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Mitochondrial Metabolism in Aging Heart

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Abstract

Altered mitochondrial metabolism is the underlying basis for the increased sensitivity in the aged heart to stress. The aged heart exhibits impaired metabolic flexibility, with a decreased capacity to oxidize fatty acids and enhanced dependence on glucose metabolism. Aging impairs mitochondrial oxidative phosphorylation, with a greater role played by the mitochondria located between the myofibrils, the interfibrillar mitochondria. With aging, there is a decrease in activity of complexes III and IV, which account for the decrease in respiration. Furthermore, aging decreases mitochondrial content among the myofibrils. The end result is that in the interfibrillar area there is an approximate 50% decrease in mitochondrial function, affecting all substrates. The defective mitochondria persist in the aged heart, leading to enhanced oxidant production and oxidative injury and the activation of oxidant signaling for cell death. Aging defects in mitochondria represent new therapeutic targets, whether by manipulation of the mitochondrial proteome, modulation of electron transport, activation of biogenesis or mitophagy, or the regulation of mitochondrial fission and fusion. These mechanisms provide new ways to attenuate cardiac disease in elders by preemptive treatment of age-related defects, in contrast to the treatment of disease-induced dysfunction.

Keywords

oxidative phosphorylation; electron transport chain; reactive oxygen species; cardiolipin; fatty acid oxidation; ubiquinol:cytochrome c oxidoreductase

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Subject Terms

Aging; Metabolism; Oxidant Stress; Ischemia; Pathophysiology; Myocardial Biology

1. Introduction

With age, the heart exhibits a decrease in the number of myocytes with a concomitant increase in the size of each cardiomyocyte and an increased accumulation of lipid and areas of fibrosis. There is increased reactive oxygen species (ROS), which are considered to be of mitochondrial origin and lead to increased cell loss. The uptake of fatty acids through the sarcolemma is augmented because of an increase in the amount of transport protein, CD36, but fatty acid oxidation is decreased.[1] Additionally, the aged heart has a reduced tolerance for stress, as exemplified by the greater injury than in the adult following ischemia/reperfusion. The heart relies on continuous production of energy and mitochondrial oxidative phosphorylation as the predominant source of this energy. Cardiac mitochondria generate 90% of the ATP in heart, but their importance is not restricted to bioenergetics, as they also act as a metabolic waystation, which contributes to the balance between glucose and fatty acid oxidation. The structure of the heart is intimately connected to the presence of two populations of mitochondria, one under the sarcolemmal membrane (SSM) and the other between the myofibrils (IFM); these two populations have different activities.

The mitochondrial defect in aging has been localized to the IFM population. Furthermore, this population of mitochondria in the aged heart generates the increased ROS. The former defect and its ROS consequence in mitochondrial energy metabolism are considered to be the underlying factors in aging, which affects an ever-growing segment of our population. Furthermore, impaired mitochondrial energy metabolism contributes to myocardial injury during stress such as ischemia and reperfusion. Defects in mitochondrial energy production in the heart during aging are now recognized as central players in impaired cellular and organ function. Extending beyond oxidative metabolism, recently described mitochondrial responses including mitochondrial dynamics of fission and fusion; the interrelated potential clearance of dysfunctional mitochondria are appreciated to potentially contribute to the aging cardiac phenotype. In this review of mitochondrial metabolism in the aged heart, we will discuss these issues and address areas where additional research is required.

2. Metabolism and Metabolic Flexibility in the Aging Heart

Mitochondrial oxidative phosphorylation (OXPHOS) is the main energy source for the myocardium. In cardiac mitochondria, fatty acids and carbohydrate are the two main classes of substrates used to generate ATP through OXPHOS.[2] Under physiologic conditions, the heart receives approximately 70% of its energy from fatty acid oxidation and 30% from metabolism of carbohydrates via pyruvate and lactate. Under different conditions, the ratio of energy supply from fatty acid and carbohydrate is dynamically adjusted, indicating that heart has a flexibility of substrate usage.[3] The impact of aging on substrate flexibility is incompletely understood.

Glycolysis yields pyruvate in the cytoplasm. To enter the mitochondria, pyruvate, first traverses the mitochondrial outer membrane via the voltage-dependent anion channel (VDAC) and then the inner membrane via monocarboxylate transporter system.[4, 5] Once in the mitochondrial matrix, pyruvate dehydrogenase (PDH) metabolizes pyruvate and generates NADH and acetyl-CoA. From acetyl-CoA, citrate synthase produces citrate using oxaloacetate from the tricarboxylic acid cycle (TCA) located in the matrix space.[4] In addition to pyruvate, other TCA substrates are regulated by the action of inner membrane transporters. Pyruvate dehydrogenase complex (PDC), a gatekeeper for mitochondrial glucose oxidation, is a key enzyme complex that modulates the competition between fatty acid and carbohydrate metabolism. PDH activity is regulated by pyruvate dehydrogenase kinases (PDKs) that respond to allosteric modifiers derived from glycolysis and fatty acid oxidation.[5] NADH and acetyl-CoA derived from fatty acid oxidation (and pyruvate oxidation) activate PDKs, which leads to PDH inhibition, whereas pyruvate generated from glycolysis deactivates the PDKs, in turn activating PDH. PDK activity is regulated via post-translational modifications including phosphorylation and lysine acetylation.[6] PDC regulation is critical to regulation of the metabolic flux of carbohydrate in pathophysiologic states.[5] In aging, post-translational modification by PDK via altered phosphorylation increases flux through PDC, favoring mitochondrial metabolism that leads to enhanced glucose oxidation.[7] Thus, there is increased capacity for glucose oxidation by mitochondria via enhanced PDC-mediated pyruvate metabolism. Also there is increased capacity for glucose oxidation by mitochondria via enhanced PDC-mediated pyruvate metabolism. When PDH activity is measured without inhibiting PDK during the assay, apparent PDH activity is decreased in aged rat hearts, but the total activity was not measured.[8]

Fatty acids are oxidized mainly within mitochondria. Beta oxidation of fatty acids occurs in the matrix following activation and transport of fatty acids into the matrix via contact sites.[3] Beta oxidation also produces NADH and $FADH_2$. Fatty acid oxidation is decreased in the aging heart.[9, 10] PDK4 content is decreased in the aged heart,[9] favoring increased glucose oxidation at the expense of fatty acid oxidation.

In contrast, the enzymes involved in the regulated transport of long-chain fatty acids into mitochondria and oxidation are unaltered despite decreases in PPARγ activity.[9] The long-chain fatty acyl-CoA is first converted to the long-chain acylcarnitine through carnitine palmitoyltransferase I (CPT-I).[11] The long-chain acylcarnitine is transported into the mitochondrial matrix across the inner mitochondrial membrane by carnitine-acylcarnitine translocase. Then the long-chain acylcarnitine is converted back to matrix long-chain acyl-CoA by carnitine palmitoyltransferase II (CPT-II).[12] Among these enzymes, CPT-I is the rate-limiting step in regulating fatty acid oxidation. There are two isoforms of CPT-I: liver type isoform and muscle type isoform. The dominant CPT-I isoform in adult heart is the muscle type isoform, which is very sensitive to malonyl-CoA inhibition. Malonyl-CoA is produced through carboxylation of acetyl-CoA by acetyl-CoA carboxylase and is converted back to acetyl CoA by malonyl-CoA decarboxylase. The increased pyruvate oxidation results in inhibition of fatty acid oxidation by increasing acetyl-CoA and subsequent malonyl-CoA generation.[3, 13] In contrast, increased fatty acid oxidation can inhibit pyruvate oxidation by generation of acetyl-CoA. Thus, there is interaction between fatty acids

oxidation and glucose metabolism. Aging does not alter CPT-I content nor phosphorylation, nor is there a shift of isoform.[3]

We found no change in CPT-I velocity or sensitivity to malonyl-CoA in either population of heart mitochondria. In contrast, the Hagen laboratory described a decrease in palmitoyl-CoA oxidation and CPT-I activity only in IFM. We believe the decreased palmitoyl-CoA oxidation occurs as a result of the IFM ETC defects; nagarse was used to isolate IFM[14] and that is associated with a decrease in CPT-I activity, whereas we employ trypsin without an effect on CPT-I.[15] Acetyl-L-carnitine supplementation reverses the age-related decline in CPT1 activity in interfibrillar mitochondria without changing the L-carnitine content in the rat heart.[14]

Fatty acid metabolism is altered in disease conditions that could interact with age-induced defects. In an in vitro working model, fatty acid oxidation is decreased with compromised cardiac function in aged mouse hearts exposed to high afterload.[16] Ischemia leads to decreased malonyl CoA content and increased FAO by increasing CPT-I activity. The circulating fatty acid concentration also is increased following ischemia.[17]

The harnessing of chemical energy by mitochondria as an inner membrane potential requires the inner membrane to have tightly-controlled permeability. The inner membrane contains ion channels and transporters that regulate proton and ion fluxes as well as the uptake of substrates and the translocation of small molecules into and out of the matrix.[2]

The phosphorylation apparatus harnesses the electrochemical energy stored in the inner membrane proton gradient to phosphorylate ADP by complex V.[2] Uncoupling of electron flux from ADP phosphorylation occurs with dissipation of the electrochemical gradient. Mechanisms to dissipate the electrochemical gradient under physiologic circumstances include activation of uncoupling proteins that span the inner membrane[18] and regulated “proton slip” within electron transport chain (ETC) complexes.[19] Membrane potential controls the rate of respiration since in complexes I and III electron flow with the complex includes a segment where electrons move counter to the membrane potential. In disease settings, dissipation of the electrochemical gradient occurs due to breaches in the inner membrane or damage to complex V that allow back-diffusion of protons down their electrochemical gradient not coupled to phosphorylation. As discussed below, damage to inner membrane phospholipids, especially cardiolipin,[20] or activation of the mitochondrial permeability transition pore are mechanisms [21, 22] that lead to pathologic uncoupling of respiration.

Respiratory defects present in mitochondria are reflected in the rate of global respiration in permeablilized muscle fibers.[23] Even though only IFM mitochondria are affected by age, global respiration in the fibers is decreased. Decreases in the rate of oxidative phosphorylation with substrates that selectively donate to complex III or complex IV are observed, supporting the impact on global respiration in the myocyte of the age-induced decrease in respiration in IFM.[23]

Interfibrillar mitochondria (IFM) isolated from 24 and 28 month aged Fischer 344 rat hearts exhibit decreased OXPHOS, whereas respiration in SSM is unaffected.[24] Although the

ADP-stimulated rates of respiration are decreased in IFM, oxygen consumption remains tightly coupled to ADP phosphorylation.[24] The decrease in uncoupled respiration localizes the defect to the ETC.[24] The decrease in respiration using selective donors to complex IV localizes a defect at least to cytochrome oxidase, the most distal complex,[24] as discussed below. Controversy regarding the presence of age-related defects was based on the fact that in some laboratories no defect was found when isolation techniques yielded only SSM[25,26] whereas combined mitochondrial populations were studied in different laboratories[27,28, 29] and in the later case, the IFM defect in fact is masked to a great extent by the presence of unaffected SSM. Age-related decreases in fatty acid oxidation [28] and cytochrome oxidase activity [30–32] were localized to IFM.[24] Previous studies of mitochondrial populations containing only SSM found, as expected, that ROS production [33] and protein import [34] were unaltered by age. The finding of defects localized to IFM is not unique to aging and is also observed in diabetes[35] and cardiomyopathy.[36] Decreased OXPHOS capacity has been observed in a variety of rat strains [37] as well as murine models of aging.[38] The study of IFM, in which key aging-related alterations in OXPHOS reside, without the confounding effects of admixed, unaltered SSM, has facilitated the study of the contributions of mitochondria to age-related dysfunction in the heart.[39]

The identification of each population is based upon location in the myocyte and the documentation of selective isolation based upon electron microscopy. Molecular markers for each population remain elusive. Unfortunately, there have not been studies of the global mitochondrial proteome to compare SSM and IFM in adult and aged hearts, unlike in the diabetic heart.[35] Nonetheless, responses at the molecular level differ between the two populations. In a proteomic study of the acetylated proteins in each population, differences between SSM and IFM were observed. The differences included outcomes from both endogenous acetylation/deacetylation as well as responses to chemical/non-enzymatic acetylation.[40] Of note, SIRT3 content was decreased in SSM but remained unaltered in IFM. [40]

It remains a possibility that the alterations in each population arise as a result of differences in regional environments within the cardiac myocyte. Global ATP content in adult and aged hearts are similar.[41] The ATP content in the subsarcolemmal region where SSM reside could differ from that in the remainder of the myocyte. IFM are in proximity to t-tubules in the sarcomeres, which may increase exposure of the IFM to calcium. The relative interaction of SSM versus IFM with sarcoplasmic reticulum via mitochondria-associated membranes also is unknown. The regional environment may provide a milieu for selective defects observed in IFM in genetic[36] and diabetic[35] cardiomyopathies as well as with aging due to the impairment of the removal of damaged mitochondria from this region. The regional differences in mitochondrial function, either as a result of damage to the mitochondria by external mechanisms or regional impairment in function, may be attenuated by the presence of connections between mitochondria in subsarcolemmal and interfibrillar regions.[42, 43]

The role of regional differences within the myocyte due to genetic deletion or overexpression of nuclear encoded mitochondrial proteins has not been considered. It would initially seem that both SSM and IFM populations would be affected by the absence or overexpression of a nuclear-encoded mitochondrial component protein to a similar extent.

Thus, the study of alteration in mitochondrial function that involved only SSM or IFM in response to gain of function or loss of function of a mitochondrial protein encoded by the nuclear genome would seem to be irrelevant. However, in light of the potential for mitochondrial damage due to regional-specific stresses, or, possibly, defective generation or removal mitochondria specific to one region of the myocyte, it is plausible that despite the similar gain or loss of a mitochondrial component, that dysfunction may be manifest in only one subpopulation of mitochondria.

3. Mitochondrial Morphology in the Aging Heart

3A. Subsarcolemmal vs. Interfibrillar mitochondria

Using transmission electron microscopy (TEM) to examine heart ventricular tissue, the presence of two spatially distinct populations of mitochondria is apparent. Using a Polytron homogenizer, skinned fibers are produced: these lack surface mitochondria, but retain mitochondria trapped between the myofibrils. This observation provides morphological evidence of the two disparate types of mitochondria: one type clustered beneath the sarcolemma (SSM), the other type, “imprisoned” by myofibrils are the interfibrillar mitochondria (IFM).[44] The content of IFM is decreased in the aged heart.[24] As supported by rigorous studies of the recovery of mitochondrial marker enzymes, the decreased content of IFM isolated from aged hearts reflects a true decrement present in myocardium.[24] In contrast, the content of SSM is unaltered with age. Thus, the decreased content of IFM magnifies the respiratory defect present in IFM. Regional differences in mitochondrial function have been observed in multiple species including mouse, hamster, guinea pig, rabbit, and dog. Aging defects in mitochondrial function have been observed in mouse and human, although studies have not yet addressed if defects are present in only one subpopulation.

3B. Mitochondrial morphology in the aging heart: Cristae and contact sites

The cartoon in Figure 3 depicts a cardiomyocyte to illustrate the location of SSM under the sarcolemmal membrane and IFM between the myofibrils (Figure 3). Structural differences of the two populations of mitochondria in adult and aged Fischer 344 rat hearts using TEM are not apparent in in situ or in isolated organelles (Figure 4a, b).[24, 44, 45] However, osmium-extracted cardiomyocytes examined by high resolution scanning electron microscopy (HRSEM) revealed differences in the three-dimensional structure of cristae. As shown in Figure 5a for adult rat heart, the sarcolemma is clearly observed, immediately beneath the sarcolemma are the SSM (shown by the arrow to Figure 5b). The empty spaces within the fiber represent areas previously containing myofibrils, which have been extracted by the osmium process. Around these voids are the organelles, the IFM, which are stacked as shown in Figure 5a. The arrow points to an IFM at greater magnification (Figure 5c). The SSM contain largely lamelliform cristae (Figure 5b), but in contrast the IFM have mostly tubular cristae (Figure 5c). Using HRSEM, in the 24-month old Fischer 344 rat the isolated SSM are mainly lamelliform (Fig. 4c) whereas the IFM contain predominantly tubular cristae (Fig. 4d).[46, 47] There are no age-related alterations in crista morphology that accompany the biochemical defects. In this study, isolated mitochondria show relatively more plasticity with rearrangement of the cristae morphology, but again there are no

structural differences between the two ages for either population of mitochondria. However, a recent study of 24 mo. aged Wistar and OXYS rats using TEM described alterations in cristae structure with age.[48] The different results could be due to the different strains of rat using different fixation.[24]

3C. Cardiolipin: Membrane building block and signaling component

Cardiolipin (CL) is a diphosphatidylglycerol lipid found largely in the mitochondrial inner membrane and that is required for optimal energy generation.[20, 49] CL is highly enriched in oxidatively-sensitive linoleic acid (C18:2).[50] Aging did not alter the content or composition of CL in SSM or IFM from elderly 24 month Fischer 344 rats compared to adult.[51] CL content was documented by analytic phosphate assay with recovery studies.[51] The acyl-group composition and molecular species of CL also was unaltered in aged hearts. In contrast, the Paradies group has described a decrease in CL content with aging estimated by UV absorbance using a mixed population of cardiac mitochondria.[30, 32] However, in our hands CL content using this approach also was unchanged in IFM with aging.[51] Thus, although others propose decreases in CL content with aging leading to altered membrane fluidity in the inner membrane,[52, 53] the role of CL content as a mechanism of age-induced defects in electron transport remains controversial.

CL undergoes age-specific modification in response to the stress of ischemia in the aged heart. [54, 55] Consistent with a lipid peroxide mechanism, the new molecular species involves the addition of 48 daltons to a single linoleic acid acyl-group, consistent with the formation of a hydroxyl-lipid peroxide. Products of linoleic acid with molecular weight increased by 48 daltons are formed during the mitochondria-driven peroxidation of linoleic acid.[56, 57] CL with a molecular weight also increased by 48 daltons is present during in situ oxidative stress in other systems.[58] The oxidation of CL provides strong support for the presence of age-enhanced oxidative damage to mitochondria during ischemic stress in the aged heart.

The formation of a single product, rather than mixture of oxygenated linoleic residues,[54] suggests that a specific, perhaps enzymatic, mechanism of peroxidation occurs in contrast to stochastic chemical peroxidation. Cytochrome *c* can alter its interaction with CL[59] leading to a conformational change that favors the oxidation of a key methionine ligand of the cytochrome *c* heme, resulting in formation of a new peroxidase activity. CL is oxidized by this newly generated cytochrome c peroxidase.[58,60] The key oxidative modification of cytochrome *c* required for acquisition of peroxidase activity occurs during ischemia,[54, 55,61] targeting oxidative modification as a plausible mechanism.

Alteration in CL species often results from defective remodeling of CL.[60] Under oxidative conditions, monolysocardiolipin (MLCL) is generated via hydrolysis of an oxidized C18:2 residue The MLCL then undergoes reacylation via a process of salvage remodeling.[60] The enzyme, tafazzin, remodels nascent CL during de novo synthesis to the required $(C18:2)_4$ species.[62, 63] Tafazzin, however, appears to contribute a minor, if any, step in salvage remodeling of CL in the mature heart in response to disease-induced CL oxidation and damage.[64] In contrast, acyl-CoA:lysocardiolipin acyltransferase-1 (ALCAT-1) is a major contributor to the salvage remodeling of CL.[60, 65, 66] In ALCAT-1 mediated remodeling, MLCL is usually reacylated using non-linoleate acyl-residues.[60, 66, 67] including very long

chain fatty acids, such as docosahexanoic acid (C22:6), creating higher mass CL species.[60, 68] Substitution of docosahexanoic acid occurs in heart failure and diabetes.[68] Thus, an alternative mechanism of formation of the new molecular species is that a higher molecular weight acyl-group has been substituted for a C18:2. However, the impact of higher molecular weight acyl residues on OXPHOS is unclear, since mitochondria with increased docosahexaenoic and arachidonic content in CL displayed unaltered rates of OXPHOS.[69] The presence of non-linoleic acyls disrupts the compact configuration of $(C18:2)_4$-CL, paradoxically increasing the oxidative sensitivity of the asymmetric CL.[20] ALCAT1 is located in the endoplasmic reticulum,[70] suggesting that secondary CL remodeling may require mitochondria-endoplasmic reticulum interactions. Since secondary remodeling of CL by ALCAT1 is extramitochondrial, it is unlikely that regional defects would be present. Thus, the lack of altered remodeling in IFM from the age heart is consistent with ALCAT1 as the major mechanism of remodeling.

Oxidation of CL decreases the function of ETC complexes,[71] disrupts the bilayer arrangement of the inner membrane,[20, 60] and favors the release of cytochrome *c* to initiate cell death.[58, 72] CL oxidation likely will continue to emerge as a key mechanism of the enhanced mitochondrial-dependent injury that occurs in the aged heart in response to pathologic stress, and is a potential mechanism of the augmented age-enhanced susceptibility to superimposed cardiac disease.

SS-31, (a synthetic peptide), can directly bind to CL on the inner membrane and improve the efficiency of the ETC. SS-31 inhibits the function of the cytochrome *c* cardiolipin peroxidase.[73] SS-31 treatment increases mitochondrial efficiency in aged skeletal muscle, decreases cardiac injury during ischemia-reperfusion, and improves mitochondrial function in heart failure.[73] These actions support the pathogenic role of cardiolipin oxidation by the cytochrome *c* peroxidase in the heart under stress.

4. Age-Induced defects in Electron Transport

The ETC is composed of four multisubunit enzyme complexes, complexes I-IV (Figure 1). Electron transfer down the redox potential gradient from NADH or $FADH_2$ to oxygen is coupled to the active transport of hydrogen ions from the matrix to the cytosolic side of the inner membrane by complexes I, III, and IV.[74] Complex I oxidizes NADH, with sequential electron flow to coenzyme Q, complex III, cytochrome *c,* and ultimately to cytochrome oxidase (complex IV), the latter reducing oxygen to water. The complexes of the ETC are organized into larger respirasomes ("supercomplexes"),[75, 76] to optimize channeling of reducing equivalents between the components. The organization of respirasomes is altered in the aged heart,[77] which can result in functional defects in electron transport in the absence of post-translational modification or decreased content of an individual ETC complex. For example, although OXPHOS of substrates that enter through complex I is decreased, the site of ETC defects in complexes III and IV (discussed below) account for the decrease. Complex I enzyme activity remained unaltered by age in rat[24] and mouse hearts.[78] Functional decreases in complex I might be related to alteration in complex I containing respirasomes,[77] potentially related to oxidative damage to CL.[79] Such alterations in supercomplex organization are observed following the onset of congestive heart failure.[80]

4A. Cytochrome oxidase (complex IV)

The aging defect in cytochrome oxidase activity occurs as a result of an altered inner membrane environment of the complex.[24, 31, 32] Activity is recovered by the addition of phospholipid liposomes,[24] including CL.[32] The finding of unaltered CL content and composition in IFM, despite the presence of a functional defect, supports the presence of a probable defect in CL in the immediate environment of cytochrome oxidase. Such a defect likely impacts the organization of cytochrome oxidase into supercomplexes,[81] with subsequent decreased enzyme activity. The altered membrane environment leads to a decrease in fluidity of the inner membrane.[52, 53] Altered phase behavior of the inner membrane with age may occur due to the depletion of n-3 acyl-groups [82] or to oxidative damage in membrane phospholipids.[83]

In addition to the phospholipid environment defect, there is a functional defect regarding the subunits of cytochrome oxidase. The content of subunits are unaltered with age, as identified by analytical immunoblotting.[84, 85] In contrast, immunoelectron microscopy revealed a 25% reduction in cytochrome oxidase subunit VIIa selective to IFM in the aged heart. The absence of immunoreaction of the VIIa subunit *in situ* assessed by immunoelectron microscopy was not due to a reduction in protein, but rather by a masking phenomenon or by an *in situ* change in protein structure affecting cytochrome oxidase.

Decreased cytochrome oxidase activity not only has the potential to impair energy production, but also to enhance cytotoxicity. Attenuated flux through cytochrome oxidase increases the relative reduction of “upstream” redox centers in complexes I and III, favoring ROS production as discussed below. Additionally, decreased cytochrome oxidase activity activates cell stress responses,[86] in part via the activation of endoplasmic reticulum stress.[87] Cytochrome oxidase content and activity are decreased in the aged human heart.[84, 85]

4B. Complex III

Complex III activity is decreased only in IFM in the aged heart.[88] Mammalian complex III is composed of two 11 subunit monomers, with three of these subunits, cytochrome *b*, cytochrome c_1, and the iron-sulfur protein (ISP) involved in electron transfer.[89, 90] Following oxidation of the substrate, ubiquinol, there is a concerted transfer of electrons to cytochromes *b* and c_1 in a bifurcated fashion mediated by motion of the ISP.[89, 90] Based upon structure-functional relationships of electron flow within complex III, [91–94] the study of mutant complex III phenotypes in bacteria,[91, 94–96] and the three-dimensional crystal structure, [89, 90] the study of partial reactions of complex III was utilized to localize the defect to the ubiquinol binding site (Qo) within cytochrome *b*. The use of specific chemical inhibitors further localizes the defect to the proximal domain of the Qo site. By comparison to phenotypes that result from site-directed mutagenesis in the bacterium *Rhodobacter spheroids* (bacteria allow straightforward site-directed mutagenesis of cytochrome *b*), the region of the age-induced defect can be deduced. The aging phenotype of complex III consists of decreased maximal activity,[88] preserved EPR signal,[88] an increased leak through myxothiazol blockade to reduce cytochrome *b*, and preserved inhibition by stigmatellin.[97] Amino acids in the Qo site are highly conserved from *R. spheroides* to mammalian cytochrome *b*.[95] Point mutations with resistance to myxothiazol but not to stigmatellin

(A126, Y132, V133, M139, G143, F275)[95] are potential sites of the age-related defect. In addition to myxothiazol resistance, mutation at Y132 also decreases complex III activity, yet maintains the EPR signal.[95] For this reason, Y132 can be considered as a potential site of aging-induced oxidative modification to the Qo site, a mechanism of the aging phenotype of complex III. Proteomic evaluation of the Qo site, including Y132, is limited by the difficultly of proteomic approaches to this most hydrophobic region of the markedly hydrophobic cytochrome *b* subunit. Current proteomic techniques should provide approaches to study this key region of age-induced alteration in electron transport. Cytochrome *b* is encoded by mitochondrial DNA, so mitochondrial DNA mutations with age could alter the composition of the Qo site with subsequent alteration in complex III activity and age-induced pathophysiology. Unfortunately, because it is a mitochondrial-encoded protein, direct molecular manipulation to test the role of Y132 is not feasible due to the multiple copies of mtDNA present.

4C. Complex V

The activity of complex V decreases with age,[98, 99] potentially decreasing the efficiency or coupling of OXPHOS. Localization of the site of the defect within complex V remains unknown. Age-induced alteration of complex V is of contemporary interest since complex V, recently identified as the likely locus of the mitochondrial permeability transition pore,[100] has an increased propensity to open, even in the baseline state, in the aged heart.[101] Nonetheless, uncoupled respiration is decreased by age in IFM in aged hearts, localizing the defects that limit the rate of OXPHOS to the ETC rather than to complex V.[24] The ADP:O ratio declines with age in the rat,[24] perhaps reflecting a defect in this complex that modestly decreases the efficiency of phosphorylation.

5. Mitochondrial DNA with age and impact on OXPHOS

Accumulation of mtDNA deletions in cells leads to respiratory chain deficiency.[102] Mitochondrial DNA copy number is decreased in aged skeletal muscle and liver compared to adults.[103] However, mitochondrial DNA copy number is not altered in the aged heart,[103,104] including SSM and IFM.[105] However, analysis of transcripts of catalytic mtDNA encoded subunits in aged hearts is suggestive of increased mtDNA mutation with age.[104] The downregulation of the 8-oxodeoxyguanosine glycosylase mouse model with defective base excision repair in mitochondria clearly supports the accumulation of oxidative damage to mtDNA with age.[106, 107] The accumulation of oxidative damage to mtDNA, likely in part from mitochondria themselves as discussed below, may be a contributing mechanism to age-induced impairment of mitochondrial metabolism. Oxidative damage to mtDNA leads to point mutations and eventual double strand breaks, resulting in large deletions.[108, 109] However, the accumulation of point mutations with age or in mice heterozygous for a proofreading-defective mitochondrial polymerase gamma ("mitochondrial mutator" heterozygotes) did not affect lifespan or cardiac function.[108] In contrast, mice homozygous for proofreading defective polymerase gamma (*Polga* $^{mut/mut}$) exhibit a marked increase in mtDNA mutation burden and cardiac dysfunction with an increase in the prevalence of cytochrome oxidase negative myocytes[109] that are likely to exhibit defective mitochondrial respiration. Of interest, aged human hearts have an increased

frequency of cytochrome oxidase negative myocytes.[84, 85] Damage and deletion of mtDNA can give rise to non-homogeneous mtDNA composition between mitochondrial populations, thus increasing mitochondrial heteroplasmy.

Accumulation of mtDNA mutations with age has functional consequences for mitochondria and the heart. There is increased protein oxidation in heart tissue and increased programmed cell death.[110] The finding of an age-related defect in IFM localized to mtDNA encoded cytochrome *b* could imply mtDNA mutation. Altered mtDNA transcription with age may also indicate defects in mtDNA regulation at levels above the mitochondrial genome itself. Recently, STAT1 localized to mitochondria was found to impact mtDNA replication.[111] Potential defects in TFAM transcription, nucleoid (the mtDNA in aggregate) function may also impact mtDNA replication and stability. Although previous studies support the lack of change in mitochondrial DNA copy number, the impact of mtDNA number and mutation burden, especially differences between the two populations of mitochondria, are worthy of reassessment in the current era of deep sequencing.

6. Production of ROS by Mitochondria in the Aged Heart

6A. Sites of Production of Oxidants within the ETC

Aging increases ROS production from mitochondria in multiple cardiac models.[112–114] Complexes I, II, and III are potential sources for ROS production by the ETC,[115–117] with complex I producing ROS by both forward and reverse electron transport.[118–120] Two mechanisms combine to increase ROS production from mitochondria in aged tissues.[121] First, decreased flux through the ETC increases the reduction of upstream complexes, especially complexes I and III, increasing the probability that reduced redox centers will directly react with molecular oxygen to generate ROS.[122, 123] Thus, the fraction of electron flux that forms ROS is greater during state 4 ADP-limited respiration than during state 3 ADP-stimulated respiration.[112, 118] Dissipation of the inner membrane potential decreases the efficiency of phosphorylation; the increased rate of electron flux through the ETC decreases the probability that a reduced redox center will directly react with molecular oxygen. ROS production from complex III is especially responsive to modulation of inner membrane potential.[124, 125] Thus, the decrease in respiration through cytochrome oxidase present in IFM from the aged heart favors the relative reduction of complexes I and III with greater ROS production. Second, aging-induced modification of individual ETC complexes alter the function of the complex to directly favor ROS production as seen with complex III in IFM.[97]

Complex III is a major site for net ROS release from mitochondria.[115122] ROS produced at the quinol oxidation site (Qo center) are generated at the cytosolic face of the inner membrane[115, 125] and are released into the intermembrane space, ultimately favoring release from mitochondria.[115, 126, 127] The aging defect at cytochrome *b* in the Qo site of complex III in IFM [97] predicts the increase in net production and release of ROS from IFM isolated from aged hearts. The net release of H_2O_2 was increased in IFM isolated from aged rat hearts compared to adult controls, whereas SSM, without the aging defect in complex III, showed no increase in ROS production.[128] In line with increased ROS production within IFM, indicators of oxidative stress increased in these organelles during aging.[128, 129] The

increased oxidative stress to mitochondria may underlie the apparent tendency of mitochondria from the aged heart to undergo permeability transition leading to cytochrome *c* release as discussed below, with subsequent initiation of programmed cell death.[128–130] The pathway of release of ROS generated by the Qo site of complex III is the mitochondrial contact site.[115, 126, 127] Contact sites are in close association with sites of mitochondria-endoplasmic reticulum interaction.[131] Thus, superoxide directed outward from mitochondria is likely to impact mitochondria-associated membranes and endoplasmic reticulum as the proximate target.[132]

ROS production from complex I can increase in two ways: by a blockade of forward electron flow from NADH-linked substrates via inhibition of enzyme activity or via blockade at distal sites in the ETC,[112, 122, 127, 133–135] including cytochrome oxidase. Alternatively, the induction of reverse electron flow from complex II to complex I using succinate as a substrate leads to ROS production.[120, 122, 136–138] Reverse electron flow requires mitochondrial membrane potential.[137] Reverse flow can occur in the presence of simultaneously available complex I and complex II substrates, thus is of probable physiologic relevance.[120]

In addition to the ETC, $p66^{shc}$ and monoamine oxidase (MAO) are sources that generate ROS in mitochondria. The $p66^{shc}$ is a ubiquitously expressed adaptor protein[139] located within the mitochondrial intermembrane space. Molecular oxygen is reduced to H_2O_2 in a redox center of $p66^{Shc}$ coupled with the oxidation of cytochrome *c*.[140] Ablation of $p66^{shc}$ increases the resistance to oxidative stress and extends the life span of mice.[139] Inhibition of $p66^{shc}$ also decreases cell injury during ischemia-reperfusion through a reduction of oxidative stress.[141–143] However, knockout of $p66^{shc}$ increases cell injury in the mouse heart subjected to reperfusion after a short period of ischemia.[144]

MAO is a flavoenzyme located in the outer mitochondrial membrane. MAO includes two isoforms (MAO-A and B) that generate H_2O_2 when MAO oxidatively breaks down neurotransmitters such as norepinephrine, epinephrine, and dopamine.[145] Oxidative stress induced by MAO contributes to cardiac injury[146] and to muscle dystrophy.[147] MAO-B, but not MAO-A, is increased in aged mouse hearts.[148] Interestingly, only inhibition of MAO-A but not MAO-B can decrease MAO-mediated H_2O_2 generation in aged heart.[149] MAO is a source of oxidative stress during aging

The increased production of ROS by mitochondria leads to greater oxidative damage within mitochondria, including protein sulfhydryl oxidation, lipid peroxidation, and mtDNA damage.[129, 150] A relative increase in the uncoupling of respiration, [118, 119, 151] as well as an increase in antioxidant enzyme contents, provides complimentary mechanisms to decrease oxidative damage. Thus, the initial target of mitochondrial ROS production is the mitochondria themselves. Next, ROS, including superoxide can be released by traversing anion channels and contact sites, [115, 126, 127] possibly targeting structures adjacent to mitochondria. Recent work has highlighted key effectors of oxidants produced by the ETC, rather than merely stochastic oxidant release from mitochondria. Examples of oxidant dependent targeted injury include activation of mitochondrial permeability transition pore

opening, generation of the cytochrome *c* cardiolipin peroxidase, and activation of the oxidant-dependent ASK-1 signaling cascade.

Overexpression of catalase within mitochondria attenuates cardiac aging, providing direct evidence that ROS generated from mitochondria is detrimental to aged hearts.[152] Although this is not always the case is best documented in invertebrate species[153] including *Caenorhabditis elegans*. Multiple genotypes that exhibit either decreased production or enhanced disposition of ROS exhibit increased lifespan.[154,155] In mammalian systems, clear evidence of the potentially protective low levels of signaling ROS production is more difficult to demonstrate. In fact, signaling ROS are critical for the mechanism of classic ischemic preconditioning, yet, as discussed below, the aged heart is relatively refractory to the protection of ischemic preconditioning, perhaps due to the excessive tonic production of ROS.

Nrf2 (nuclear factor-erythroid 2-related factor 2) is a redox-sensitive transcription factor. Nrf2 is activated in response to oxidative stress and leads to subsequent up-regulation of antioxidants.[156] Surf1 (surfeit locus protein 1) is a nuclear encoded chaperone protein and aids the assembly of the subunits of Complex IV. Knockout of the Surf1 leads to decreased activity of complex IV and the OXPHOS rate. The defect of complex IV in Surf1 knockout mice activates mitochondrial biogenesis and Nrf2 expression,[157] in line with a cytochrome oxidase defect that impairs energy production and enhances ROS production. Expression of Nrf2 is decreased in elderly patients.[158] Strategies to stimulate Nrf2 expression and enhance endogenous antioxidants may decrease the severity of cardiovascular disease in the elderly.[159] Thus, the lack of endogenous activation of this redox sensitive transcription factor does not support an upregulation in cytoprotective ROS signaling in the aged heart.

6B. MPTP Opening in the Aged Heart: Relationship to ROS Production

The susceptibility to mitochondrial permeability transition pore opening is increased in the aged heart[101] increasing the potential of augmenting disease-enhanced permeability transition. Although the precise identity of the permeability transition pore remains a matter of debate, emerging data supports complex V as a key component;[100] and contributions of the phosphate carrier [160] and contact sites [161] still deserve consideration.

In contrast to the severe metabolic stress of ischemia-reperfusion with attendant calcium overload and oxidative storm, the role of enhanced permeability transition pore opening in the aged heart during baseline metabolism is possibly related to reversible, transient opening (“flickering”) of the pore. This may participate in the regulation of mitochondrial calcium content [162] and can affect membrane potential. Calcium overload in the mitochondria does not appear to be a primary mechanism of pore opening during basal metabolism, although transient calcium release from sarcoplasmic reticulum via mitochondria-associated membranes, especially in response to mitochondrial driven oxidant production as discussed above, deserves consideration. A well-known stimulus for pore opening is oxidative stress,[163] especially that from the ETC. ROS production from complex I [164] and possibly complex III [165] favors pore opening in line with the sites of its production in the aged heart. Furthermore, blockade of the ETC proximal to complex III decreases the likelihood of pore

opening.[166] Thus, increased susceptibility to pore opening is a potential effector of ETC-mediated enhanced ROS production.

6C. Mitochondria-Metabolism Based Signaling in Response to ROS Production

Mitochondria serve as important signaling nodes for cell survival and death. In addition to serving as stochastic sources of ROS production, mitochondrial homeostasis is important for cell survival and death. Oxidant-mediated damage to proteins within mitochondria leads to activation of new, reinforcing signaling in response to cell stress. The potential of ETC-mediated mitochondrial oxidative damage increasing the susceptibility to permeability transition pore opening was discussed above. Signaling-mediated readouts of mitochondrial metabolism and oxidant production are critical to cardiomyocyte survival in the aged heart. Enhanced ROS production from IFM may indeed drive regional redox signaling. The combination of increased ROS production with relative blockade at cytochrome oxidase would support the preferential activation of the cytochrome *c* cardiolipin peroxidase in IFM from aged hearts.

Cytochrome *c*, a carrier component of the ETC, can undergo oxidative modification and activate oxidant-dependent signaling for cell death. It contains binding sites for CL.[62] In the normal state, all the coordination positions of the heme iron are occupied. The heme iron has two axial ligands, His_{18} and Met_{80}. Oxidation of Met_{80} to a methionine sulfoxide opens the axial coordination site allowing acquisition of a peroxidase function of electron transfer from H_2O_2 to CL.[167] The peroxidation and depletion of CL signals the activation of cell death programs.[58] An increase in intra-mitochondrial ROS generation coupled with the electron flow through the cytochrome *c* segment of the ETC oxidizes cytochrome *c*, resulting in depletion of CL.[61] The oxidative stress of ischemia-reperfusion increases cytochrome *c* methionine sufoxide, indicative of peroxidase formation in the *in situ* heart.[61] The role of cytochrome *c* peroxidase formation in the CL alterations that occur with aging requires further study.

The activation of oxidant-sensing signaling systems for cell survival includes the redox-sensitive protein kinase apoptosis signal-regulating kinase 1 (ASK-1). ASK1 is present in an inactive complex with the reduced form of thioredoxin (Trx). When oxidative stress is increased, ASK1 dissociates from Trx, resulting in ASK1 activation.[168] ASK1 activates the mitogen-activated protein kinase cascade. In liver, aging leads to activation of ASK-1 in the baseline state.[169] In response to stress, there is evidence in brain of age-enhanced activation of ASK-1.[170] ASK-1 is a mediator of cell death in the heart in response to ischemia-reperfusion,[171] but its role in the aging heart in response to baseline stress remains uncertain. ASK-1 activation in response to oxidative stress can impact OXPHOS and susceptibility to permeability transition.[172] Thus, oxidant production from mitochondria might activate ASK-1, which interacts with cardiac stress responses,[171] and potentially further alters mitochondrial function.

7. Regulation of Mitochondrial Metabolism by Transcription Factors

The ability to acutely regulate oxidative metabolism concomitant with a redirection of transcriptional programs is an appealing mechanism for a concerted cell stress response,

especially for aging tissues susceptible to age-enhanced disease. Transcription factors have been identified within mitochondria.[173] Some transcriptional factors localize to the outer membrane and regulate outer membrane permeability and susceptibility to cell death.[173] Modulation of mtDNA expression by “nuclear” transcription factors is an elegant expression of mitochondria-nucleus cross-talk,[111, 173] and likely contributes to the regulation and stability of mtDNA as discussed above. Regulation of mtDNA can impact metabolism via regulation of mtDNA-encoded catalytic subunits of complexes I, III, IV, and V. A more direct modulation of ETC activity and OXPHOS is evident for STAT3 [174] and perhaps for p53.[175] These latter two peptides are incorporated into mitochondria.

STAT3 provides a central contribution to stress response[176] with a cardioprotective role.[177] In addition to its canonical actions as a transcription factor, a pool of STAT3 resides within the mitochondria[111, 174, 178] in the matrix of both SSM and IFM.[179] STAT3 binds to GRIM-19, a subunit of complex I.[180] Mitochondria-localized STAT3 modulates activity of the ETC, although it clearly is not present in a 1:1 stoichiometry with metabolic targets.[181, 182] In STAT3-null hearts the activities of complex I and II are decreased, leading to decreased OXPHOS.[174] Reconstitution of STAT3-null cells with mitochondria-targeted STAT3 containing an inactive DNA-binding domain restored deficits in OXPHOS.[174] STAT3 deletion and inhibition mitochondria are more sensitive to calcium-induced opening of the MPTP,[178] likely reflecting the association of STAT3 with cyclophilin D.[178] The role of STAT3 mediated processes in relationship to age-related OXPHOS defects as well as the response to exogenous stress of mitochondria from aged hearts remain areas of active investigation.

Stress-induced translocation of p53 to the mitochondria involves mono-ubiquitination of a distinct cytoplasmic pool of p53 by the E3 ligase Mdm2.[183] The translocation of p53 to the outer membrane modulates members of the bcl-2 family and impacts susceptibility to programmed cell death.[184] p53 also accesses the matrix compartment..[185] p53 is activated in senescent tissues, including endothelial cells, and thus may affect vascular function and perhaps even metabolism in the aged heart.

8. Mitochondrial Fission and Fusion

A number of excellent reviews have highlighted the importance of fission and fusion processes in cardiac injury during ischemia-reperfusion and subsequent heart failure development.[186–190] Mitochondrial dynamic processes are proposed to be involved in the aging process.[191] The blockade of fission or promotion of fusion contributes to cell senescence. Knockout of fis1 in cultured cells results in elongation of mitochondria accompanied by cell morphological changes of senescence including enlargement and flattening as well as staining for senescence-associated acidic β-galactosidase activity and increased expression of Mitofusin-1.[192, 193] Cell senescence is observed in dysfunctional endothelial cells during aging.[194] However, the impact for cardiac myocytes, which do not exhibit a classic senescent transcriptional program including β-galactosidase activity, is less clear. Nonetheless, this body of work supports the notion that enhanced mitochondrial fission may attenuate age-induced cellular dysfunction, likely via isolation and removal of

dysfunctional mitochondria as discussed below. In fact, fission-associated autophagy[191] is emerging as an important mechanism of mitochondrial homeostasis in the heart.

Post-translational modification of mitofusin-1 modulates cell responses during oxidative stress.[195] In antimycin A-treated cells, the Mitofusin-1 level is markedly increased with augmented cell death. Antimycin-A is a complex III inhibitor that is used to increase ROS production from from the Qo site of complex III,[122] favoring ROS production in a similar manner to the age-related defect discussed above.[97] Proper regulation of Mitofusin-1 content leading to a balance of mitochondrial fission and fusion needs to be maintained to promote cell survival during oxidative stress. Mitofusin- ubiquitination is increased in antimycin-A treated cells, leading to proteasome degradation and decreased fusion in response to oxidative stress. Inhibition of Mitofusin-1 degradation increases cell death in antimycin-A-treated cells. These studies indicate a net protective role of mitochondrial fission in cell senescence and aging.[193]

The content of Mitofusin-1 and Mitofusin-2 is increased in skeletal muscle in aged mice whereas the Fis1 level is decreased. Beclin 1 content is also decreased. These results suggest that aging increases mitochondrial fusion and attenuates mitophagy.[196] The findings in skeletal muscle again support the concept that fission favors protection against oxidative stress. Manipulation of mitochondrial dynamics is a new strategy to decrease cardiac injury during ischemia-reperfusion.[197, 198] Thus, the enhanced oxidant production present in the aged heart may impair protective mitochondria fission-based responses to superimposed disease. Modulation of mitochondrial dynamic changes may be a strategy to attenuate the deleterious impact of age-related defects in the ETC. Decreased fission in the baseline state may predispose to an increased susceptibility of the aged heart to injury.

9. Mitochondrial Biogenesis, Dynamics and Removal

Age-related decreases in mitochondrial yield and OXPHOS are limited to IFM. The decrease in IFM may reflect a population with content decreased due to impaired production or a more rapid turnover due to increased removal. Mitochondrial biogenesis and removal are altered by age.[199], In contrast to the neonatal heart, the activation of PCG1 alpha mediated mitochondrial biogenesis in the adult heart did not lead to evident region specific proliferation within the myocyte.[200] The differential responses in adult and neonatal hearts to activation of a constitutively active PGC1alpha transgene might be related to the preexisting developed cardiomyocyte cellular architecture in the adult. Targeted removal of IFM may be enhanced in the aged heart. The defective IFM, with increased ROS production[97] and potential for membrane depolarization due to enhanced susceptibility to permeability transition,[101] should be targeted for elimination by mitophagy.[201] Such removal would attenuate the oxidative-driven injury mechanisms discussed above. Membrane depolarization may activate Parkin-mediated mitophagy,[202, 203] or, alternatively, appropriate activation of fission may activate fission-driven autophagy.[191] However, the localization of age-related defects to IFM suggests that due to their siting among the myofibrils, that removal of these mitochondria may be impaired. In this case, reduced removal would point to decreased mitochondrial biogenesis as the mechanism of the decreased content of IFM with age. In fact, rates of replacement and half-lives of the two

populations in vivo is a key question relevant to the aged heart, and can be addressed by stable isotope approaches and the analysis of mitochondrial proteins and phospholipids.[204] It is critical to understand the activation of mitochondrial removal mechanisms in vivo in the aged wild-type heart, both at baseline and in disease states. Transgenic models provide key insights into mechanisms of removal, but may be less informative in identifying the pathway of activation for removal of dysfunctional mitochondria in aging with or without superimposed disease. An impaired response of mitophagy could contribute to the increased susceptibility of the aged heart to cardiac stress, and impaired biogenesis of mitochondria could enhance susceptibility of the aged heart to superimposed stress.

Proteolytic enzyme mediated protein degradation impacts cardiovascular dysfunction in acquired heart disease.[201, 205, 206] An increase of Omi/HtrA2 (an intermembrane space protease) content enhances cardiac injury in aged hearts compared to adult hearts.[207] In contrast, loss of HtrA2/Omi causes premature aging.[208] In aged rat hearts, Lon protease activity is decreased even despite an increase in protein content.[209] However, the downregulation of Lon can decrease cell injury during hypoxia-reoxygenation.[210]. The decreased Lon activity may contribute to suppressed autophagy during aging.[211]

10. Harnessing the Mitochondria: Treatment of Age-Enhanced Cardiac Disease by Modulation of Aging-Induced Defects

Mitochondria-directed strategies to limit cardiac injury are highly relevant to the protection of the aged heart and to the ultimate improvement of clinical outcomes in high-risk elderly patients. Relevant to mitochondrial metabolism in the aged heart and treatment of cardiac disease in elders is the goal to modulate or mitigate age-related defects in mitochondrial metabolism in order to reduce injury from subsequent cardiac disease. Novel therapies are required because classical approaches widely used in experimental studies in the adult heart are of limited benefit when applied to the aged heart. Activation of cytoprotective signaling cascades that center on mitochondria in ischemic preconditioning and postconditioning are clearly less effective in the aged heart.[212–217] It remains unclear if the ineffectiveness of signaling-based cytoprotection in the aged heart is at the level of the signaling cascades upstream of the mitochondria or the inability of dysfunctional mitochondria to respond to the cytoprotective modulation. It appears that mitochondria in the aged heart retain the capacity to respond to cytoprotective modulation since endogenous protective mechanisms can be restored in aging hearts by treatments including caloric restriction (CR)[213] and exercise.[218]

Compared to young rats, the rate of oxidation is decreased in permeabilized cardiac fibers isolated from aged rat hearts using pyruvate-/malate and succinate as complex I and complex II substrates.[219] CR improves OXPHOS with complex I but not complex II substrates. CR also improves complex I activity in aged hearts through induction of ND6 expression (a complex I subunit encoded by mitochondrial DNA).[219] CR does not affect ETC enzyme activity nor mitochondrial respiration in young rats.[219] CR can improve mitochondrial bioenergetics through reduction of oxidative stress and activation of sirtutins.[220] Resveratrol supplementation also provides benefit in delaying aging-mediated cardiovascular

dysfunction. CR, exercise, and resveratrol all activate sirtuin 1 and 3. The details can be found in many excellent reviews.[221–223]

In Fisher 344 rats, wheel running exercise decreases mitochondrial production of H_2O_2 with reduced MnSOD activity in both SSM and IFM from aged rat hearts.[129] However, acute prolonged exercise paradoxically may impair OXPHOS and lead to decreased respiratory coupling.[224] Thus, proper exercise may provide a beneficial effect in improvement of mitochondrial oxidative metabolism.[224]

Furthermore, isolated mitochondria from aged hearts respond to pharmacologic effectors including direct agonists of the mitochondrial potassium-ATP channel.[214, 225] Thus, the potential for direct mitochondria-targeted intervention remains. The aged heart exhibits a major clinical challenge for protective therapy. First, not only myocardial injury increases compared to younger hearts for similar ischemic stress, but the ability to muster endogenous protective pathways is impaired. This challenge in the aged heart makes a strong case for the consideration of novel, direct mitochondrial-centered treatments to mitigate cardiac damage during acute and chronic cardiac stress.

If the aging defect in mitochondrial respiration could be diminished or removed, would the aged heart now sustain less injury? Acetylcarnitine treatment improved aging-induced decreases in OXPHOS, complex III and complex IV.[226,227] The content of cytochrome *b* in IFM from aged hearts was increased.[227] In the aged heart, acetylcarnitine treatment improved tolerance to ischemia-reperfusion injury to that observed in adult controls, with decreased infarction and improved contractile recovery.[227] Acetylcarnitine increased transcription of mtDNA linked to a greater content of ETC subunits,[228] indicating stimulation of mitochondrial protein synthesis.[229] Increased transcriptional responses may occur via activation of mitochondrial sirtuins [230, 231] or alternatively, via increases in intracellular acetyl-CoA. The latter may activate nuclear transcription via modulation of nuclear epigenetic responses. The acetylcarnitine studies provide proof of concept that modulation of aging-induced defects in mitochondrial metabolism can reduce cardiac injury from cardiac disease. The approach to rejuvenate mitochondrial function in order to directly address age-enhanced cardiac disease remains an attractive and potentially useful clinical strategy.

In addition to the defect in the ETC of the aged hearts, post-translational modification can also contribute to the altered mitochondrial metabolism. Sirtuins can affect mitochondrial metabolism through reversible lysine-residue acetylation.[221, 223, 232] In female Wistar rats, the activities of complex I-IV are decreased in middle and aged hearts compared to young rats. The NAD^+ content in middle and aged hearts is decreased compared to young, whereas NADH content is increased in middle and aged hearts. The sirtuin1 activity is also decreased in aged hearts.[233] Thus, the altered sirtuins may contribute to altered metabolism in aged hearts. As an alternative, therapy to directly increase NAD+ content remains an option, having been utilized in skeletal muscle and brain, but the impact on heart remains to be seen.[234–236]

Supplemental coenzyme Q10 in PUFA (polyunsaturated fatty acids) diet extends life span in 24 month old rats with decreased H_2O_2 generation and increased catalase activity.[237] The coenzyme Q10 supplementation in PUFA diet also improves the activity of cytochrome oxidase in 24 month aged mouse heart mitochondria.[238] However, addition of coenzyme Q10 does not affect the respiratory chain activities, superoxide generation, and antioxidants in C57BL/6 mice.[239] Coenzyme Q10 may attenuated aging defect in aged heart mitochondria.

Rapamycin is known to extend life span in animal species.[240] Feeding of rapamycin in late life extends life span in C57BL/6 mice with decreased metabolite rate.[241] In aged female C57BL/6 mice, rapamycin feeding for 10 weeks transiently induces autophagy, increases mitochondrial biogenesis, improves energy metabolism, especially fatty acid metabolism, and alters the myocardial metabolome.[10] Rapamycin therapy started even late in life exerted beneficial effects in cardiac function and cardiac gene and protein expression.[242, 243] The response to superimposed stress of ischemia-reperfusion or heart failure remains an area of major interest.

11. Summary and Conclusions

Aging results in defects in cardiac mitochondrial metabolism centered on the electron transport chain. IFM, located amongst the myofibrils, sustain the greatest alteration with age, exhibiting a decrease in both number and mass and a depressed capacity for OXPHOS because of defects in ETC complexes III and IV. The presence of dysfunctional mitochondria reflects age-induced alterations in mitochondrial dynamics involving altered mitochondrial fission and. Age-altered mitochondria produce greater fluxes of ROS, leading to damage of mitochondrial components, injury to adjacent organelles, and finally and fatally to the oxidant-activation of signaling pathways for cell death. Reduced electron transport may in part be due to age-related dysregulation of transcription factors that regulates mitochondrial metabolism, an understudied area. Impaired metabolism of fatty acids, dysregulated glucose metabolism, and mitochondrial defects are critical in metabolic responses of the elderly heart to disease-imposed stress. Aging defects in mitochondria represent new therapeutic targets, whether by manipulation of the mitochondrial proteome, modulation of electron transport, activation of biogenesis or mitophagy, or the regulation of fission and fusion. The study of aging defects in cardiac metabolism is not a sterile one, rather an area that presents an opportunity for developing novel therapies that could benefit cardiac disease, and perhaps slow the pace of age-induced changes In the heart, with the happy result of lengthening life spans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ALCAT1, Acyl-CoA	lysocardiolipin acyltransferase-1
ASK-1	Activation of apoptosis signal-regulating kinase 1
CL	Cardiolipin
CPT-I	Carnitine palmitoyltransferase I
CPT-II	Carnitine palmitoyltransferase II
FAO	Fatty acid oxidation
HRSEM	High resolution scanning electron microscopy
IFM	interfibrillar mitochondria
ISP	Iron-sulfur Protein
MAO	Monoamine oxidase
MLCL	Monolysocardiolipin
MPTP	Mitochondrial permeability transition pore
OXPHOS	Mitochondrial oxidative phosphorylation
PDH	Pyruvate dehydrogenase
PDC	Pyruvate dehydrogenase complex
PDK	Pyruvate dehydrogenase kinase
ROS	Reactive oxygen species
SSM	subsarcolemmal mitochondria
TEM	transmission electron microscopy
Trx	thioredoxin
VDAC	Voltage-dependent anion channel

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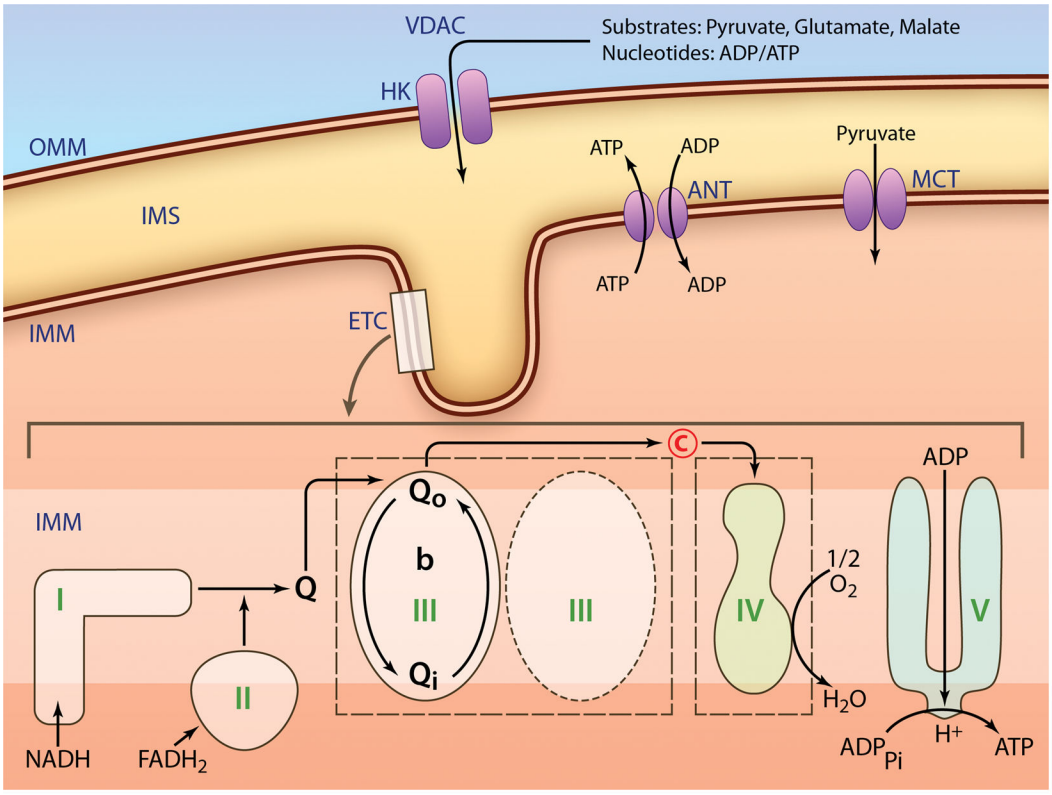

Figure 1.

Schematic of mitochondrial metabolism. OM, mitochondrial outer membrane; HK, hexokinase; VDAC, voltage-dependent anion channel; IMS, intermembrane space; IM, mitochondrial inner membrane; MCT, monocarboxylate transporter; ANT, adenine nucleotide translocase; IMM, mitochondrial inner membrane; ETC, electron transport chain; Q, ubiquinone; C, cytochrome c. (Illustration Credit: Ben Smith).

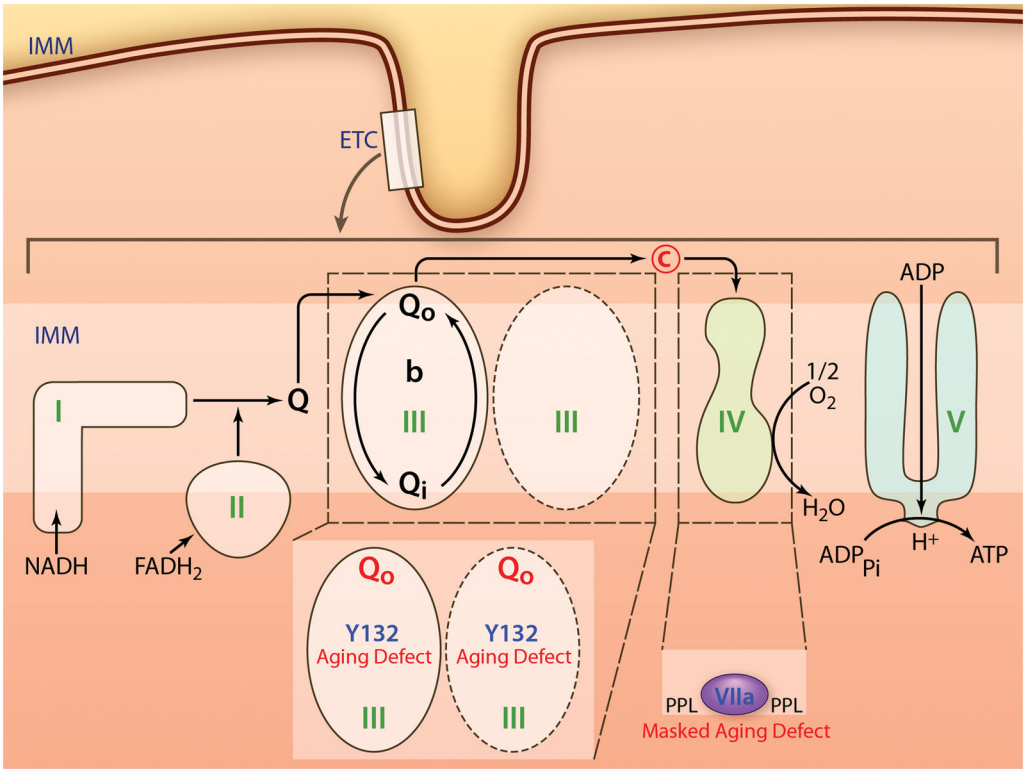

Figure 2.

Schematic of the interfibrillar mitochondrial defects in the aged heart. Abbreviations similar to Figure 1. Q_o site, ubiquinol binding site on cytochrome b_l with proposed defect in Y132; PPL, phospholipid, proposed defect affecting subunit VIIa in complex IV. (Illustration Credit: Ben Smith).

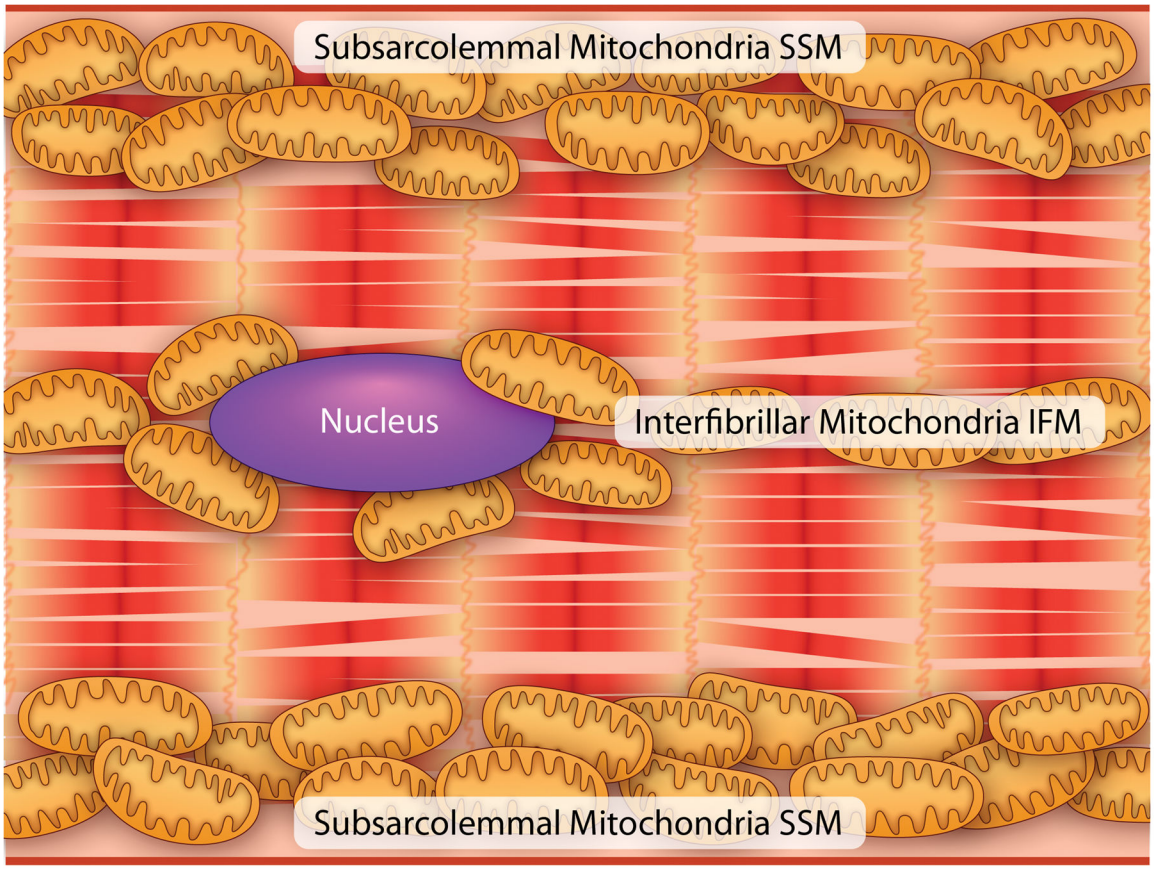

Figure 3.

A schematic of a cardiomyocyte to highlight the location of subsarcolemmal (SSM) and interfibrillar mitochondria (IFM). (Illustration Credit: Ben Smith).

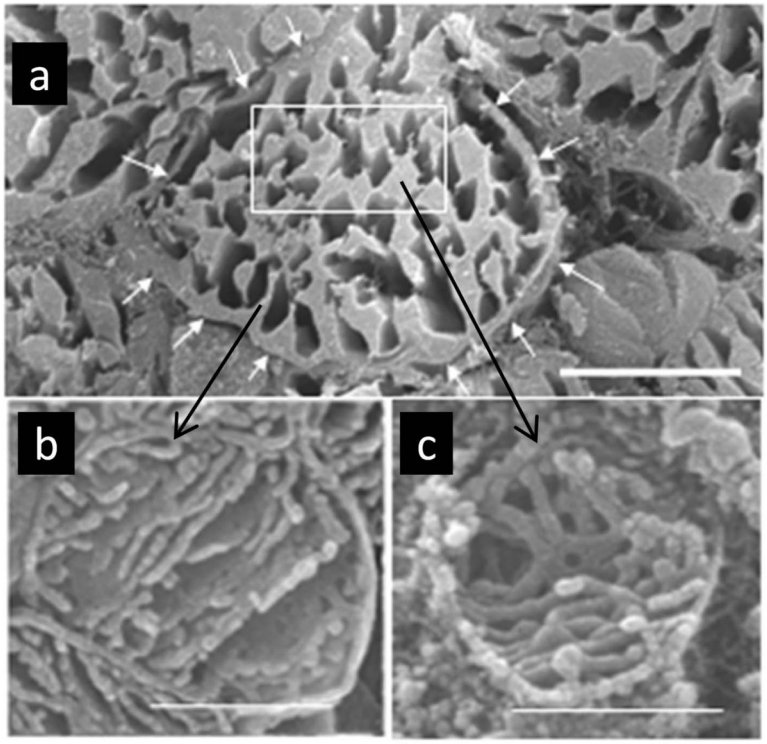

Figure 4.

Ultrastructure of young and aged Fischer 344 rat hearts. (a) Electron micrograph of myocardium of a 6-month rat. (b) Myocardium of a 28-month rat. SSM are clustered in groups under the sarcolemmal membrane; IFM are between the myofibrils. There are no structural differences noted between the two ages.[24] (c) Isolated SSM from a 24-month rat, osmium-extracted. The cristae morphology is mixed between lamelliform and tubular. (d) Isolated IFM from a 24-month rat, osmium-extracted. Cristae are primarily tubular.[47] Magnification in (a) and (b) is 6000 and the bar in (c) and (d) is 1 μm.

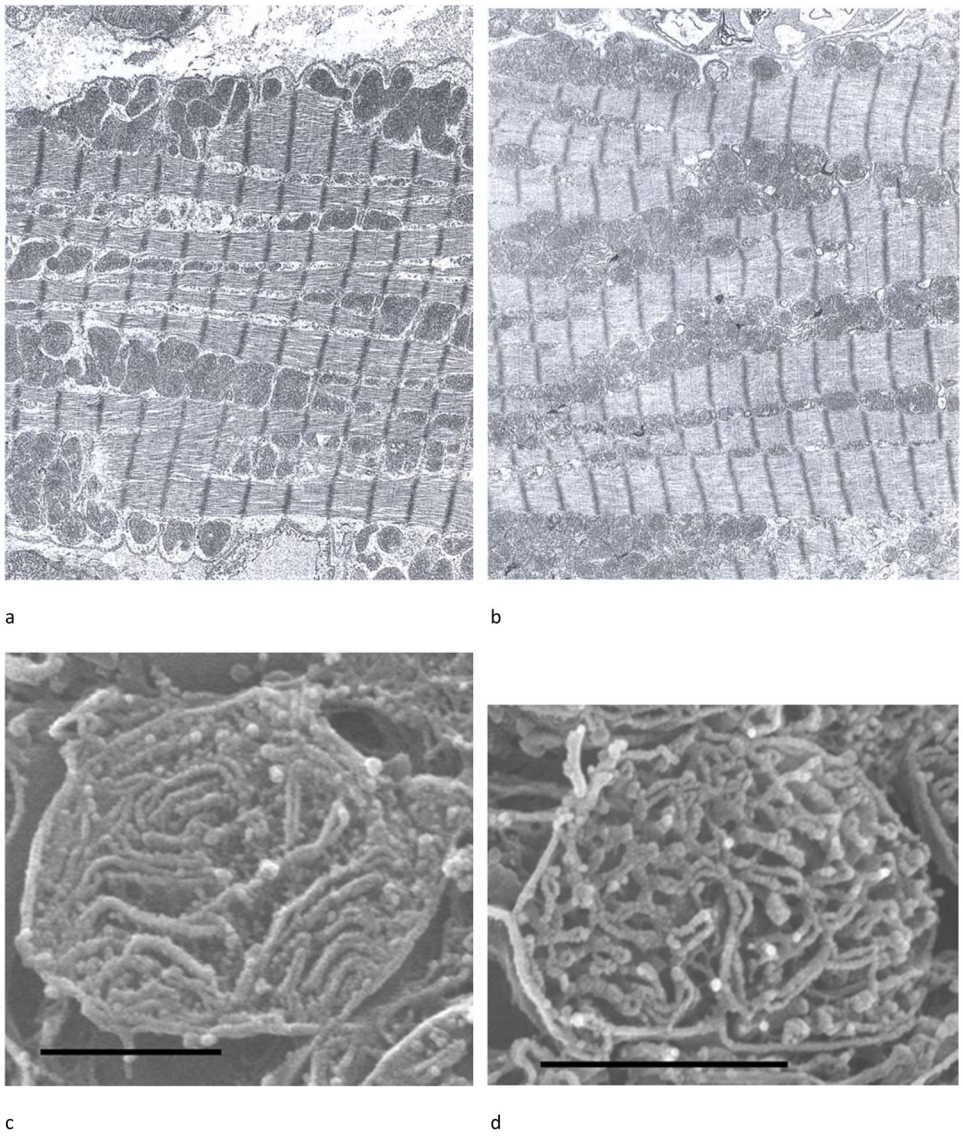

Figure 5.

(a) Osmium-extracted cardiomyocyte in situ from an adult rat heart observed in cross-section by HRSEM. The sarcolemma is pointed out by the series of white arrows. The SSM are under the sarcolemma; the black arrow to Fig. 1b where a typical SSM is shown at higher magnification. The box identifies the more central area where the myofibrils have been extracted by the osmium treatment exposing the IFM. The arrow to Fig. 1c shows an IFM in higher magnification. (b) A SSM containing lamelliform cristae exclusively. (c) An IFM with tubular cristae forming a lattice. Scale line for (a) = 4 μm and for (b) and (c) = 0.5 μm.

The images are taken from Figure 1a and Figure 2a and 2d from reference 46.

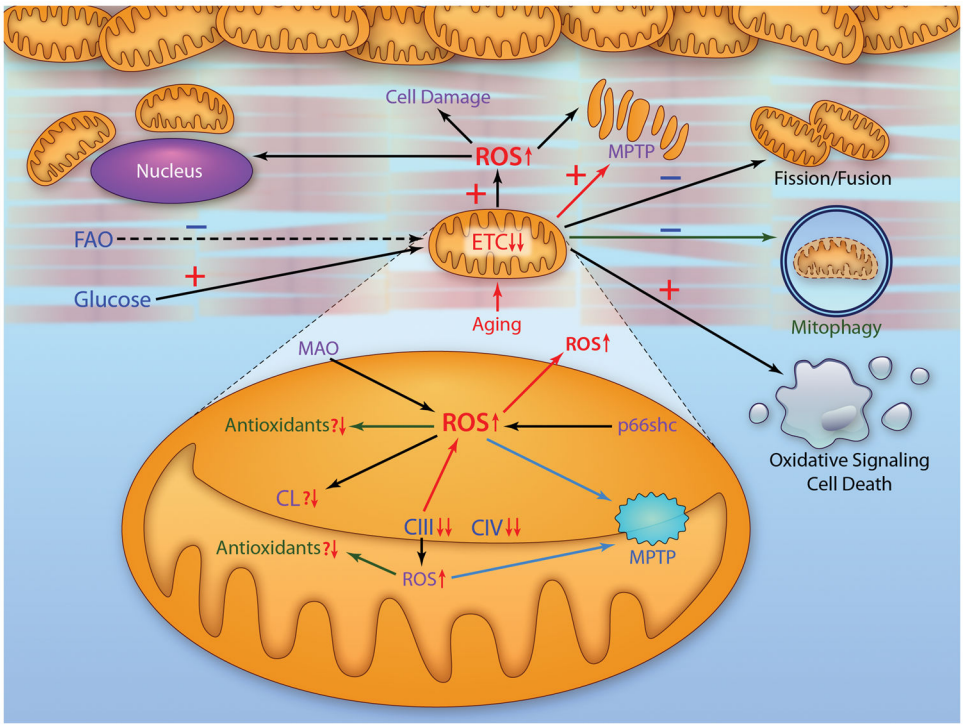

Figure 6.

A schematic of a cardiomyocyte to show defects present in global and mitochondrial metabolism with age in concert with mechanisms of mitochondria-driven cellular injury. The aged heart exhibits impaired fatty acid oxidation (FAO) and preserved oxidation of glucose. Aging leads to defects in the electron transport chain that involve complexes III and IV. Monoamine oxidase (MAO) and $p66^{shc}$ are significant sources of oxidants. There is a decrease in cardiolipin (CL). Antioxidant contents are relatively unchanged, although a decrease in matrix antioxidant capacity leads to mitochondrial damage. Age-related damage enhances the production of ROS that lead to mitochondrial and cell damage. Additionally, effector mechanisms of age-induced mitochondrial damage discussed include an increased susceptibility to mitochondrial permeability transition pore opening (MPTP) (Section 6B); impaired mitochondrial dynamics favoring fusion over fission (Section 8); likely impaired mitophagy (Section 9); and enhanced oxidant derived signaling to activate cell death programs (Section 6C). (Illustration Credit: Ben Smith).