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Physiological and structural differences in spatially distinct subpopulations of cardiac mitochondria: influence of cardiac pathologies

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Submitted 30 September 2013; accepted in final form 24 April 2014

Hollander JM, Thapa D, Shepherd DL. Physiological and structural differences in spatially distinct subpopulations of cardiac mitochondria: influence of cardiac pathologies. Am J Physiol Heart Circ Physiol 307: H1–H14, 2014. First published April 28, 2014; doi:10.1152/ajpheart.00747.2013.—Cardiac tissue contains discrete pools of mitochondria that are characterized by their subcellular spatial arrangement. Subsarcolemmal mitochondria (SSM) exist below the cell membrane, interfibrillar mitochondria (IFM) reside in rows between the myofibrils, and perinuclear mitochondria are situated at the nuclear poles. Microstructural imaging of heart tissue coupled with the development of differential isolation techniques designed to sequentially separate spatially distinct mitochondrial subpopulations have revealed differences in morphological features including shape, absolute size, and internal cristae arrangement. These findings have been complemented by functional studies indicating differences in biochemical parameters and, potentially, functional roles for the ATP generated, based upon subcellular location. Consequently, mitochondrial subpopulations appear to be influenced differently during cardiac pathologies including ischemia/reperfusion, heart failure, aging, exercise, and diabetes mellitus. These influences may be the result of specific structural and functional disparities between mitochondrial subpopulations such that the stress elicited by a given cardiac insult differentially impacts subcellular locales and the mitochondria contained within. The goal of this review is to highlight some of the inherent structural and functional differences that exist between spatially distinct cardiac mitochondrial subpopulations as well as provide an overview of the differential impact of various cardiac pathologies on spatially distinct mitochondrial subpopulations. As an outcome, we will instill a basis for incorporating subcellular spatial location when evaluating the impact of cardiac pathologies on the mitochondrion. Incorporation of subcellular spatial location may offer the greatest potential for delineating the influence of cardiac pathology on this critical organelle.

Structural and Functional Differences

With the advent of advanced imaging techniques, insight into cellular and organellar structure of the cardiomyocyte has...
provided a wealth of information for the cardiovascular researcher. Among these insights has been the recognition that in cardiac muscle, mitochondria exist in a number of different subcellular locales. This phenomenon has been corroborated in numerous mammalian species including mouse, rat, muskrat, guinea pig, hamster, rabbit, dog, pig, monkey, cow, and human (22, 37, 46, 77, 84, 87, 118, 119, 124, 134). This phenomenon is consistent with the evaluation of other noncardiac cells such as neurons where functional heterogeneity between dendritic, somatic, axonal, and presynaptic segments due to variations in energy demands and calcium (Ca$^{2+}$) signaling dynamics are associated with structural and biochemical differences in mitochondria situated in a particular neuronal region. Ultimately, this heterogeneity may dictate differences in pathophysiological response of various neuronal regions (60). As with neuronal tissue studies suggest that mitochondrial spatial location within the myocyte may be associated with a particular response to physiological and pathological stimuli (43, 55, 65, 83, 131).

With the pioneering development of mitochondrial isolation techniques designed to sequentially fraction spatially distinct subpopulations of mitochondria using both mechanical and enzymatic procedures (96), efforts to define their individual roles in various pathological conditions has been actively pursued. With the use of ultrastructure analyses, several mitochondrial subpopulations have been identified in the cardiomyocyte, including those residing below the sarcolemma termed subsarcolemmal mitochondria (SSM) and those residing between the myofibril contractile apparatus termed interfibrillar mitochondria (IFM) (Fig. 1A) (118). Additionally, a specific population of mitochondria, isolated as IFM, reside in the perinuclear region. For the purpose of this review, attention will be paid particularly to both the SSM and IFM populations.

**Structural differences.** Structural alterations between mitochondria located in different subcellular locales have been reported using a number of diverse experimental techniques. With the use of thin sections from left ventricular tissue of Japanese Monkeys (*Macaca fuscata*), scanning electron microscopy and transmission electron microscopy revealed distinct populations of mitochondria including perinuclear mitochondria, IFM, and SSM (118). Perinuclear mitochondria were clustered at the nuclear poles and mostly spherical in shape with lengths ranging from 0.8 to 1.4 μm. These mitochondria contained well-developed curved cristae with relatively little matrix area. In addition, IFM were identified and observed in longitudinal rows between the myofibrils, occupying the entire space between Z-lines (33) and bookended by the junctional sarcoplasmic reticulum (79) (Fig. 1B). IFM were elongated in shape with usually one mitochondrion existing per sarcomere. IFM were ~1.5–2.0 μm in length, and their cristae structures also displayed curved configurations. Finally, SSM were located beneath the sarcolemma (plasma membrane) and were somewhat more variable in length (0.4–3.0 μm), possessing closely packed cristae. Overall, perinuclear and mitochondria were smaller than IFM in size and possessed a more rounded shape (79), whereas SSM were varied in size and shape displaying oval, spherical, polygonal, and horse-shoe patterns (118). Others have reported similar ultrastructural patterns using confocal imaging of HL-1 cells in the perinuclear region, which contain mitochondria clustered around the nucleus (66).

En bloc staining of human papillary muscle revealed differences in staining patterns between SSM, IFM, and perinuclear mitochondria, suggesting differences in chemical properties and metabolic activities (24). These findings are consistent with flow cytometric analyses, which use membrane-dependent dyes coupled with flow cytometry size calibration microspheres to determine absolute mitochondrial size and internal granulation in both SSM and IFM (20–22, 143). Insight into subcellular mitochondrial distribution has also been afforded by probability density analyses. With the use of a three-dimensional modeling approach coupled with MitoTracker Red staining enabling probability density and distribution, isolated rat cardiomyocytes revealed a highly ordered crystal-like pattern in which IFM were arranged in longitudinal rows in clefts between myofibrils (5). We have observed similar staining patterns in adult mouse cardiomyocytes stained with MitoTracker Deep Red 633 (Fig. 2). The periodicity with neighboring strands was consistent such that IFM were located primarily at the level of the A-band of the myofilaments. In contrast, similar analyses on rainbow trout (*Oncorhynchus mykiss*) cardiomyocytes revealed one single cylinder-shaped layer of myofibrils situated beneath the sarcolemma in which the mitochondrial arrangement was random and chaotic (5). Thus species differences may be associated with differential mitochondrial spatial patterns in a comparative physiological context.

![Fig. 1](http://ajpheart.physiology.org/)

Fig. 1. **A:** Longitudinal ultrathin (70 nm) electron microscopy sections from left ventricular mouse heart imaged at ×6,000. **B:** Longitudinal ultrathin (70 nm) electron microscopy sections from left ventricular mouse heart imaged at ×10,000. The bar equals 0.5 μm. SSM, subsarcolemmal mitochondria; IFM, interfibrillar mitochondria.
To gain insight into structural differences in cristae morphology, Riva and colleagues used high resolution scanning electron microscopy to examine left ventricular tissue from rat. SSM and IFM displayed distinct morphological patterns in terms of cristae structure with SSM containing primarily (77%) lamelliform cristae, which are broad and flat. In contrast, IFM cristae morphology was somewhat mixed and variable with some mitochondria (55%) possessing only tubular cristae, whereas others (24%) possessing some lamelliform cristae mixed with tubular cristae. Furthermore, 21% of IFM possessed only lamelliform cristae. These authors concluded that most of the cristae structure in SSM were lamelliform with occasional tubular cristae, whereas IFM possessed a reciprocal pattern in which some lamelliform cristae exist despite an abundance of tubular cristae. These authors speculate that the individual cristae morphological patterns may contribute to the functional differences observed between the two subpopulations, including a reduction in intracristal space of tubular cristae. Furthermore, these authors speculate that a reduction of intracristal space could lead to a higher concentration of protons within the structure and enhance ATP synthase activity, which is consistent with IFM functional differences (108). In addition, biochemical composition of the two cristae structures may differ in terms of lipid or protein contents, which could ultimately influence structure (108). Indeed, examination of the sphingolipid pool in cardiac SSM and IFM has revealed differences in the content of ceramide, which was higher in the SSM (88).

Although spatially distinct subpopulations of mitochondria have been identified in cardiac tissue, it is still unclear as to whether interactions between SSM and IFM occur in vivo. Skulachev has proposed that SSM are joined to each other by intermitochondrial junctions with the innermost layer of SSM connected to the IFM via mitochondrial filaments. This hypothesis suggests that SSM and IFM interact with one another such that consumption of oxygen by the SSM from the capillary coupled with active respiration leads to the transmission of protons, via mitochondrial filaments, to the IFM enabling ATP generation to be used by the contractile apparatus. If such a model were to exist, it would provide distinct advantages including enhancing the ability for IFM to generate ATP despite lower oxygen content in the core of the cell. As a result, the lower oxygen tension in the interior of the cell could limit oxidative stress in this locale, and reactive oxygen species (ROS) production would remain primarily at the cell periphery, preserving the cell core from damage (122). Thus SSM may serve as a protective barrier to the cell, maintaining permissive oxygen levels within (66, 122). Similar findings have been reported in mouse skeletal muscle using electron microscopy, suggesting physical continuities between SSM and IFM (101). Additionally, considerable evidence from network modeling studies suggests that cardiac mitochondria communicate across the cell (at distances of up to ~100 μm), providing a platform for synchronized mitochondrial behavior (147). Thus it is conceivable that cross talk between SSM and IFM populations exists to provide a dynamic network between subcellular locales to generate energy and transmit critical intracellular signals.

Functional differences. In conjunction with structural differences between spatially distinct mitochondrial subpopulations, numerous reports have indicated that SSM and IFM possess distinct functional differences, which may result from their particular subcellular locales. Mitochondria residing in the perinuclear region of the cell have been suggested to generate ATP that drives mitochondrial metabolism close to the nucleus. In contrast, it has been hypothesized that IFM supply ATP for contraction, whereas SSM are involved primarily in the provision of ATP for active transport of electrolytes and metabolites across the sarcolemma (89, 96, 111, 118). Although these postulations have not been definitively determined experimentally, they are consistent with the concept that the spatial location of a given mitochondrion is reflective of the processes in which it supplies ATP and the given locale in which it resides.

Oxidative phosphorylation/substrate utilization. Functional differences between mitochondrial subpopulations relating to oxidative phosphorylation and TCA cycle activities have been reported. Palmer et al. (96) described functional differences between spatially distinct mitochondrial subpopulations, which included higher respiration rates, as well as succinate dehydrogenase and citrate synthase activities in IFM compared with SSM. However, carnitine palmitoyltransferase and α-glycerophosphate activities were similar between SSM and IFM. Moreover, IFM oxidized all lipid and nonlipid substrates 1.5 times faster than the SSM. These authors suggest that the two mitochondrial subpopulations possess differences in some biochemical properties, which may indicate distinct metabolic roles for each in the cell (96). It should be pointed out that the

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**Fig. 2.** Z-series fluorescent images of Mitotracker (633 nm laser at 2.0% power, LP 650 nm emission filter) and 4’,6-diamidino-2-phenylindole (405 nm laser at 6.1% power, BP 420–480 nm filter) stained adult mouse cardiomyocytes, which were collected sequentially through the entire cell. Three different focal planes from the center of the cell are shown. Images were acquired on a Zeiss LSM510 confocal with a 63×/1.40 Oil DIC Plan-Apochromat objective at 1.7× zoom.
use of some proteases such as Nagarse, which has been used for the isolation of IFM, may impact enzymes located at or in direct contact with the outer mitochondrial membrane (98). A study by Matlib et al. (81), examining isolated mitochondrial subpopulations in different ionic strength buffers, indicated that differences in state 3 respiration rates were dependent on the ionic balance of the media, which led to changes in total cytochrome c release from polytron-treated mitochondria (SSM). These authors conclude that the observed difference in state 3 respiration rates between the two subpopulations may be a function of mitochondrial isolation conditions, which precipitate loss of cytochrome c into the medium, and not the result of physiological differences between the two subpopulations (81). In contrast, Palmer et al. (95) observed no biochemical or morphological differences in either subpopulation following exposure of each subpopulation to the preparative procedure used to isolate the alternate population. In their analyses, these authors report faster complex I, II, and III oxidation rates in IFM compared with SSM, with no differences observed in complex IV oxidation rates. Moreover, the cytochrome concentrations and activities of NADH oxidase and complex IV were similar in both subpopulations, whereas the concentration of cytochrome aa3 was significantly lower in the SSM subpopulation. These authors conclude that the functional performance of the two populations cannot be explained by the experimental manipulation (95). Similar findings have been reported in mitochondrial subpopulations from mouse cardiac tissues indicating that IFM display higher complex I, III, and IV activities relative to SSM (2, 21). In addition, ATP synthase (complex V) activities have been reported to be higher in IFM compared with SSM (4, 19). Confirmation of higher state 3 respiration rates has been reported by others evaluating isolated mitochondrial subpopulations from human papillary muscle and preruminant calves (102, 139). A recent study investigating the susceptibility of oxidative phosphorylation machinery to oxidative modifications revealed a predisposition of SSM respiratory chain complexes to carbonylation, whereas IFM respiratory chain complexes were more susceptible to nitration. Moreover, ATP synthase subunits α and β were carbonylated in both subpopulations, albeit more intensely in SSM. These authors highlight a localization dependence of cardiac mitochondria oxidative phosphorylation activity and susceptibility to posttranslational modifications (93). Findings from our laboratory are confirmative of higher nitrotyrosine content in the IFM subpopulation (21). To examine the effect of inorganic phosphate on mitochondrial oxidative phosphorylation, Duan and Karmazyn (25) treated isolated IFM and SSM with different inorganic phosphate concentrations and observed decrements in SSM oxidative phosphorylation at an earlier time point compared with IFM. However, equal decrements were observed in both subpopulations when a long incubation period with inorganic phosphate was imposed. These authors conclude that quick increments of inorganic phosphate may contribute to decreased ATP synthesis, specifically in the SSM population, influencing early ischemic events (25).

Calcium handling. Mitochondrial Ca2+ has been recognized as a potential mediator of a variety of mitochondrial metabolic and pathological processes (114). Mitochondrial Ca2+ dynamics are a function of their relative proximity to constituents of the Ca2+-handling apparatus. Analysis of rat ventricular myocytes revealed localization of ryanodine receptors at the middle of the sarcomere and in the zone of the perinuclear mitochondria, which displayed a 25% longer spark duration (80, 113). Furthermore, IFM are located in very close proximity to known Ca2+ release sites, the junctional sarcoplasmic reticulum, which is located between IFM and T-tubule (79). Although no compelling functional linkage between the mitochondria and sarcoplasmic reticulum or endoplasmic reticulum has been provided, the possible existence of direct adenine nucleotide funneling between the cardiac junctional sarcoplasmic reticulum and IFM has been hypothesized (59). Indeed, due to their intracellular positioning IFM are located closest to the microdomains of highest elevated Ca2+ and, as a result, display a central involvement in the process of mitochondrial Ca2+ cycling (79). Isolation of mitochondrial subpopulations from thyroparathyroidectomized rats revealed a selective increase in the maximal ability of the SSM subpopulation to accumulate Ca2+, which was accompanied by a proportionate increase in maximal respiratory rates. Moreover, release rates of stearic acid and oleic acid were reduced in the SSM subpopulation in both the presence and absence of Ca2+. Furthermore, the rate of release of arachidonic acid was decreased in the SSM subpopulation only in the presence of Ca2+, suggesting that a lower level of Ca2+-activated phospholipase A2 is present in the SSM of thyroparathyroidectomized rats (18). A study by Papanicolaou et al. examined the ability of mitochondrial subpopulations to uptake Ca2+ in cardiomyocyte-specific mitofusin-1 knockout (Mfn-1 KO) mice. These authors observed a reduction in the ability of Mfn-1 KO IFM to uptake extra-mitochondrial Ca2+, whereas SSM displayed no change. To assess ROS-induced mitochondrial permeability transition pore (mPTP) dynamics in mitochondrial subpopulations, tert-butyl hydroperoxide was introduced to isolated mitochondria, which resulted in a release of mitochondrial Ca2+. SSM displayed similar release dynamics of Ca2+ in both Mfn-1 wild-type and knockout animals, whereas Ca2+ release was significantly blunted in the IFM of Mfn-1 KO mice. These authors conclude that tolerance against ROS-induced mitochondrial damage resulting in mPTP opening is localized more to IFM than SSM (99). Our laboratory has previously reported that mPTP opening resulting from a tert-butyl hydroperoxide and Ca2+ stimulus was slower in IFM compared with SSM, suggesting differences in apoptotic propensity between the two subpopulations (143). Direct and indirect Ca2+ movement was monitored to examine the Ca2+ uptake capacity of cardiac mitochondrial subpopulations in rat. These authors observed increased accumulation of Ca2+ in the IFM population. Moreover, the maximal amount of Ca2+ uptake in both subpopulations was followed by increased proton permeability, as well as a subsequent release of accumulated Ca2+. Upon morphological examination, the SSM subpopulation displayed disrupted morphology coupled with a release of cytochrome c and mitochondrial marker enzymes. In contrast, IFM subpopulation morphology and marker enzyme release was unaffected (97). These findings suggest differences between the two mitochondrial populations in terms of ability to accumulate Ca2+, as well as resist the damage associated with Ca2+ overload. Changes in total Ca2+ content in mitochondrial subpopulations upon electrical stimulation were examined using electron probe microanalysis in isolated guinea-pig ventricular myocytes. Unstimulated myocytes displayed similar Ca2+ contents in both

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subpopulations. However, potentiation using electrical stimulation resulted in a significant increase in Ca\(^{2+}\) content in the SSM subpopulation. Moreover, removal of extracellular K\(^+\) coupled with electrical stimulation increased Ca\(^{2+}\) content in the SSM to a greater degree than in the IFM. Significantly higher concentrations of mitochondrial Na\(^+\) were also observed in the SSM of electrically stimulated myocytes. These authors conclude that differences in mitochondrial Ca\(^{2+}\) and Na\(^+\) contents are attributed to subsarcolemmal cytosolic microdomains of elevated Ca\(^{2+}\) and Na\(^+\) generated during electrical stimulation (36).

**Protein synthesis.** To assess proteome dynamics of mitochondrial genome-encoded proteins, mitochondrial subpopulations were isolated from rats that were given heavy water (\(\text{D}_2\text{O}\)) for up to 60 days. Introduction of \(\text{H}_2\text{O}\) enabled labeling of amino acids with \(\text{H}_2\text{H}^2\)O and was followed by mass isotopomer distribution analysis to calculate synthesis rates of individual proteins. Upon measurement of protein synthesis rates, the IFM subpopulation displayed a lower synthesis rate than the SSM. These authors suggest that slower protein synthesis rates in the IFM compared with the SSM may contribute to functional differences observed between these subpopulations residing in different subcellular locales (61).

Table 1 summarizes some of the structural and functional differences reported in spatially distinct mitochondrial subpopulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SSM</th>
<th>IFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>Beneath sarcolemma</td>
<td>Between myofibrils</td>
</tr>
<tr>
<td>Organization</td>
<td>Random</td>
<td>Longitudinal rows</td>
</tr>
<tr>
<td>Shape</td>
<td>Oval, spherical, horse-shoe</td>
<td>Elongated</td>
</tr>
<tr>
<td>Length, (\mu)m</td>
<td>0.4–3.0</td>
<td>1.5–2.0</td>
</tr>
<tr>
<td>Cristae structure</td>
<td>Lamelliform</td>
<td>Predominantly tubular</td>
</tr>
<tr>
<td>ATP generated for</td>
<td>Active sarcolemma transport</td>
<td>Muscle contraction</td>
</tr>
<tr>
<td>SDH activity</td>
<td>Higher</td>
<td>Higher</td>
</tr>
<tr>
<td>CS activity</td>
<td>Higher</td>
<td>Higher</td>
</tr>
<tr>
<td>Oxidative metabolism</td>
<td>Higher</td>
<td>Higher</td>
</tr>
<tr>
<td>Lipid substrates</td>
<td>Higher</td>
<td>Higher</td>
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<tr>
<td>Nonlipid substrates</td>
<td>Higher</td>
<td>Higher</td>
</tr>
<tr>
<td>Oxidative phosphorylation</td>
<td>Higher</td>
<td>Higher</td>
</tr>
<tr>
<td>Electron transport chain</td>
<td>I–V</td>
<td></td>
</tr>
<tr>
<td>Posttranslational</td>
<td>Carboxylation</td>
<td>Nitration</td>
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<tr>
<td>modification overload</td>
<td>Higher</td>
<td></td>
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SSM, subsarcolemal mitochondria; IFM, interfibrillar mitochondria.

**Pathological Influence**

**Ischemia.** Ischemia and reperfusion injury lead to increased mitochondrial ROS production and apoptosis initiation, all of which can impact heart function (7, 34, 123). The ischemia/reperfusion (I/R) phenomenon is composed of two distinct phases, ischemia and reperfusion, both of which directly impact the mitochondrion. Ischemia alone, with no reintroduction of oxygenated blood, has been associated with deleterious effects on the SSM. Canines subjected to 60 min of ischemia via occlusion of the circumflex coronary artery displayed significant structural alterations to the SSM, accompanied by a decrease in membrane fluidity. These data support the contention that cardiac mitochondrial subpopulations are affected differently during ischemia while providing evidence for damage to mitochondrial membranes during the ischemic period (119). Rabbits subjected to 30 and 45 min of global ischemia displayed decreased oxidative phosphorylation through cytochrome oxidase dysfunction, as well as decreased mitochondrial cytochrome c and cardiolipin contents in the inner mitochondrial membrane (IMM) of SSM. These studies indicate that cardiolipin is a direct target for mitochondrial damage, culminating in decreased mitochondrial cytochrome c content leading to diminished oxidative phosphorylation (11, 77, 78).

Interestingly, rat hearts submitted to a 15-min or 30-min warm ischemia protocol (37°C) displayed negative effects to both mitochondrial subpopulations. However, a cold ischemia protocol (0° to 4°C) influenced the respiratory control index of SSM without effect to IFM (140). These findings indicate that the ambient conditions surrounding the ischemic portion of the heart may influence mitochondrial subpopulation functionality differently. With the use of an isolated perfused rat heart model, 25 min of global ischemia led to damage to the electron transport chain (ETC), which was associated with an increase in the production of hydrogen peroxide (\(\text{H}_2\text{O}_2\)) from complexes I and III specifically in the SSM, highlighting a potential mechanism for cardiac injury (12). However, blockade of the ETC at complex I with amobarbital attenuated ROS generation from ischemia damaged mitochondria (126). Interestingly, usage of amobarbital to block a proximal site (complex I) in the ETC of isolated rabbit hearts undergoing 30 min of stop-flow ischemia enabled preservation of cytochrome c content specifically in the SSM. In contrast, blockade of electron transport at cytochrome oxidase (complex IV) with azide did not retain cytochrome c in the SSM during ischemia (16). Taken together, these studies indicate that cardiac ischemia is associated with damage to the SSM, although ischemic modality may influence the subpopulation at risk.

**Hypoxia.** Hypoxia has been shown to decrease mitochondrial respiration in the cardiomyocyte, which is associated with cellular dysfunction (50). Nevertheless, literature addressing mitochondrial subpopulation differences in hypoxic models remains scarce (45). Using a rat model of chronic hypoxia (11% oxygen exposure for 14 days), Heather et al. (45) observed a decrease in state 3 respiration rates of both SSM and IFM when using fatty acid and pyruvate as substrates. However, decreases in ETC complexes I, II, and IV activities in hypoxic SSM were associated with attenuation in ROS production, ultimately providing protection against mPTP opening (45). This study indicates that hypoxic adaptation may not necessarily be deleterious to the cardiac mitochondrion. Additionally, others have demonstrated enhanced permeability of SSM to NADH during hypoxia using a model in which isolated rat mitochondria are presented with a hypoxic challenge (26). Ultimately, hypoxia appears to predominately influence the SSM, although the effects may not be entirely negative in nature. Such a finding is not unexpected inasmuch as mitochondria located near the sarcolemma are exposed to a substantially higher oxygen tension compared with those located near the center of the cell where core oxygen pressure is...
near zero (129). As a result, large changes in oxygen tension during hypoxic conditions are more likely to be experienced by SSM compared with IFM.

Myocardial infarction. When an area of the heart experiences a loss in blood perfusion, that region becomes ischemic causing irreversible necrosis to the myocardium. A common experimental model for myocardial infarction is coronary artery ligation, which creates an ischemic myocardial region (62). Rats subjected to a coronary artery ligation treatment displayed decreased respiration rates and ETC complex III protein contents and activities, as well as mitochondrial cytochrome c levels in both SSM and IFM, inadvertently causing an increase in H$_2$O$_2$ production (44). Similarly, Rosca et al. using a canine microembolization-induced HF model reported that supercomplexes consisting of complex I/complex III dimers/complex IV were decreased in both mitochondrial subpopulations of the failing heart despite changes in phosphorylation profiles for some supercomplex constituents (109, 112). A coronary artery ligation model in rats coupled with a high fat feeding protocol resulted in an increase in fatty acid availability, state 3 respiration rates, and ETC complex II and IV activities as well as acyl-CoA dehydrogenase activities in SSM and IFM leading to an improvement in both cardiac contractile and mitochondrial function (104–106). In addition, rats fed a diet supplemented with eicosapentaenoic acid and docosahexaenoic acid displayed altered mitochondrial membrane phospholipid fatty acid composition of SSM and IFM in myocardial infarcted hearts via coronary artery ligation; however, no effect on mitochondrial respiration nor mPTP opening were noted (92). Taken together, myocardial infarction appears to influence both mitochondrial populations.

I/R. Mitochondrial dysfunction has been suggested to play a pivotal role in tissue injury during I/R (123). Studies investigating I/R injury and its impact on the mitochondrion indicate that both mitochondrial subpopulations may be affected. Depressed oxidative phosphorylation rates and mitochondrial ADP/ATP translocase activity has been shown to occur in both mitochondrial subpopulations following 20 min of ischemia and 30 min of reperfusion in rat heart (30). To determine whether a relationship exists between mitochondrial dysfunction and myocardial performance, a canine model was employed, which used a global ischemic insult for 2 h. The respiratory function of both mitochondrial subpopulations was correlated with myocardial contractility such that maximal rates of contraction displayed a 27% reduction from the preischemic value, which was associated with similar declines in state 3 respiration rates and respiratory control indexes. Moreover, 1 h of reperfusion restored maximal rates of contractility, as well as reversed the decline in state 3 respiration rates and respiratory control indexes to baseline in both subpopulations (142). Similarly, using a global I/R model in transgenic mice overexpressing a mitochondrial-specific phospholipid hydroperoxide glutathione peroxidase (mPHGPx) isoform, which functions to lessen lipid peroxidation generated by phospholipid oxidation, we have observed cardioprotection to both mitochondrial subpopulations as evidenced by preservation of ETC complexes. Interestingly, this cardioprotection manifested differently dependent upon subpopulation type such that mPHGPx provided protection to ETC complexes I, III, and IV in SSM, while providing protection to ETC complexes I and III in IFM (22). In contrast with the above studies, ischemia alone in a perfused rabbit heart model revealed loss of cardiolipin content with decrements in oxidative metabolism. These authors suggest that the mechanisms involved occur through cytochrome oxidase and loss of cytochrome c in SSM independent of reperfusion damage (70). Taken together, these studies suggest that cardiac I/R influences both mitochondrial subpopulations.

Preconditioning. Ischemic preconditioning is an approach designed to protect the heart against a greater subsequent ischemic insult (62). Isolated rat hearts treated with phosphatidylcholine before zero-flow ischemia show improved oxidative phosphorylation in SSM (28). In addition, rat hearts preconditioned with isoflurane have shown myocardial protective effects as indicated by attenuation of cytochrome c loss from the IMM of SSM (103). In a study by Lesnfsky et al. (69) examining perfused rabbit hearts treated with rotenone before 45 min of ischemia, mitochondria displayed functional protection distal to the site of rotenone block as exhibited by preservation of cardiolipin and cytochrome c contents, as well as the rate of oxidation through cytochrome oxidase, specifically in the SSM. Similarly, reversible blockade of electron transport by amobarbital has been shown to preserve bcl-2 content and attenuate Ca$^{2+}$-stimulated mitochondrial swelling specifically in the SSM of a rabbit model (10), whereas administration of amobarbital in rat hearts before ischemia has been shown to protect oxidative phosphorylation and cytochrome c content in both mitochondrial subpopulations (9, 13). Thus the majority of studies investigating cardiac ischemic preconditioning indicate that the SSM subpopulation is primarily affected, which may be a function of greater sensitivity to the initial ischemic condition and Ca$^{2+}$ overload. One could speculate that this phenomenon is an indirect result of the milieu associated with the subsarcolemmal and extracellular spaces. Finally, connexin 43, which has been linked to cardioprotection from ischemic preconditioning, has been reported as existing only in the SSM of the cardiomyocyte, indirectly implicating SSM in the mechanism (6). These findings are interesting and suggest that the ischemic preconditioning phenomenon may be most effective in addressing pathologies that impart deleterious effects to the SSM subpopulation.

Postconditioning. Cardiac postconditioning has been shown to be a viable strategy for cardioprotection due to its clinically feasible application, which can be predictable and under the control of a clinician (146). A postconditioning protocol consisting of 4 cycles of 1 min ischemia followed by 1 min of reperfusion or an intravenous injection of cyclosporine A in a rabbit model of I/R revealed improved Ca$^{2+}$-retention capacity in both subpopulations. However, postconditioning, but not cyclosporine A, reduced total heart oxidative stress. These authors suggest that during early minutes of reperfusion, postconditioning reduces oxidative stress and inhibits mPTP opening independent of alteration in oxidative phosphorylation and mitochondrial membrane potential (94). Fischer 344 rat hearts undergoing 25 min of global ischemia and 30 min of reperfusion when treated with amobarbital for 3 min at the onset of reperfusion display decreased cardiac injury as well as improved IMM potential in both subpopulations, indicating preserved IMM integrity (15). Using a Langendorff perfused rat heart model of 25 min of global ischemia followed by 30 min of reperfusion, Chen et al. (14) reported improved Ca$^{2+}$ tolerance and IMM potential in the SSM subpopulation. These
effects were observed when electron transport was blocked by amobarbital during ischemia as well as postconditioning (6 cycles of 10-s I/R) applied at the onset of reperfusion (14). Thus studies investigating I/R postconditioning reveal SSM to be primarily affected with a minority of studies revealing similar effects in the IFM. As with preconditioning, one could speculate that this phenomenon is an indirect result of the milieu associated with the subsarcolemmal and extracellular spaces. These reports also suggest that a cardiac postconditioning treatment may be best suited for cardioprotective strategies geared toward pathologies in which SSM are primarily impacted.

Heart failure/pressure overload/volume overload. Mechanical dysfunction of the myocardium in heart failure (HF) leads to an inability to sufficiently supply oxygenated blood to the body to meet its metabolic requirements (62, 109). The interplay between metabolism and ATP production is fundamental in the disease progression, and studies have shown that mitochondrial energetics and morphology are centrally involved (110). Interestingly, enlarged megamitochondria, found sporadically in between myofibrils, display augmented cristae number in cardiomyocytes from iron-deficient Sprague-Dawley rats, which develop marked cardiac hypertrophy (130, 132). Although it is unclear how the megamitochondria develop, the authors suggest that they may arise as a result of the fusion process (130). Both mitochondrial biogenesis and electron transport chain enzymes are impacted during heart failure (40, 58, 116). Mitochondrial biogenesis is enhanced during the cardiomyocyte compensated hypertrophy phase in an effort to match energy demand, but is subsequently decreased during the pathological decompensation phase (111). δ-Sarcoglycan null hamsters, a rodent model of dilated cardiomyopathy that exhibits decreased mitochondrial oxidative capacity and is responsive to nutritional and metabolic therapies, displayed distinct effects to the IFM subpopulation. These effects included a decrease in mitochondrial yield and direct impact on Ca$^{2+}$-induced mPTP (37, 38). These data are in agreement with others using cardiomyopathic hamsters in a time course analyses in which defective oxidative phosphorylation was confined to the IFM subpopulation. These authors suggest that the IFM dysfunction observed may be related to alterations in IMM transport properties involved with adenine nucleotides and/or ATP synthesis (54). Because oxygen tension in the center of the cell is much less than at the cell periphery (129) and because cardiac hypertrophy would potentially enhance the distance for oxygen and metabolites to diffuse, hypertrophic conditions are more likely to negatively impact IFM compared with SSM. Additional evidence of mitochondrial subpopulation biogenesis effects as a result of heart failure can be gleaned from studies using desmin-null mice, which display clumping of SSM and abnormal proliferation of IFM, as well as swelling of the mitochondrial matrix, all of which is exacerbated with an exercise intervention (85). The disordered nature of the enhanced mitochondrial density raises the question of whether the mitochondrion makes the subpopulation or is myocyte regionalization of greater importance. Studies using rat models of pressure and volume overload heart failure suggest that both subpopulations may be involved in the disease development. Using an aortic-constricted spontaneously hypertensive rat model of pressure overload, Sparagna et al. (124) observed a progressive loss of tetralinoleoyl cardiolipin in both SSM and IFM subpopulations. These findings were complemented by similar findings using human left ventricular tissue from patients with dilated cardiomyopathy. These results differ from van Empel et al. (136) using mice deficient in apoptosis-inducing factor, which were subjected to pressure overload using an aortic banding protocol. These authors reported a reduced capacity for free radical scavenging that was specific for SSM. In contrast, rats that underwent transverse aortic constriction for 20 wk displayed decreased total mitochondrial content, as well as decreased state 3 and state 4 respiration rates, which impacted IFM to a greater extent (115). It is unclear as to why the results differ, but it may be related to the differences in pathological model used and/or animal model of choice. Examination of animals subjected to aortocaval fistula, a model of volume overload, revealed decreased levels of ETC complexes I-V in SSM, as well as decreased state 3 respiration rates (41, 135). Other studies have suggested that dysfunction in the IFM during volume overload may not be observed because of its ability to respond to increased myocardial demand by improving mitochondrial efficiency through a reduction in state 4 respiration (82, 141). Taken together, these studies suggest that mitochondrial subpopulations are impacted differentially in heart failure dependent on model used, such that IFM appear primarily affected with the exception of volume overload models in which SSM appear to be primarily affected.

Aging. Mitochondrial dysfunction has long been considered as a central contributor to the development of the aged heart due to its essential role in cellular processes including the generation of ROS (23). Comparing an aged rat model (24 mo and 28 mo) to a young adult rat model (6 mo), Fannin et al. observed age-related alterations in mitochondrial function confined solely to the IFM subpopulation (32). Specific alterations included decreased oxidative phosphorylation rates and cytochrome oxidase enzyme activities (32). Similar observations have been reported for IFM in aged rat heart including decreased rates of oxidative phosphorylation, enhanced oxidant production, and decreased complex IV enzyme activity, with no changes in SSM (42, 127). These findings complement those of Judge et al. (56) who observed increased oxidative stress and antioxidant enzyme activities in the IFM of the aged rat heart. In addition, aging has been associated with a decrease in ETC complex III activity in the IFM of aged rat heart, which has been linked in part to a defect in the cytochrome c binding site of complex III (72), without effect on the IMM lipid environment or cristae morphology (53, 68, 87, 107). Electron leakage at complex III, leading to increased superoxide production (86) and reduction in cytochrome oxidase subunit VIIa, was detected only in the IFM of aged rat hearts (35). Additionally, IFM displayed an increased susceptibility to Ca$^{2+}$-induced mPTP opening in aged rat heart (48). In addition to functional effects, aging has been associated with IFM structural alterations including size and internal morphology in a mouse model (17). In contrast, evaluation of the aged heart in a canine model revealed alternate results. Assessment of Ca$^{2+}$-induced mPTP opening in 1-year-old and 8-year-old female beagles with hypertension and left ventricular wall thickening indicated that SSM were affected to a greater extent than IFM (1). Ultimately, mitochondrial dysfunction associated with the aged rat heart appears to be limited predominantly to the IFM subpopulation leading to decrements in mitochondrial function, whereas in large animal models, effects occur primarily to

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SSM. These results suggest that experimental model differences may be predictive of mitochondrial subpopulation dysfunction profiles in the aged heart.

**Aging and I/R.** The aged heart is more sensitive to additional cardiac events including I/R, due in part to a decline in overall mitochondrial function (76, 144). Using an aged rat model, Lesnefsky et al. (74, 75) identified a new oxidized molecular cardiolipin species that arises in both mitochondrial subpopulations mainly during ischemia rather than reperfusion due to changes in the oxidative milieu. Furthermore, aged rat heart undergoing 25 min of ischemia displayed decreased ETC complex III activity in both cardiac mitochondrial subpopulations with the effects being greatest in the IFM (71). Additionally, these authors report that the damage to ETC complex III observed in the aged rat heart is a result of alteration of the cytochrome c binding site, whereas the addition of an ischemic insult leads to a marked decrease in its electron paramagnetic resonance signal of the iron-sulfur protein. The net result is an enhanced mitochondrial derived injury in the aged/ischemic rat heart at ETC complex III that ultimately influences IFM functionality and provides a mechanism for enhanced injury to aged/ischemic IFM (71). Inherently, aging has been shown to decrease IFM oxidative metabolism; however, Lesnefsky et al. (73) observed restoration of ETC complexes III and IV activities and oxidative phosphorylation in the IFM following 25 min of ischemia and 30 min of reperfusion in the aged rat heart pretreated with acetylcarnitine. Using a similar I/R protocol, Chen et al. (15) observed preservation of IMM potential and integrity in both SSM and IFM when amobarbital treatment was presented at reperfusion. Taken together, these studies indicate that pharmacological modulation of electron transport may present an avenue for decreasing mitochondrial injury brought about by I/R in the aged heart. In summary, the IFM are predominantly affected in the aged heart; however, both cardiac mitochondrial subpopulations are affected when the aged heart undergoes I/R injury.

**Exercise.** Physical exercise has been attributed to the prevention of cardiovascular diseases (100, 133). Furthermore, exercise training is associated with the induction of mitochondrial biogenesis (47, 51). Moreover, increased mitochondrial enzyme proteins and activities have been observed in tissues of exercise-trained animals (125, 128). However, studies examining exercise training and its effects on cardiac mitochondrial subpopulations are sparse. Aged C57BL/6J mice subjected to a treadmill training protocol displayed increased IFM hypertrophy and loss of matrix and cristae, as well as formation of giant mitochondria (17). However, Fischer 344 rats subjected to long-term voluntary wheel running coupled with caloric restriction showed a reduction in H$_2$O$_2$ production and lower manganese superoxide dismutase (MnSOD) activities in both SSM and IFM subpopulations. Interestingly, these authors observed a significant increase in protein carbonylation in SSM, which was not observed in IFM (57). These authors suggest that the increased protein carbonylation observed in SSM may be related to the decreased MnSOD activity in this subpopulation. In contrast, because IFM possess higher antioxidant enzyme activities, a resultant increase in protein carbonylation was not observed despite also displaying decreased MnSOD activities (57). A treadmill training protocol with gradually increased running times was used to examine proteomic changes in rat mitochondrial subpopulations. Significant alterations in mitochondrial proteomic make-up were observed with 11 proteins (7 upregulated and 4 downregulated) in IFM and two proteins (1 upregulated and 1 downregulated) in the SSM subpopulation. Monoamine oxidase A, a principal enzyme involved in catalyzing the oxidative deamination of several monoamines resulting in ROS, was decreased in both subpopulations. These authors conclude that downregulation of monoamine oxidase A by endurance exercise represents a physiological and practical approach to prevent cardiac oxidative stress, cell death, and apoptosis in situations where ROS production increases (63). A study using an exercise training protocol with rats in which running times were gradually increased revealed increases in protein levels of primary antioxidant enzymes accompanied by lower maximal rates of mPTP opening, as well as prolonged times to $V_{\text{m}}$ in both SSM and IFM subpopulations. These authors conclude that endurance exercise promotes biochemical alterations in both cardiac mitochondrial subpopulations resulting in a phenotype that resists apoptotic stimuli (64). Hence, studies examining exercise and cardiac mitochondrial subpopulations reveal proteomic as well as functional changes in both mitochondrial subpopulations.

**I/R and exercise.** Hearts from Sprague-Dawley rats, subjected to a 5-day treadmill training protocol with increasing duration, underwent a global I/R protocol consisting of 40 min of ischemia followed by 45 min or reperfusion. After reperfusion, both mitochondrial subpopulations displayed an increase in H$_2$O$_2$ production, which was prevented by exercise training solely in the SSM subpopulation. Furthermore, I/R-induced decrements in state 3 respiration rates in both mitochondrial subpopulations were reversed only in the SSM when using complex I driven substrates, but in both subpopulations when using complex II driven substrates. Respiratory control ratio was decreased in both subpopulations regardless of substrate used and preserved with exercise training only in the SSM (67). Although studies are limited, findings suggest that mitochondria from exercise-trained hearts that undergo a subsequent I/R protocol display greater protective effects to SSM compared with IFM.

**Drugs.** Numerous groups have investigated the effects of specific drugs on cardiac mitochondrial subpopulations. In a study by Duan and Karmazyn (27) investigating the effects of verapamil in rat cardiac mitochondrial subpopulations, the authors report significant reversal of oxidative phosphorylation depression in SSM, which was induced by treatment with phosphate. In contrast, these same authors incubated isolated cardiac mitochondrial subpopulations with phosphate and D,L-carnitine, which led to a prevention in the reduction of oxidative phosphorylation without affecting the ADP/ATP translocation system. These authors conclude that the mitochondrial IMM instability induced by phosphate is prevented by D,L-carnitine in a dose-dependent manner (29). Four weeks of aldosterone/salt treatment in rats elicited an increase in H$_2$O$_2$ and 8-isoprostane production in SSM, which was accompanied by enhanced opening of the mPTP and an increase in SSM free Ca$^{2+}$. However, cotreatment with Carvedilol and Nebivolol attenuated the increase in SSM Ca$^{2+}$ while preventing mPTP opening and oxidative stress. Thus cardiomyocyte necrosis observed with aldosterone treatment can be favorably regulated by cotreatment of these two compounds and may involve protection via SSM-specific mechanisms (8). A recent
study investigating the differential responses of rat ventricles to diazoxide revealed decreased ATP production, reduced Ca\(^{2+}\)-accumulating capacity, and an increased sensitivity of the mPTP to opening specifically in SSM (52). Interestingly, acute intraperitoneal administration of ethanol (75 mmol/kg body wt) combined with a flooding dosage of L-[4–3H] phenyl alanine in Wistar rats revealed a depressed fractional rate of protein synthesis in both subpopulations. These authors conclude that the reduced synthesis rate of mitochondrial proteins in response to ethanol exposure may be partly connected to depression in myocardial contractility and associated with functional damage of mitochondrial metabolism (120). In contrast, cocaine-induced cardiac dysfunction in rats revealed increased ROS production and decreased ATP synthesis in the IFM (137), which may occur through a xanthine oxidase-mediated mechanism (138). Hence, the majority of studies investigating the effects of drugs on mitochondrial subpopulations implicate SSM as being primarily affected. Because of their distal location within the cell, IFM may not possess the ability to respond to drug treatment as readily compared with SSM, which reside at the cell periphery.

**Diabetes mellitus.** Studies have examined cardiac mitochondrial subpopulations in different metabolic diseases including diabetes mellitus. Cardiovascular complications and heart disease are the leading cause of morbidity and mortality in patients with diabetes mellitus (39). Diabetic cardiomyopathy, characterized by contractile dysfunction independent of atherosclerosis, is the leading cause of heart failure in patients with diabetes mellitus (54, 117, 145). Diabetes mellitus can be characterized by lack of insulin production (type 1 diabetes mellitus) or resistance to insulin (type 2 diabetes mellitus). Mitochondrial dysfunction has been shown to be central to the pathogenesis of both types of diabetes mellitus.

**Type 1 diabetes mellitus.** We have previously shown that IFM subpopulations isolated from Swiss-Webster mice rendered diabetic by multi low-dose streptozotocin injections display decreases in size and internal complexity as well as decrements in ETC complex I and III function. Moreover, superoxide production and oxidative damage to proteins and lipids are significantly higher in the type 1 diabetic IFM (21). Indeed, we have observed decreases in cardiolipin content of type 1 diabetic IFM (19, 21) which was associated with decrements in cardiolipin biosynthetic pathway constituent cardiolipin synthase, as reflected by decreased protein and enzymatic activity in the IFM (19). Furthermore, we have observed enhanced apoptotic propensity in type 1 diabetic IFM, which was associated with increased caspase-3 and -9 activities, mPTP opening, Bax, and cyclophilin D protein contents, with decrements in mitochondrial cytochrome c content and Bcl-2 levels (143). Proteomic approaches using isobaric tags for relative and absolute quantitation and two-dimensional-differential in-gel electrophoresis reveal a greater impact in IFM proteomic make-up during type 1 diabetic insult characterized by a decreased abundance of fatty acid oxidation and electron transport chain proteins. Furthermore, mitochondrial protein import is compromised in type 1 diabetic IFM, providing a potential mechanism accounting for proteomic dysregulation associated with the content of nuclear-encoded mitochondrial proteins (3). Overexpression of mPHGPx has been shown to increase ETC complex function and attenuate H\(_2\)O\(_2\) production and lipid peroxidation in type 1 diabetic IFM. Moreover, reversal of protein import dysfunction and lessened proteomic loss in type 1 diabetic IFM was observed with preservation of oxidative phosphorylation, tricarboxylic acid cycle, and fatty acid oxidation processes as indicated by Ingenuity Pathway Analysis (2). Slc25a3, an inner membrane protein transporter involved in the provision of inorganic phosphate to the mitochondrial matrix, is decreased specifically in type 1 diabetic IFM, which is associated with decreased ATP synthase activity and ATP production (4). Fancher et al. (31), who investigated the effects of type 1 diabetes mellitus on the function and expression of ATP-dependent K\(^+\) channels in FVB mice, observed a decrease in pore-forming subunit Kir6.1 in both subpopulations, whereas diazoxide-sensitive sulphonylurea receptor SUR1 was only decreased in IFM. In summary, the majority of studies indicate that type 1 diabetes mellitus primarily affects the IFM. It is unclear as to why this phenomenon occurs but may be related to higher respiration rates, membrane potential, and inherent protein import rates in the IFM subpopulation.

**Type 2 diabetes mellitus.** Examination of type 2 diabetes mellitus has revealed diametrically opposed results from studies of type 1 diabetes mellitus. Using a db/db mouse model, we have reported alterations in mitochondrial morphology, including decrements in size and internal complexity, as well as decreased state 3 respiration rates, ETC complex activities, ATP synthase function, and membrane potential, specifically in the SSM subpopulation. These results were complemented by increased oxidative damage in the form of enhanced lipid peroxidation by-products and nitrotyrosine residues (21). Proteomic evaluation in db/db mouse heart revealed greater loss of SSM proteins as a result of the pathology, which was consistent with functional and structural alteration to this specific subpopulation. Hence, SSM is most affected in the type 2 db/db mouse heart.

It is unclear as to why type 1 and type 2 diabetes mellitus would influence mitochondrial subpopulations differently such that type 1 diabetes mellitus impacts the IFM while type 2 diabetes mellitus influences the SSM. Although both pathologies impart similar deleterious effects on the mitochondrion, the milieu resulting from the pathology are somewhat different. For instance, enhanced free fatty acid content is more pronounced in the type 2 diabetic phenotype. Examination of intracellular lipid volume in type 2 diabetic patient vastus lateralis revealed enhanced accumulation (3-fold increase) specifically in the SSM locale without impact on intermyofibrillar lipids. These authors suggest the potential that lipids in the SSM region may more readily interfere with key processes involved in metabolic signaling including insulin signaling, based upon their location, compared with IFM (90). It is important to note that these studies were performed in skeletal as opposed to cardiac tissue. Interestingly, although both type 1 and type 2 diabetes mellitus primarily impact different mitochondrial subpopulations in the heart, some commonalities in mitochondrially associated etiologies exist. Correlation between the loss of function and proteomic dysregulation observed in a specific mitochondrial subpopulation is consistent based upon the diabetes mellitus type examined. For instance, during type 1 diabetic insult, cardiac IFM display extensive structural and functional changes, which are correlated with pronounced mitochondrial proteome dysregulation. In contrast, the oppo-
site is observed in the type 2 diabetic setting such that SSM display extensive structural and functional changes, which are correlated with pronounced mitochondrial proteome dysregulation. Additionally, proteomic loss in the IMM, which contains proteins involved in oxidative phosphorylation as well as protein import machinery, appears to be specifically impacted in both the IFM of type 1 diabetic heart as well as the SSM of type 2 diabetic heart, suggesting that this submitochondrial locus is particularly prone to proteomic alteration resulting from diabetic insult (49). Furthermore, key processes including the import of nuclear-encoded mitochondrial proteins and the loss of essential constituents in that process appear to be most impacted in the IFM of type 1 diabetic mitochondria and the SSM of type 2 diabetic mitochondria. Thus the most negatively impacted mitochondrial subpopulation, independent of diabetes type, incurs deleterious effects on the same key mitochondrial processes. As a result, both pathologies provide complementary information including identification of proteins and/or processes to target for therapeutic intervention that can treat both diabetic phenotypes.

Hypermetabolism. Thyroid hormones regulate metabolic activity in a number of tissues, including the heart. Hyperthyroidism can influence heart rhythm and its energy demand. Furthermore, chronic hyperthyroidism can influence the oxidative capacity of heart mitochondria (91). To determine the oxidative capacity in cardiomyocytes from control and hypermetabolic (hyperthyroid) rats, SSM and IFM subpopulations were isolated. These authors report greater oxidative capacity, as well as higher concentrations of cytochrome aa3, in the IFM subpopulation in hypermetabolic rats without effects on the SSM (121). Evaluation of mitochondrial volume density revealed no changes as a result of the hypermetabolism condition. Nevertheless, due to the increase in IFM oxidative potential, the oxidative capacity in the IFM zone (oxidative capacity × volume density) was 80% higher than that of the SSM zone as a result of hypermetabolism. Thus hypermetabolism further increases the oxidative capacity of IFM without significantly impacting the SSM subpopulation. Hence, hypermetabolism in the form of hyperthyroidism primarily affects the IFM subpopulation.

Table 2 summarizes some of the subpopulation-specific differences in response to cardiac pathologies reported in spatially distinct mitochondrial subpopulations.

Conclusions

In conclusion, the central role of the mitochondrion in cardiac pathologies is becoming more appreciated by the cardiovascular researcher due in part to its participation in a multitude of cellular processes critical for cardiac function. Macrostructural imaging of cardiac tissue using a multitude of methodological approaches indicates that distinct pools of mitochondria exist in various subcellular locales including under the sarcolemma, between the myofibrils and in close proximity to the nuclear region. These observations coupled with microstructural imaging techniques reveal differences between spatially distinct mitochondrial subpopulations in many of their morphological features including shape, size, and internal cristae structure. Furthermore, studies assessing functional features of spatially distinct populations indicate differences which may be a result of the local milieu presented in a given subcellular niche and/or the functional role it plays based upon this location. As a result, spatially distinct subsets of mitochondria appear to be influenced differently during pathological insult, which may reflect specific disparities in the etiology of a given pathology such that the stress elicited may differentially impact subcellular locales and the mitochondria contained within. Thus incorporation of subcellular spatial location when evaluating mitochondria should be considered to assure the greatest potential for delineating the influence of cardiac pathology on this critical organelle.

Future Directions

Although a wealth of studies have provided a foundation for understanding the structural and functional features of mitochondrial subpopulations, understanding why spatially distinct subpopulations respond differently to disease states remains crucial and may lend greater insight into the development of cardiac pathologies. This information would enhance our ability to design therapeutics that take into consideration the

Table 2. Differences in SSM and IFM response to cardiac pathologies

<table>
<thead>
<tr>
<th>Stimuli/Factor</th>
<th>Species</th>
<th>Primary Subpopulation Affected</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia</td>
<td>Rat, rabbit, canine</td>
<td>SSM</td>
<td>11, 12, 70, 77, 78, 119, 126, 140</td>
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<td>Hypoxia</td>
<td>Rat</td>
<td>SSM</td>
<td>26, 45</td>
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<tr>
<td>Myocardial infarction</td>
<td>Rat, dog</td>
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<td>44, 92, 104–106, 109, 112</td>
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<td>I/R</td>
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<td>Both</td>
<td>22, 30, 70, 142</td>
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<tr>
<td>Preconditioning</td>
<td>Rat, rabbit</td>
<td>SSM</td>
<td>6, 9, 10, 13, 28, 69, 103</td>
</tr>
<tr>
<td>Postconditioning</td>
<td>Rat, rabbit</td>
<td>SSM</td>
<td>14–16, 94</td>
</tr>
<tr>
<td>Heart failure</td>
<td>Rat, hamster</td>
<td>SSM</td>
<td>37, 38, 54, 130, 132</td>
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<td>Pressure overload</td>
<td>Mouse, rat</td>
<td>IFM</td>
<td>82, 115, 124, 136</td>
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<tr>
<td>Volume overload</td>
<td>Rat</td>
<td>SSM</td>
<td>41, 82, 135, 141</td>
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<tr>
<td>Aging</td>
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<td>IFM</td>
<td>1, 17, 32, 35, 42, 48, 53, 56, 68, 72, 86, 87, 107, 127</td>
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<tr>
<td>Aging and I/R</td>
<td>Rat</td>
<td>IFM</td>
<td>15, 71, 73–75</td>
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<td>Exercise</td>
<td>Mouse, rat</td>
<td>Both</td>
<td>17, 57, 63, 64</td>
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<tr>
<td>Exercise and I/R</td>
<td>Rat</td>
<td>Both</td>
<td>67</td>
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<tr>
<td>Pharmacological interventions</td>
<td>Rat, hamster</td>
<td>SSM</td>
<td>8, 27, 29, 52, 120, 137, 138</td>
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<td>Diabetes mellitus</td>
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<td>2–4, 19, 21, 31, 143</td>
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<tr>
<td></td>
<td>Type II Mouse</td>
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<tr>
<td></td>
<td>Hypermetabolism</td>
<td>IFM</td>
<td>121</td>
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</table>

I/R, ischemia-reperfusion.

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spatially distinct subpopulations of mitochondria

subcellular location of dysfunctional mitochondria. Ultimately, implementation of this information into therapeutic design would enable the development of treatment strategies with greater specificity and less off-target effects. Prophylactic strategies that take advantage of the inherent spatial, structural, and functional differences between mitochondrial subpopulations offer a unique opportunity to do so. Because alterations in the mitochondrial proteome dictate downstream structural and functional effects on the mitochondrion and ultimately cardiac function, strategies designed to lessen or reverse mitochondrial proteome loss from nuclear and mitochondrial genome-encoded components as well as limiting deleterious post-translational modifications to constituents of the mitochondrial proteome may offer the opportunity for developing treatment options for the cardiac patient.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: J.M.H., D.T., and D.L.S. prepared figures; J.M.H., D.T., and D.L.S. edited and revised manuscript; J.M.H., D.T., and D.L.S. approved final version of manuscript.

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GRANTS
This work was supported by the National Institutes of Diabetes and Digestive and Kidney Diseases Grant DP-2DK083095 (to J. M. Hollander). D. L. Shepherd is a recipient of a National Institutes of Health Predoctoral Fellowship (T32HL090610).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: J.M.H., D.T., and D.L.S. prepared figures; J.M.H., D.T., and D.L.S. edited and revised manuscript; J.M.H., D.T., and D.L.S. approved final version of manuscript.

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