the healthy heart.[4] The contribution of glucose to the acetyl CoA pool in the heart is increased by insulin during the postprandial period and during exercise. Substrate switching also is achieved by acute regulation of transcription of metabolic enzymes in response to changes in substrate level,[54] oxygen availability,[55] and the intrinsic circadian clock within cardiomyocytes.[56,57]

3.2 'Metabolic remodelling'[58] in the failing heart

Most clinical[59–61] and experimental[62–64] studies find that both hypertrophied and failing heart have an intrinsic metabolic inflexibility characterized by a shift toward glucose oxidation at the expense of FA oxidation. However, most studies report that the decrease in

FA oxidation is not compensated for by an increase in glucose oxidation.[63,65] As a consequence, the failing heart is an energy-compromised organ.

Does mitochondrial remodelling underlie this metabolic remodelling?

Defects at specific sites of the mitochondrial ETC, decrease in oxidative phosphorylation, as well as decrease in mitochondrial density may impair the capacity of myocardium to oxidize FAs as energetic substrates, whereas glycolysis is unaffected. Impairment of FA oxidation also may be secondary to changes in the activity and kinetic properties of carnitine palmitoyltransferase I (CPT I), the controlling step in the entry of long-chain FAs to the mitochondria. CPT I is inhibited by malonyl-CoA. The results reported by various studies differ regarding the changes in the content of malonyl-CoA in the failing myocardium.[66−68] In moderately severe HF in dogs, our group did not observe any difference in the activity of CPT I and its sensitivity toward inhibition by malonyl-CoA. However, malonyl-CoA content was decreased by 22% with HF, suggesting less *in vivo* inhibition of CPT I activity.

Alternatively, the decline in FA oxidation in the failing heart is due to the reduction in the expression of genes involved in mitochondrial transport and oxidation of FAs as a consequence of the decline in the activity of the nuclear receptor PPARα. However, changes reported in patients with HF lack consistency.[69,70] Moreover, discrepancies between the expression of PPARα, mRNA, and protein content of FA oxidation enzymes, and FA oxidation rates were reported in HF,[71] suggesting that postranslational modifications may play a role in the regulation of FA oxidation enzymes and FA oxidation rates.

Since sustained activation of the β-adrenergic receptor-stimulatory GTP-binding protein-adenylyl cyclase (AC) signalling pathway in HF has deleterious effects on the heart, treatment with β-blockers is beneficial.[72] However, it was reported that the β-blocker, metoprolol, inhibits mitochondrial FA β-oxidation via a direct effect on the catalytic activity and malonyl-CoA sensitivity of CPT I.[73] CPT I stably interacts and is directly controlled by phosphorylation induced by cAMP-protein kinase A (PKA) pathway as an effector of the β1-adrenergic receptor signalling.[74] PKA enhances the binding between the anchoring protein, AKAP149, and both liver and heart CPT I isoforms on heart mitochondria, increases the phosphorylated state, and decreases malonyl-CoA sensitivity without affecting the catalytic activity of CPT I.

4. New aspects of mitochondrial function and dysfunction in HF

4.1 Oxidative phosphorylation and respirasomes

Mitochondrial oxidative phosphorylation forms the basis for ATP production. In mammalian mitochondria, the oxidative phosphorylation system is composed of four oxidoreductase complexes (complexes I, II, III, and IV) and the ATP synthase (complex V). Three of the four oxidoreductase complexes couple electron transport with translocation of protons from the mitochondrial matrix to the intermembrane space, thus generating an inner membrane proton gradient[75] that drives the synthesis of ATP from ADP and P_i by complex V.

According to the structural model of the mitochondrial inner membrane, initially proposed more than 50 years ago by Chance and Williams[76] and expanded and amplified by Schagger's group,[9] the structural support for oxidative phosphorylation is provided by assemblies of the ETC complexes into supercomplexes. The supercomplex consisting of complex I, dimeric complex III, and one copy of complex IV ($I_1III_2IV_1$) found in rodent,[77] dog,[10] and bovine[9] heart mitochondria, also contains coenzyme Q and cytochrome c, and functions as a cohesive respiratory unit (respirasome) because it transports electrons from NADH to reduce oxygen.[78] According to the three-dimensional map of the bovine heart $I_1III_2IV_1$ supercomplex,[79] the individual complexes physically interact in this assembly, and the electron carriers have short diffusion distances between complexes, supporting the concept of channelled electron transfer that decreases electron leakage and superoxide production.[80]

The ETC complexes, whether unincorporated in respirasomes or organized in supercomplexes, are embedded in the phospholipid bilayer of the mitochondrial inner membrane. CL is an anionic phospholipid present almost exclusively in the mitochondrial inner membrane of eukaryotic cells. Tetra-linoleoyl-CL [$(C18:2)_4$-CL] is the predominant form of all CL species and provides structural and functional support to components of both the mitochondrial ETC and phosphorylation apparatus.[81−85] Recent studies suggest that CL plays a central role in the higher order organization of mitochondrial ETC in supercomplexes. It was reported that CL is essential for either formation[86] or stabilization of respiratory supercomplexes in both yeast[87,88] and humans.[89]

4.2 Regulation of mitochondrial function

As the main ATP provider for cardiomyocytes, mitochondrial function is regulated according to cellular energetic needs via signal transduction pathways that involve second messengers, such as cAMP, calcium, or ROS.

4.2.1 Reversible phosphorylation

Reversible phosphorylation of proteins is a main cellular regulatory mechanism. The largely impermeable inner mitochondrial membrane keeps mitochondrial proteins out of reach of signalling cascades initiated by cytosolic kinases. However, a computational analysis predicted that 5% of protein kinases are targeted to mitochondria in yeast.[90] A recent review estimated that 18 out of 63 mitochondrial phosphoproteins participate in oxidative phosphorylation.[91] However, the list of mitochondrial phosphoproteins has markedly increased since then.

There is accumulating evidence that reversible phosphorylation at serine and threonine amino acid residues induced by cAMP-activated PKA changes the function of mitochondrial proteins. The cAMP/PKA signalling pathway is enhanced by sympathetic stimulation. Cardiomyocytes β1 receptors couple to the stimulatory G protein, activate the AC, and increase cytosolic cAMP. Binding of cAMP to the regulatory subunits of PKA induces the dissociation of the holoenzyme and phosphorylation by the catalytic subunits of sarcolemmal L-type Ca channels and sarcoplasmic phospholamban, with increase in the cytosolic calcium responsible for the positive chronotropic and inotropic effects. There is a pronounced activation of the sympathetic system in animals and patients with HF that is inversely correlated with survival. The use of β1-selective receptor blockers is clinically beneficial,[72] leading to a reduction by a third or more in the risk of death, a benefit considered greater than that yielded by any other drug used in HF. These data suggest that the regulation of the cAMP/PKA pathway may be responsible for the protective effect of β1-receptor antagonists in HF.

Besides the transmembrane AC, soluble AC regulated by pH, bicarbonate, and calcium, and localized mainly to mitochondria and nucleus[92] has been identified. The two distinct ACs suggest subcellular pools of cAMP that can be regulated differently in response to either extra- or intracellular signals. Fluorescent resonance energy transfer analysis revealed a rapid increase in cAMP within mitochondria of cells stimulated with a β agonist,[93] despite the restricted diffusion of cAMP in the cytosol (<1 μm). These data suggest either a preferential accumulation of cAMP within the mitochondria or an increase in the activity of mitochondrial soluble AC induced by β-adrenergic stimulation. Recently, it was shown that the cAMP that activates mitochondrial PKA and increases oxidative phosphorylation via phosphorylation of complex IV subunits is generated within the mitochondria by mitochondrial AC activated by the tricarboxylic acid cycle-generated CO_2.[94] Thus, cAMP represents a regulator of oxidative phosphorylation, ensuring that electron transport responds to both extracellular and mitochondrial stimuli.

Additional evidence shows that mitochondria have a complete cAMP-PKA signalling pathway: (i) using both immuno-gold electron microscopy and ELISA[95] in tissues and isolated mitochondria, respectively, PKA holoenzyme has been identified at the inner membrane and matrix of heart, skeletal muscle, brain, and kidney mitochondria; (ii) there is functional evidence for the presence of PKA in the mitochondrial inner membrane;[96–100] (iii) PKA is recruited to the outer mitochondrial membrane by A-kinase anchoring proteins (AKAPs);[101–103] (iv) signal-terminating mechanisms, such as phosphodiesterases[94] and phosphatases[104,105] also, are present within the mitochondria.

cAMP/PKA-induced phosphorylation events that change the function of the proteins occur on components of all mitochondrial ETC complexes and phosphorylation apparatus (Table 1). However, the mere identification of a phophorylation event in relation to a mitochondrial protein is insufficient to conclude that the post-translational modification regulates mitochondrial function. At least two other criteria must be met. First, the existence of these phopshorylation events must be confirmed in vivo. For example, the phosphorylation of the ESSS subunit in complex I was not identified in vivo,[106] and the site of phosphorylation not conserved in mammals.[107] Therefore, the significance of this post-translational modification in the regulation of complex I assembly at present is not established in mammalian mitochondria. Second, solid evidence of the regulatory effect of these phosphorylation events on the function of the protein is mandatory.

Recent studies suggest that cytochrome c oxidase is a main target for the regulation of oxidative phosphorylation by cAMP-dependent phosphorylation.[108,109] Phophorylation of serine 441 in subunit I of isolated kidney and bovine heart cytochrome c oxidase switches on the allosteric inhibition of the enzyme at high intramitochondrial ATP,[108,110] whereas calcium-activated dephosphorylation turns it off. These data led to the proposal of the second regulatory mechanism of oxidative phosphorylation by high intramitochondrial ATP/ADP ratios.[110,111] In contrast to the first mechanism of respiratory control, mediated through the proton gradient across the mitochondrial inner membrane, the second mechanism is independent of proton motive force, but based on the exchange of bound ADP by ATP in subunit IV at high intramitochondrial ATP ratios. The dependency of ATP synthase on membrane potential ($\Delta\psi_m$) leads to high intramitochondrial ATP at a $\Delta\psi_m$ of 100–120 mV followed by phosphorylation and inhibition of cytochrome c oxidase, which prevents further increase in membrane potential. Because at $\Delta\psi_m$ values above 140 mV, mitochondrial ROS formation increases exponentially with increasing $\Delta\psi_m$,[112] it is proposed that the second mechanism of respiratory control maintains low $\Delta\psi_m$ values and prevents ROS production.

The allosteric regulation of complex IV by ADP/ATP ratio is overruled by a cAMP-dependent tyrosine phosphorylation of subunit I at tyrosine 304 induced by an as-yet unknown tyrosine kinase. This modification causes alterations in the kinetic properties of cytochrome c oxidase, with decrease in V_{max} and increase in K_m and full inhibition at physiological concentration of the substrate cytochrome c.[113] This tyrosine residue is conserved in all eukaryotes examined and in some bacteria. Moreover, glucagon and TNFα have similar inhibitory effects on cytochrome c oxidase,[114] suggesting that this regulation has significance in vivo. It is suggested that the phosphorylation of tyrosine 304 in subunit I located at the interface between the two complex IV monomers may regulate their interaction.[113]

Phosphorylation of complex IV subunits emerges as either detrimental or beneficial for complex IV activity in both normal cardiac mitochondria (Table 1), as well as in mitochondria isolated from pathologic myocardium. Experimental hypoxia and cardiac ischaemia cause increased PKA-dependent phosphorylation of subunits I, IVi1, and Vb of complex IV[115] associated with lower activity of complex IV and increased ROS production.[116] In contrast to these studies, upregulation of mitochondrial soluble AC improves COX activity and mitochondrial respiration,[94] as well as mitochondrial biogenesis in a ROS-dependent manner, in cultured cells and in animals with COX deficiency.[117]

4.2.2 Calcium

Mitochondria are targets of calcium signalling in cardiomyocytes. Close apposition with the endoplasmic reticulum (ER) allow mitochondria to rapidly accumulate calcium upon its release from the ER via ryanodine receptors through the opening of the inositol 1,4,5-triphosphate-gated channels.[118] It was shown that the increase in mitochondrial calcium concentration leads to increased NADH production[119–122] and upregulation of ATP production.[123]

Calcium directly activates key mitochondrial metabolic enzymes (pyruvate, α-ketoglutarate, and isocitrate dehydrogenases). Recent work has shown that there are additional calcium-dependent signalling pathways operative in mitochondria which may affect oxidative phosphorylation, such as calcium-dependent protein kinases and calcineurin.

The calcium- and phospholipid-dependent signalling pathway is represented by the classical members of the protein kinase C (PKC) family. Activation of PKC-α leads to a sustained decrease in oxidative phosphorylation and membrane depolarization preceded by an inhibition of ATP synthase activity.[124] In a different study, Liu et al.[125] showed that the α, β, and ε subunits of ATP synthase have PKC consensus motifs, and the PKC-α-induced phosphorylation of β subunit of ATP synthase on a serine residue inhibits the activity of complex V. PKC-dependent phosphorylation of subunit IV of complex IV increases complex IV activity in neonatal cardiac myocytes.[126]

The effect of mitochondrial calcium-activated serine/threonine phosphatase calcineurin signalling pathway on mitochondria was uncovered by studies of transgenic mice with cardiac-specific expression of an active calcineurin cDNA which exhibit cardiac hypertrophy that progresses to HF.[127] Cardiac SSM from such mice have decreased ADP-dependent oxidative phosphorylation rates with complex II and IV substrates, protein contents of ND3 subunit of complex I and subunit I of complex IV (encoded by mitochondrial DNA), as well as subunit IV of complex IV (encoded by nuclear

Table 1 Mitochondrial phosphoproteins and their potential role in regulation of mitochondrial function

Protein	Location	Role	Source	Phosphorylation (P*) sites	Effect of P* on function
C I					
ESSS	Matrix arm of complex I	C I assembly[145]	Bovine heart[107] Mouse fibroblasts[90]	Serine 20[107]	Increases C I activity[90]
10 kDa subunit	Intermembrane site in C I	C I assembly[146]	Bovine heart[107] Rat heart[147]	Serine 55[107,147]	Increases C I activity and decreases ROS production[147]
42 kDa subunit	MIM[148]	NADH-binding[149]	Bovine heart[106]	Threonine?[106] Serine 59[150]	Unknown
C II					
Flavo protein subunit	Matrix side of C II	FAD binding and succinate-ubiquinone reduction	Tumour cells[151]	Unknown	Decreases succinate-ubiquinone reductase and increases fumarate reductase activities[151]
C III					
Core I	MIM		Bovine heart[150] Human sperma[152]	Unknown	Unknown
Core II	MIM		Bovine heart[150] Yeast[153]	Unknown Serine 141	Unknown Unknown
Cyt c	Mitochondrial intermembrane space	Transfer of electrons from C III to C IV	Bovine heart[154] Bovine liver[155]	Tyrosine 97[154] Tyrosine 48[155]	Reduces oxidation of P*-cytochrome c by C IV[154,155]
C IV					
Subunit I	MIM	Cyt c oxidation, oxygen reduction, proton translocation	Bovine heart and liver[113,114] Bovine heart[156]	Tyrosine 304[113,114] Suggested serine 441[110]; threonine?[156]	Inhibits complex IV activity[113,114] Turns on the allosteric inhibition of complex IV by high ATP[156]
Subunit II	MIM	Cyt c oxidation, oxygen reduction, proton translocation	Human osteoclasts Murine fibroblasts	Tyrosine?[157] Tyrosine?[158]	Increases C IV activity[157] Decreases COX activity and ATP level[158]
Subunit IV	MIM	Regulation of C IV by the ATP/ADP ratio	Bovine liver[159] Bovine heart[156] Yeast[153]	Unknown[159] Serine 34[156] Threonine 55[153]	Unknown Unknown Unknown
Subunit Va	Matrix side of C IV	Regulation of complex IV by thyroid hormons	Bovine heart[156]	Serine 4[156] Threonine 35[156]	Unknown
Subunit VIb	Intermembrane side of C IV	Cooperativity between the two cyt c binding sites within the C IV dimer	Yeast[153]	Serine 82[153]	Unknown
C V					
Subunit α	Matrix part of C V (F1)	ATP synthesis from ADP and P_i	Bovine heart Potato[160] Yeast[153]	Unknown; Serine 178	Unknown Unknown
Subunit β	Matrix part of C V (F1)	ATP synthesis from ADP and P_i	Human skeletal muscle[161] Yeast[153]	Threonine? Serine 373; Threonine 237[153]	Unknown Unknown
Subunit δ	Matrix part of C V (F1)	ATP synthesis from ADP and P_i	Potato	Unknown	Unknown
Subunit γ	Matrix part of C V (F1)	ATP synthesis from ADP and P_i	Yeast	Serine 62[153]	Decreases the dimerization of C V[153]
Adenine nucleotide translocase	MIM	ADP, ATP transport	Yeast[153]	Serine 42, 155, 157; Threonine 39, 156[153] Tyrosine 190, 194[162]	Unknown Facilitates nucleotide transport and increases cellular respiration
Phosphate carrier	MIM	Phosphate transport	Bovine heart[106] Yeast[153]	Unknown;[106] Serine 4, 145; Threonine 297[153]	Unknown Unknown

C I, Complex I; C II, Complex II; C III, Complex III; C IV, Complex V; MIM, mitochondrial inner membrane; Cyt c, cytochrome c; ?, sequence not given.

DNA) and increased superoxide production. These calcium-dependent changes in mitochondrial function might contribute to the development of HF.

4.3 Defects in individual components of the ETC and phosphorylation apparatus in HF

A variety of mitochondrial defects has been described in the ETC complexes and components of the phosphorylation apparatus in both humans and experimental models of HF, and was evaluated by us in a recent review.[128] Most of these mitochondrial defects have not been confirmed by other groups. Moreover, a direct causal relationship between the purported mitochondrial defects and HF either has not been studied or has proven elusive.[129] In addition, the link between the reported specific defects and the decrease in mitochondrial oxidative phosphorylation has not been studied. Why is this link important? Mitochondria achieve the maximal oxidative capacity when the limitation by the phosphorylation system is released experimentally by the addition of an uncoupler.[10,130] These data suggest that there is an apparent excess in the activity of ETC complexes relative to the phosphorylation apparatus and the oxidative phosphorylation requirement.[131,132] The sites of control of respiration in normal heart-isolated mitochondria are located at complex I in the ETC[133] and at adenine nucleotide translocase and complex V in the phosphorylation apparatus.[134] In contrast, in a large variety of intact cells, the site of control of oxidative phosphorylation is located in complex IV[135] due to the ATP-allosteric inhibition of the complex.[136] Therefore, flux control analysis is necessary in both heart mitochondria and cardiac fibres isolated from failing hearts to establish a link between the ETC defects and the decreased oxidative phosphorylation.

4.4 Abnormalities in integrated mitochondrial function

The analysis of oxidative phosphorylation assesses the integrated function of the ETC coupled to ATP synthesis, membrane transport, dehydrogenase activities, and the structural integrity of the mitochondria. Decreased ADP-dependent respiratory rates were reported when oxygen consumption[28] was measured in saponin-permeabilized cardiac fibres isolated from rat,[28,137] dog,[19] and human[138] hearts with dilated cardiomyopathy as well as pressure overload and ischaemic HF.[138,139] In contrast, oxygen consumption by swine myocardium with pressure overload congestive HF was unchanged.[140] The reduced respiratory rates in cardiac fibres may be due to either a drop in mitochondrial function or a decrease in mitochondrial density. Citrate synthase activity and mitochondrial DNA copy number have been considered markers of mitochondrial density. However, discrepancies between these parameters were reported in HF, suggesting that these parameters may not be reliable to indicate changes in mitochondrial biogenesis in the failing cardiomyocytes.[28] Also, respiratory studies on cardiac fibres do not differentiate between the two distinct mitochondrial populations, SSM and IFM mitochondria.[38,42] Some of the reported disparate findings regarding defects in mitochondrial respiration in HF[141–143] were reconciled when the two distinct populations of cardiac mitochondria were separated and investigated.[40,144] These data were evaluated by us in a previous review.[128] We conclude here that studies on oxidative phosphorylation in freshly isolated cardiac SSM and IFM give a more accurate picture of the topology of bioenergetic defect in cardiac pathology.

4.5 Decrease in oxidative phosphorylation and respirasomes in HF

In a follow-up to the work of Sharov et al.[19] on oxidative phosphorylation in saponin-permeabilized cardiac fibres, we performed a comprehensive study of respiratory properties of the two populations of heart mitochondria[10] in a canine model of intracoronary microembolization-induced HF of moderate severity. Both populations of heart mitochondria were equally affected in this model of HF. We found a dramatic decrease in state 3 respiratory rates with substrates donating electrons at different sites in the ETC that was not relieved by an uncoupler, indicating that the defect in oxidative phosphorylation is localized in the terminal part of the ETC at the level of complex IV. However, neither complex IV activity nor the amount of the substrate cytochrome c was altered in HF. We concluded that the decrease in oxidative phosphorylation of heart mitochondria can be explained by the decrease in the amount of functional respirasomes.

We further asked whether this defect in the supramolecular assembly is due to changes in the phospholipids of the mitochondrial inner membrane or modifications of the subunits of the ETC complexes. The content of the main phospholipid species, including CL, as well as the molecular species of CL, was unchanged in cardiac mitochondria in HF. In heart mitochondria isolated from HF, complex IV not incorporated into the respirasomes contained an increased content of threonine phosphorylation. This suggested that cAMP-dependent phosphorylation may have a role in decreasing the level of supercomplexes. In saponin-permeabilized heart muscle fibres, cAMP caused a decrease in oxidative phosphorylation, at least at the level of complex IV (unpublished observations). We suggest that phosphorylation of specific complex IV subunits either limits the incorporation of complex IV in supercomplexes or decreases supercomplex stability.

According to our findings,[10] we propose a mechanistic pathway by which the decrease in mitochondrial functional respirasomes drives the progression of HF. The decrease in respirasomes in HF is the primary event causing the decrease in mitochondrial oxidative phosphorylation, and is coupled with an increase in electron leakage and superoxide generation in complexes I and III that are not incorporated within respirasomes. Complex I-generated superoxide is responsible for the oxidative alterations of mtNDA and the adjacent mitochondrial inner membrane lipids and proteins including subunits of the ETC complexes. These modifications cause the decreased activity of ETC complexes reported by others in severe HF, potentially decrease oxidative phosphorylation, and finally lead to the degradation of the damaged complexes. Complex III-generated superoxide is responsible for the oxidative alterations of the cytosolic myofibrillar proteins. This sequence of events signals the transition to irreversible heart damage.

5. Conclusions, future direction

HF is a multifactorial syndrome characterized by mechanical dysfunction of the myocardium. Recent studies identify mitochondria as both the target and the origin of major pathogenic pathways which lead to the progression of myocardial dysfunction. As the main process that drives the cardiac pump, mitochondrial oxidative phosphorylation is regulated by reversible phosphorylation induced by cAMP, which is

either synthesized within the mitochondria or generated by the stimulatory G protein-coupled AC as an effector of the β-receptor activation. Therefore, excessive sympathetic stimulation may dysregulate mitochondrial function by affecting either individual complexes or their supramolecular assemblies (respirasomes). Mitochondrial defects lead to metabolic remodelling, deficit in cardiac energetics, and increased oxidative stress. Therefore, the elucidation of mitochondrial cytopathy in HF is crucial and forms the basis of a therapeutic strategy to maintain mitochondrial integrity, optimize substrate metabolism, alleviate oxidative stress, and improve the contractile function of myocardium.

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