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Mitochondrial ROS and mitochondria-targeted antioxidants in the aged heart

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ABSTRACT

Excessive mitochondrial ROS production has been causally linked to the pathophysiology of aging in the heart and other organs, and plays a deleterious role in several age-related cardiac pathologies, including myocardial ischemia-reperfusion injury and heart failure, the two worldwide leading causes of death and disability in the elderly. However, ROS generation is also a fundamental mitochondrial function that orchestrates several signaling pathways, some of them exerting cardioprotective effects. In cardiac myocytes, mitochondria are particularly abundant and are specialized in subcellular populations, in part determined by their relationships with other organelles and their cyclic calcium handling activity necessary for adequate myocardial contraction/relaxation and redox balance. Depending on their subcellular location, mitochondria can themselves be differentially targeted by ROS and display distinct age-dependent functional decline. Thus, precise mitochondria-targeted therapies aimed at counteracting unregulated ROS production are expected to have therapeutic benefits in certain aging-related heart conditions. However, for an adequate design of such therapies, it is necessary to unravel the complex and dynamic interactions between mitochondria and other cellular processes.

1. Introduction

Aging hearts show a progressive decline in structure, function and metabolism [1]. In large part this is due to the impairment in mitochondrial function and accumulating mitochondrial DNA (mtDNA) mutations/deletions, features that have been closely correlated with an excessive reactive oxygen species (ROS) formation. Indeed, ROS are central to the Mitochondrial Free Radical Theory of Aging postulating that oxidative damage to mitochondrial proteins and DNA is the key event leading to organelle dysfunction and further exacerbation of ROS formation [2]. Aged hearts show impairments in redox status, metabolic flexibility and organelle dynamics, and a decrease in mitochondrial respiration and content [1]. Supporting the central role of mitochondria in aging is the observation that accelerated aging is driven by mtDNA mutations [3,4]. Although those studies did not find a clear-cut correlation between mutations in mtDNA and oxidative stress, the importance of mitochondrial ROS in cardiac aging was demonstrated by studies using mice overexpressing mitochondria-targeted catalase. These mice show improved organelle redox status, attenuated cardiac aging and increased lifespan [5]. Similarly, deletion of $p66^{Shc}$, a cytosolic protein that can translocate to mitochondria and lead to hydrogen peroxide (H_2O_2) formation, results in increased resistance to stress and a 30% extension in lifespan [6].

While the key contribution of mitochondria to the aging process remains unequivocal, the Mitochondrial Free Radical Theory of Aging has been expanded in more recent years to accommodate the possibility that ROS can also exert protective and signaling roles [7]. In this regard, the mitohormesis hypothesis proposes that, rather than being simply deleterious byproducts, ROS may act as signaling molecules and have

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Abbreviations

- 4-HNE 4-hydroxynonenal
- AGEs advanced glycation end products
- Ang-II angiotensin II
- BH4 tetrahydrobiopterin
- CaMKII calcium/calmodulin-dependent protein kinase II
- CMA chaperone-mediated autophagy
- CML carboxymethyl lysine
- Drp1 dynamin related protein 1
- EC excitation-contraction
- EPR electro-paramagnetic resonance
- ETC electron transport chain
- GLO1 glyoxalase-1
- GPCR G-protein-coupled receptor
- GPX glutathione peroxidase
- GR glutathione reductase
- GSH reduced-glutathione
- H2O2 hydrogen peroxide
- HDAC4 histone deacetylase 4
- HF heart failure
- HIF-1α hypoxia inhibitory factor-1α
- IDH isocitrate dehydrogenase
- IPC ischemic preconditioning
- IR ischemia/reperfusion
- lncRNAs long non-coding RNAs
- LV left ventricle
- MAO monoamine oxidase
- MCU mitochondrial calcium uniporter
- MDA malondialdehyde
- ME malic enzyme
- MEF2 myocyte enhancer factor 2
- Mfn mitofusin
- MitoPQ MitoParaquat
- MnSOD manganese superoxide dismutase
- mPTP mitochondrial permeability transition pore
- mtDNA mitochondrial DNA
- NADPH nicotinamide adenine dinucleotide phosphate
- NCX sodium/calcium exchanger
- NKA sodium-potassium ATPase
- Nnt nicotinamide nucleotide transhydrogenase
- NO nitric oxide
- NOS nitric oxide synthase
- NOX NADPH oxidases
- O2 superoxide
- OH hydroxyl radical
- Opa1 optic atrophy 1
- PGC1α peroxisome proliferator-activated receptor-gamma coactivator 1 alpha
- PKA protein kinase A
- PRX peroxiredoxin
- RAAS renin-angiotensin-aldosterone system
- RAGE receptor for advanced glycation end products
- RET reverse electron transport
- RIRR ROS-induced ROS release
- ROS reactive oxygen species
- RyR2 ryanodine receptor 2
- SAMP8 senescence-accelerated mouse model
- SAMR1 senescence-resistant mouse model
- SDH succinate dehydrogenase
- SR/ER sarco/endoplasmic reticulum
- TPP triphenylphosphonium
- TRX2 thioredoxin 2
- TRXR thioredoxin reductases
- XO xanthine oxidases
- β-AR β-adrenergic receptor
- ΔΨm mitochondrial membrane potential

beneficial effects on cell functions, resulting in the extension of health/lifespan [7,8]. This concept holds true also in the context of cardioprotection induced by ischemic preconditioning (IPC), whereby ROS produced during short and intermittent periods of ischemia/reperfusion (IR) induces a series of adaptive changes that lead to protection against the main ischemic insult [9]. The key role for ROS in this process is supported by the observation that antioxidants abolish this cardioprotective effect [10]. On the other hand, excessive oxidant production in the aged and failing hearts begs for the development of therapies that can counteract deleterious ROS formation and re-establish an optimal redox balance. However, clinical studies employing general antioxidant therapy did not report any beneficial effects in that regard [7,11]. This suggests that the relationship between mitochondria, ROS and other cellular processes is more complex than what was previously thought.

In this review, we will discuss the involvement of mitochondria and mitochondrial ROS formation in aging and aging-related cardiac pathologies. We will also illustrate the intricate interplay between mitochondria, mitochondrial ROS and other cellular processes and organelles, such as calcium and sarco/endoplasmic reticulum (SR/ER), and how alterations in this equilibrium may impact on cardiac physiology and disease. Finally, we will argue how a better understanding of these processes is required for an appropriate design of successful therapeutic strategies to combat aging-related alterations in the heart.

2. Aging, mitochondria and cardioprotection

From a biochemical viewpoint, aging can be defined as a time-dependent decline in the efficiency of cells to maintain their homeostasis, leading to deleterious and cumulative effects on the organism and, ultimately, increasing the likelihood of disease and death. It follows an irreversible progression through a multifactorial process that shares common molecular hallmarks among species [12], although our understanding of the cause-effect relationship between them remains incomplete and controversial. In the heart, aged cardiomyocytes develop cytoarchitectural and biochemical alterations that may underlie their reduced adaptive reserve capacity to respond to exercise, stress and damage [13]. Due to their uninterrupted contractile activity, which relies on an enormous and dynamic energy supply, cardiomyocytes are particularly dependent on the adequate maintenance of mitochondrial integrity and fitness. Indeed, some of the age-dependent functional changes in cardiomyocytes, i.e., mismatch between energy demand and supply under metabolically demanding conditions, altered calcium handling and increased pro-oxidative status, largely involve mitochondria and resemble those of failing cardiomyocytes [14,15]. Although the causative role of mitochondria in cardiomyocyte senescence is still a matter of debate, they appear to be important mediators of the transition between health and disease in different age-related pathological conditions.

In the context of ischemic heart disease, one of the main responsible for the impact of cardiovascular diseases on death and disability among the elderly, the aged heart becomes less tolerant to IR injury, both in males and females, by mechanisms that go beyond the higher burden of comorbidities usually present in aging [14,16]. After an acute thrombotic coronary occlusion, the gold-standard treatment is the timely reopening of the culprit artery by an emergency angioplasty. However,

and even though restoring blood flow is the most efficient way to salvage an ischemic myocardium, the abrupt normalization of oxygen and pH, as well as the osmotic gradient secondary to metabolite washout, impose an additional tissue damage, known as reperfusion injury [17]. Even after a successful reperfusion, advanced age is considered one of the most prominent confounding factors, negatively influencing the extension of the myocardial infarction and the efficiency of some therapeutic interventions [18]. Mitochondria are important players in IR injury, and they specifically contribute to this age-dependent heart vulnerability to damage, both as triggers and targets of other cell deleterious processes [19]. On the one hand, they determine cardiomyocyte survival after normalization of oxygen by reactivating the ATP generation and through their participation in primary and secondary signaling cascades involved in some cardioprotective strategies, like ischemic and pharmacological conditioning [20]. On the other hand, mitochondria can themselves precipitate cell death through a series of complex and interrelated mechanisms that eventually lead to energy collapse and sarcolemmal rupture (see section 5) [21].

The contribution of mitochondrial ROS to cardiomyocyte death during myocardial IR has been established by studies in which interventions addressed to alleviate the initial burst of mitochondrial ROS upon reperfusion resulted in a significant reduction of myocardial infarct size and better contractile recovery [22]. However, the disturbed redox status already present in aging may synergistically contribute to IR-induced mitochondrial ROS production and aggravate cardiomyocyte death in the aged hearts [23]. Some endogenous cardioprotective pathways mediated by mitochondria become less effective or ineffective in the aged heart [13]. Thus, ischemic preconditioning and other pharmacological conditioning strategies trigger a cascade of signaling events that are at least partially mediated by the release of mitochondrial ROS [24]. Whether this age-dependent loss of cardioprotective efficiency is due to changes in mitochondrial ROS production is still a matter of debate. This is because the complexity of both spatial organization and functional specialization of mitochondria within cardiomyocytes makes the interpretation of their pathophysiological contribution more difficult. Several lines of evidence are coincident in demonstrating that mitochondrial sensitivity to injury (either ischemic or toxic) and cardioprotection varies depending on mitochondrial subcellular location (subsarcolemmal or interfibrillar) [25,26] (see section 4.1). Therefore, although the role of mitochondria as therapeutic targets in cardioprotection is beyond doubt, especially in elderly patients [27], the intersection between aging, mitochondrial ROS and cardioprotection is much more complicated than was initially considered.

3. ROS in the aged heart

3.1. Mitochondrial ROS production and elimination

ROS have important roles in cellular homeostasis and signaling pathways in mammals. Intracellular ROS are generated in multiple compartments through the contribution of various macromolecules and their production increases with aging. Some of those macromolecules, such as the family of NADPH oxidases (NOXs), are located within cell membranes, while others such as cyclooxygenases are in the cytosol. Although these membrane and cytosolic contributors participate in the overall oxidative processes of the cells, up to 90% of physiologically generated ROS are of mitochondrial origin (mitochondrial ROS) [28].

Within cardiomyocytes, ROS are generated in different compartments by different enzymes, including NOX2 and NOX4 [29], uncoupled nitric oxide synthase [30], and xanthine oxidases (XO) in the cytosol [31], the $p66^{Shc}$ in the mitochondrial intermembrane space and monoamine oxidase (MAO) in the mitochondrial outer membrane [32]. The $p66^{Shc}$ may generate ROS through the catalysis of electron transfer from cytochrome *c* to oxygen. MAO is responsible for the degradation of endogenous monoamine neurotransmitters and dietary amines in a process that generates H_2O_2 and aldehydes as intermediate products [33]. MAO overactivation has been recognized as an important cause of oxidative stress in the heart during aging [34,35]. Nevertheless, the mitochondrial electron transport chain (ETC) is considered the most relevant source of cellular ROS by many authors [36–38], but not all [39]. At the ETC, mitochondrial ROS are mainly generated as by-products of oxidative phosphorylation at complexes I (NADH: ubiquinone-oxidoreductase) and III (ubiquinol: cytochrome c-oxidoreductase; cytochrome *bc*1-complex) [40] and contribute to retrograde redox signaling from the organelle to the cytosol and nucleus. Although these respiratory complexes are generally considered as the main producers of superoxide ($O_2^{\bullet-}$) radicals released into the mitochondrial matrix and the intermembrane space, other components of the respiratory chain, especially complex II (succinate dehydrogenase, SDH) and the redox state of the ubiquinone pool (Q-pool) also contribute to $O_2^{\bullet-}$ production [40,41]. Mitochondrial ROS are generated in large part from single electrons escaping the ETC complexes. Here, the $O_2^{\bullet-}$ is produced when the reduced forms of flavin mononucleotide or ubiquinone pass an electron to O_2, thereby generating $O_2^{\bullet-}$ [42,43]. $O_2^{\bullet-}$ can react with and damage many macromolecules, including components of the Krebs cycle compromising mitochondrial energetics [44,45]. The rate of $O_2^{\bullet-}$ formation is increased when damage to the respiratory chain or low ATP demand increases the ratio of $NADH/NAD^+$ [46,47]. Furthermore, mitochondria may potentiate ROS from neighboring mitochondria and other sources through a process termed "ROS-induced ROS release" (RIRR) [48] (Fig. 1). This concept involves ROS-induced activation of the mitochondrial inner membrane anion channel [49], the mitochondrial permeability transition pore (mPTP) [50], and ATP-sensitive K^+-channels [51]. Dynamic balances between production and consumption regulate the levels of $O_2^{\bullet-}$ and H_2O_2 in the mitochondrial matrix and the cytosol. When ROS production increases over physiological levels, exceeding the detoxification capacity of the cells, molecular damage arises. This phenomenon may underlie different pathophysiological contexts, including aging [52,53]. Therefore, overproduction of $O_2^{\bullet-}$ and H_2O_2 by mitochondria is an important player of the altered mitochondrial redox signaling [54].

Mitochondria are protected against mitochondrial ROS overproduction by several mitochondrial antioxidants and other defense systems, such as glutathione peroxidases, thioredoxin peroxidases, dismutases, peroxiredoxins, oxidized cytochrome *c*, glutathione, thioredoxin 2 (TRX2), glutaredoxin 2, complex IV, coenzyme Q, ascorbic acid, tocopherol, vitamin E, and carotene [55]. Specifically, loss of endogenous oxidized cytochrome *c* after mitochondrial outer membrane permeabilization has been shown to increase oxidative stress during IR in the heart [55]. Manganese superoxide dismutase (MnSOD) converts $O_2^{\bullet-}$ to H_2O_2 in the mitochondrial matrix, while copper- and zinc-SOD detoxifies $O_2^{\bullet-}$ in the mitochondrial intermembrane space and/or cytosol [56,57]. The H_2O_2 generated in the mitochondrial matrix is converted to hydroxyl radical by mitochondrial aconitase [57]. The $NADPH/NADP^+$ ratio is important for antioxidant defense through its role as a donor of redox potential to glutathione and thioredoxin reductases (TRXR) and for the regulation of the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase [58]. Glycolysis and the pentose phosphate pathway are also involved in mitochondrial ROS detoxification [59]. Some studies suggest that ROS generated by Nox4 can induce oxidation of mitochondrial proteins, thereby exacerbating mitochondrial ROS formation through RIRR [58]. During aging, Nox4 is upregulated while the mitochondrial antioxidant mechanisms are downregulated. Excessive ROS production via Nox4 exceeds the capacity of the antioxidants, a mechanism that contributes to further ROS accumulation and mitochondrial damage during aging. In addition, the peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC1α), which is a master regulator of energy metabolism and mitochondrial biogenesis, also controls the expression of mitochondrial antioxidant genes [60,61]. Its dysregulation has been shown to exacerbate oxidative stress and inflammatory

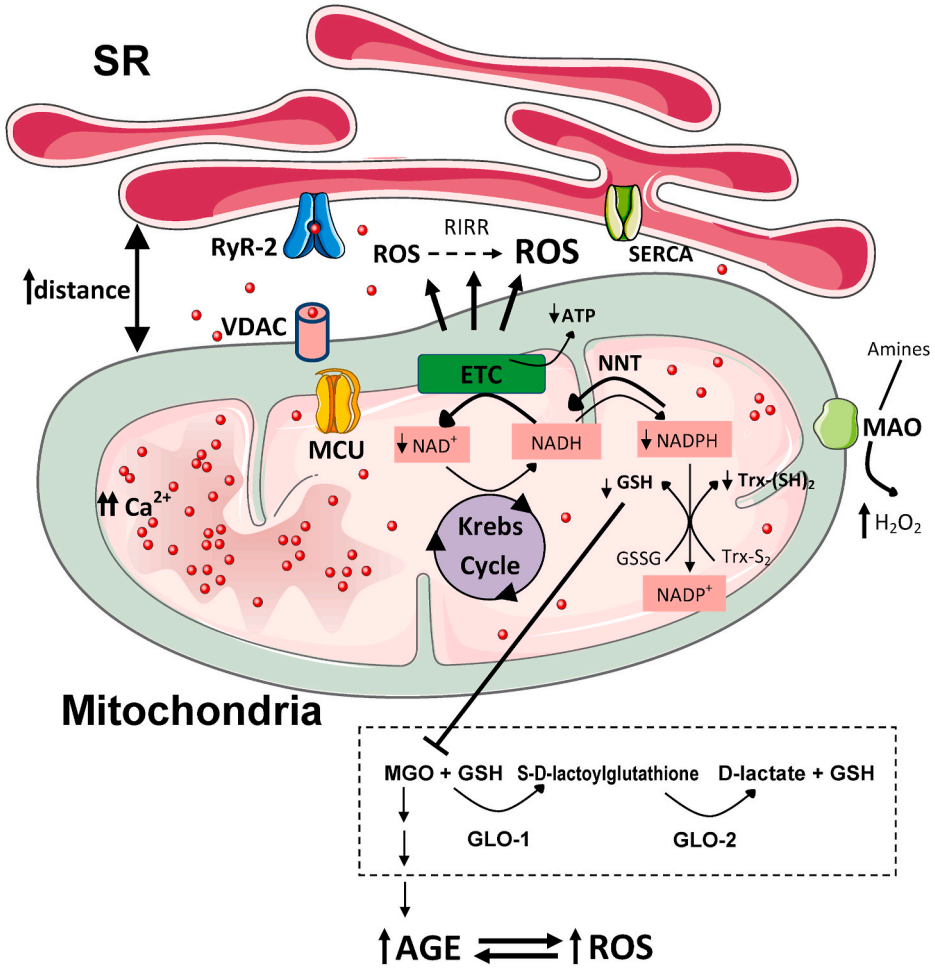

Fig. 1. Altered mitochondrial calcium cycling, ROS production and bioenergetics during aging. Functionally deficient ETC, overactive MAO, decreased GSH and TRX regeneration and defective SR-mitochondria communication are proposed as prominent players of the uncoupling between calcium cycling, mitochondrial energy production and ROS balance present in aged cardiomyocytes. The deleterious pro-oxidant environment creates a self-amplifying loop and contributes to glycative damage (glycooxidation stress). AGE: advanced glycation end products; ETC: electron transport chain; GLO: glyoxalase; GSH: glutathione; MAO: monoamine oxidase; MCU: mitochondrial calcium uniporter; Nnt: nicotinamide nucleotide transhydrogenase; RIRR: ROS-induced ROS release; RyR2: type 2 ryanodine receptor; SERCA: sarcoplasmic reticulum calcium ATPase; SR: sarcoplasmic reticulum; VDAC: voltage-dependent anion channel.

response in some metabolic disorders such as obesity and diabetes [61].

There is also a close relationship between cellular oxidative stress and intracellular levels of some cations, although the underlying mechanism for cation-induced mitochondrial ROS generation is not fully understood. Early studies demonstrated a cross-talk between transition metals and increased oxygen and nitrogen species in biological systems [62]. Some metals, like iron, copper, chromium, vanadium, and cobalt, undergo redox cycling reactions (usually through Fenton reaction and generation of superoxide radical and the hydroxyl radical, while others, like mercury, cadmium, and nickel lead to depletion of antioxidants and protein damage through bonding to sulfhydryl groups [63]. Moreover, redox metals can react with lipid peroxides producing toxic dicarbonyl intermediates (malondialdehyde [MDA], 4-hydroxynonenal [4-HNE]) that eventually form irreversible chemical adducts with proteins. Other cations, like calcium, interact with ROS through different cell pathophysiological pathways. Available evidence indicates that mitochondrial membrane depolarization contributes to the loss of calcium homeostasis, which in turn increases the sensitivity of mitochondria to undergo mPTP opening and to increase ROS production [64]. Other studies have demonstrated that increased mitochondrial calcium and zinc [65,66] (with an interactive role among them), mPTP opening, alterations in function and/or expression of ion transporters located in mitochondrial membranes and opening of mitochondrial KATP-channels [64] can exacerbate mitochondrial ROS generation. All this evidence indicates that most of these alterations could be reversed and/or prevented with antioxidant treatments targeting mitochondrial ROS.

Apart from direct cardiac stressors that increase oxidative stress in aging, coexisting non-hemodynamic alterations and other extra-cardiac triggers can also play a role. For example, low-grade inflammation observed in elderly patients has been related to several sources of ROS over-production secondary to the senescence of immune cells, mainly neutrophils and macrophages [67,68]. Also, obesity and diabetes are well documented triggers of oxidative stress and the pathophysiology of some age-related conditions, such as chronic kidney disease and hypertension, is also related to a pro-oxidant status [69].

3.2. ROS (dys)regulation and oxidative stress

The free radical theory of aging proposed by Harman (1956) and further refined by Miquel and colleagues (1980) suggests that mitochondrial ROS emanating from ETC damages macromolecules, especially mtDNA. In turn, this can cause mitochondrial dysfunction and contribute to pathologies associated with aging, including cardiovascular diseases [70,71]. This ROS imbalance can affect nuclear gene expression, ion handling, and mitochondrial metabolism [72,73]. In addition, oxidative stress can cause the activation of an inflammatory response, apoptotic signaling, and endoplasmic reticulum (ER) stress in different tissues, including the heart [74]. Many studies [75,76], but not all [77,78], suggest that antioxidant capacity becomes compromised with age. However, despite discrepancies among different studies, most data agree that there is an increase in oxidative damage in the heart associated with aging. Some of the factors reported to cause uncontrolled mitochondrial ROS production during aging are summarized in the Table.

To limit the oxidative damage, the MnSOD converts $O_2^{\bullet-}$ into H_2O_2, which can be further reduced to hydroxyl radical ($OH^{\bullet}$) in the presence of reduced metal atoms unless H_2O_2 is transformed to water (H_2O) by

glutathione peroxidase (GPX) and peroxiredoxin (PRX). The scavenging reaction of PRX requires other cellular dithiol proteins such as TRX, whereas the enzymatic reaction of GPX requires reduced-glutathione (GSH) [43,79]. In turn, TRX and GSH are regenerated by thioredoxin reductase (TR) and glutathione reductase (GR), respectively, using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as an electron donor [79]. Three enzymes regenerate NADPH, including nicotinamide nucleotide transhydrogenase (Nnt), NADP + -dependent isocitrate dehydrogenase (IDH), and malic enzyme (ME). These enzymes in turn require Krebs cycle products to exert their reaction [80]. Therefore, the Krebs cycle is important not only for providing the reducing equivalents NADH and $FADH_2$ necessary for ATP production at the FoF1 ATP synthase, but also to provide substrates to regenerate NADPH and therefore, to maintain the antioxidative capacity of the mitochondrial [81]. Despite compartmentalization, ROS scavenging systems of the mitochondrial matrix significantly contribute to cytoplasmic ROS handling [51].

Numerous studies underscore the role of mitochondrial ROS production and oxidative stress in cardiac aging [43,79]. An age-related increase in the expression and activity of both XO and MAO has also been described in the hearts of aged rats [82]. However, even though these enzymes play an important role in cardiovascular ROS generation, the most important sources of ROS in the aged heart are NOX and the mitochondrial ETC [36–38]. The role of NOX-derived ROS production and oxidative stress in the aged heart was demonstrated in Fischer 344 (F344) rats. In this study, the authors reported an age-dependent increase in the expression and activity of NOX2 isoform. These changes were accompanied by increased renin-angiotensin-aldosterone system (RAAS) activation and oxidative stress that could be attenuated by the pharmacological inhibition of NOX activity [83]. A similar mechanism has been suggested to play a causal role in endothelial dysfunction observed in the aged heart [84]. For instance, Outdo and colleagues [85] reported an increased NOX activity and expression in aortas from aged rat heart as well as decreased cardiac function and oxidative damage. Elevated mitochondrial ROS production and enhanced mitochondrial NOX4 expression were reported in vascular smooth muscle cells from aged mouse hearts, and this was accompanied by increased aortic stiffness and cardiac dysfunction. Besides, ROS can activate fibrogenic pathways in cardiac fibroblasts and modulate matrix metalloproteinase activity and collagen synthesis, suggesting an important role of oxidative stress in age-related cardiac fibrosis [86–88]. Besides these mechanisms, loss of the capacity to undergo mild IMM depolarization appears to be responsible for enhanced ROS formation with age [89]. Mitochondrial membrane-bound hexokinases or creatine kinase activities result in the formation of ADP that is transported to the mitochondrial matrix to drive ATP synthesis. This causes a small drop in the mitochondrial membrane potential ($\Delta\Psi m$) and reduced ROS generation by the respiratory complexes [90,91]. A recent study showed that this mechanism of mitochondrial redox control is lost in short-lived mice vs long-lived animals [89], suggesting that it may represent a universal anti-aging mechanism characterizing several tissues, including the heart.

In cardiomyocytes, a large fraction of ROS (up to 90%) is produced by the leakage of electrons from the mitochondrial ETC [92]. Age-related changes in mitochondrial function have been correlated with a decline in the activities of ETC complexes and a higher rate of ROS production associated with oxidative stress and thus contributing to cardiac aging [93,94]. For instance, Kumaran and colleagues [95] reported a decrease in the activity of complex I, III, and IV in heart mitochondria from aged rats. The authors suggested that age-related decline in the activity of these enzymes results in partial blockage of electron flow, which alters the reducing potential of electron carriers favoring their autoxidation and further ROS generation, thus establishing a vicious cycle that aggravates oxidative stress and promotes cardiac aging. In support of a central role of oxidative stress in cardiac aging are studies examining hearts from a mouse model that display a phenotype of accelerated or resistant aging. Rodriguez and colleagues [96] demonstrated an age-dependent oxidative stress in the heart of the senescence-accelerated mouse model (SAMP8) compared to senescence-resistant (SAMR1) mice, as evidenced by an increased level of lipid peroxidation products (malondialdehyde [MDA] and 4-hydroxynonenal [4-HNE]). Moreover, aged hearts from SAMP8 mice exhibited a lower mitochondrial GSH/GSSG ratio compared to aged hearts from SAMR1 mice, suggesting a pro-oxidative redox environment because of depleted ROS scavengers pool [96,97]. In addition, the increase in oxidative stress markers was accompanied by reduced activity of the respiratory complexes II, III, and IV in heart mitochondria from aged SAMP8 mice.

Oxidative stress can contribute to cardiac aging through oxidative modification of intracellular proteins, which ultimately may lead to defective mechano-energetic coupling and age-related cardiac dysfunction. Hence, the activities of the Krebs cycle enzymes IDH, a-ketoglutarate dehydrogenase, SDH and malate dehydrogenase have been shown to be decreased in aged rat hearts due to the loss of thiol groups (-SH) [95,98]. Furthermore, the authors report reduced enzyme activity of mitochondrial complex I [98]. Because several subunits of complex I are encoded in mtDNA [99], and the mtDNA is highly vulnerable to oxidative damage, the authors proposed that decreased complex I activity might be explained, to some extent, by mtDNA mutations mediated by oxidative stress. Further evidence supporting a direct role of oxidative stress in cardiac aging was obtained using transgenic mice overexpressing mitochondria-targeted catalase [5], in which age-related changes (accumulation of oxidized mitochondrial proteins oxidation and increased mtDNA damage) could be significantly attenuated, supporting the role of chronic ROS exposure and mitochondrial ROS in cardiac aging. Increased ROS/RNS levels have also been correlated with changes in mitochondrial morphology [59]. In an experimental study performed in fibroblasts and skeletal muscle myoblasts, cell exposure to H_2O_2 led to ubiquitination in mitochondrial fusion/fission proteins (Mfn1 and Mfn2), loss of mitochondrial $\Delta\Psi m$ and mitochondrial fragmentation [100]. It has been demonstrated that two of the fusion/fission proteins, dynamin related protein 1 (Drp1) and optic atrophy 1 (Opa1), are post-translationally modified by redox regulation (via both ROS and RNS), a mechanism that may also be involved in mitochondrial fragmentation [101]. Whether this mechanism takes place in cardiac aging deserves further investigation.

3.3. *Can mitochondrial ROS be protective?*

Given that ROS may act as second messengers, it is likely that this feature could be exploited to induce activation of cardioprotective signaling pathways and adaptive changes that could protect the heart from damage in pathological circumstances. Intermittent and/or moderate ROS formation is the key mechanism underlying the cardioprotective effects elicited by IPC against IR injury [102]. Yet, whether a primary increase in ROS within the mitochondrial compartment can yield the same result is less clear. A recent study has shown that a careful titration of the mitochondrial redox cycler MitoParaquat (MitoPQ), leading to a gradual $O_2^{\bullet-}$ generation, can induce diametrically opposite effects in cardiomyocytes or hearts subjected to stress *in vitro* or *in vivo* [103]. Low concentrations of MitoPQ (i.e., 0.01 μM) caused a moderate increase in mitochondrial ROS formation that had no impact on mitochondrial function or the frequency of calcium transients, but it did increase the SR calcium load. This mild increase in mitochondrial $O_2^{\bullet-}$ protected isolated cardiomyocytes from anoxia/reoxygenation injury *in vitro* and reduced infarct size in mice subjected to IR injury *in vivo*. In contrast, higher doses of MitoPQ (>0.05 μM) led to a severe increase in mitochondrial ROS, loss of $\Delta\Psi m$, mPTP opening and impairment in calcium homeostasis. Therefore, an excessive mitochondrial ROS formation is a determining and primary cause of cardiac loss of function and viability. These findings demonstrate that different amounts of $O_2^{\bullet-}$ produced within mitochondria may lead to utterly

opposite effects not only at the mitochondrial level, but also in the cytosol and SR thereby affecting cardiomyocyte survival. It is also important to bear in mind that mitochondrial ROS exert profoundly different effects in young vs aged subjects. Indeed, overexpression of mitochondria-targeted catalase is beneficial in pathological conditions or in aged mice [104,105], but the cardiac proteome of young mitochondrial catalase overexpressing mice closely resembles that of older wild-type animals [106]. This suggests that reducing mitochondrial ROS levels at a young age when basal ROS are already at low levels, is likely not beneficial.

Mitochondrial ROS contribute to the maintenance of tissue homeostasis also by regulating autophagy, a process responsible for the lysosomal removal of damaged or unnecessary proteins or organelles [107]. If this mechanism of quality control is impaired, the accumulation of damaged/undegraded material may lead to cell dysfunction and premature aging [108,109]. In fact, deletion of Atg5 in cardiomyocytes results in left ventricle (LV) dilation, contractile dysfunction and early mortality [110], while whole-body Atg5 overexpression attenuates signs of aging and prolongs lifespan [111]. Interestingly, autophagy attenuation is associated with cardiac senescence [112], while stimulation of the autophagic process significantly reduces cardiac aging and extends lifespan by removing dysfunctional mitochondria through mitophagy [113]. Also, chaperone-mediated autophagy (CMA) is a selective form of autophagy critical for the maintenance of hepatic tissue homeostasis during aging or in conditions of oxidative stress, among others [114]. However, whether CMA plays a role in cardiac pathophysiology remains unknown. ROS-dependent modulation of autophagy may be direct, through oxidation of Atg4 cysteine residue that allows for autophagosome formation, or indirect, by inducing lysosomal calcium release that in turn stimulates autophagy [107,115]. Furthermore, cytosolic calcium can inhibit autophagy as well [116]. Thus, elucidating the intricate interplay between calcium and ROS-dependent autophagy modulation will allow identifying efficient therapeutic strategies to target these processes in aging or disease.

4. The interplay between ROS and calcium

4.1. Mitochondrial spatial heterogeneity and interaction with sarcoplasmic reticulum

Adult cardiomyocytes are organized by a complex cytoarchitecture for efficient and finely controlled con-traction so that mitochondrial movements are significantly restricted.

Because the heart is the organ with the highest energy requirement of the entire organism, cardiac mitochondria occupy as much as 40% of cardiomyocyte volume and are differentiated into specific subpopulations (i.e., subsarcolemmal, interfibrillar and perinuclear mitochondria) with distinct biochemical, morphological and functional properties [117]. The high intracellular compartmentalization present in adult cardiomyocytes and the restrictions in mitochondrial movements imposed by cell cytoarchitecture, has led to the development of a significant subcellular mitochondrial specialization and heterogeneity that has consequences in their ROS production capacity, participation in cardioprotective signaling, sensitivity to injury and development of the aging phenotype. Thus, subsarcolemmal mitochondria have a greater contribution to the overall ROS production within the cells than interfibrillar mitochondria [118], are less tolerant to calcium overload and more vulnerable to IR injury [16,119], and appear to be more responsive to the protective effect of some pharmacological strategies [25]. Indeed, connexin 43, a protein whose translocation to mitochondrial membranes has been implicated in cardioprotection [120] is only present in subsarcolemmal mitochondria [121]. On the other hand, the deleterious consequences of aging are preferentially manifested in interfibrillar mitochondria, in which a more pronounced reduction of the respiratory capacity has been described [26,122], as well as an increased amount of calcium precipitation in their matrix [123], and changes in the inner membrane lipid composition that favor unregulated ROS production at complex III associated with aging [19].

Interfibrillar mitochondria contribute more to the cellular ATP production due to higher respiratory capacity [117], and unlike subsarcolemmal mitochondria, are functionally and anatomically linked to the SR (10–50 nm interorganelle distance) through several bridging proteins [124]. This close communication allows a rapid and localized calcium exchange from SR to mitochondria, necessary for the coupling of mitochondrial energy production with the highly variable cardiac energy demand [106]. Importantly, SR-dependent mitochondrial calcium uptake also regulates the rate of GSH regeneration (see section 4.2) [125]. Therefore, adequate SR-mitochondria communication provides the basis for the balance between mitochondrial ROS generation and mitochondrial ROS scavenging capacity and contributes to the existence of spatially restricted ROS generation areas within the cells ("ROS microdomains") involved in cell signaling processes [126]. The fact that age-dependent functional decline preferentially impacts on interfibrillar mitochondria [26,127] has led to the assumption that SR may contribute to it. Previous studies have demonstrated that SR-mitochondria communication is partially disrupted in the cardiomyocytes of aging mice [26] resulting in a defective interorganelle calcium exchange and a concomitant mismatch between mitochondrial ROS production and GSH regeneration under energy demanding conditions [26]. More recently, glycation of ryanodine receptor 2 (RyR2), one of the proteins involved in SR-mitochondria bridging, has been proposed to mediate, at least in part, the functional mismatch between calcium homeostasis, energy coupling and antioxidant regeneration at the SR-mitochondria microdomains in the myocardium of aged mice and humans [128] by a mechanism involving an increased non-regulated SR-dependent calcium leak and a subsequent mitochondrial calcium accumulation. Such excess of mitochondrial calcium can, in turn, disrupt the reciprocal and necessary balance between ROS and calcium, following a positive feedback where more ROS production aggravates calcium dysregulation [128] (Fig. 1). In agreement with this, it has been described that excessive mitochondria-derived ROS production can contribute to aberrant calcium handling during aging through thiol oxidation of RyR [129]. Indeed, the pro-oxidative status present in the microenvironment around the SR-mitochondria contact sites has been linked to several age-related cardiac pathologies, including atrial fibrillation [130] and heart failure (HF) [131], and may have a role in IR injury, in which abnormal SR-dependent calcium oscillations have been shown to precipitate mPTP opening and cell death [132].

Overall, these observations support the concept that preservation of the functional coupling between SR and mitochondria might be an important biological target to reduce oxidative stress and maintain calcium homeostasis in aged cardiomyocytes.

4.2. ROS, calcium and mitochondrial bioenergetics

During physiological workload transitions, the heart needs to increase cardiac output to match demand, and this is accomplished by sympathetic activation through β-adrenergic receptor (β-AR) stimulation, which increases the amplitude and frequency of cytosolic calcium transients, resulting in more ATP hydrolysis at the myofilaments [133,134]. At the same time, the increased ADP flux to mitochondria accelerates the FoF1 ATP synthase to regenerate ATP, therefore, dissipating the proton-motive force. To counterbalance this, the ETC hastens the oxidation of NADH and $FADH_2$ and consequently, accelerates electron flux and O_2 consumption [135]. To match the elevated demand for NADH and $FADH_2$ during cardiac workload transition, the increase in cytosolic calcium facilitates mitochondrial calcium uptake via the mitochondrial calcium uniporter (MCU). In the mitochondrial matrix, calcium stimulates rate-limiting enzymes of the Krebs cycle: pyruvate dehydrogenase, IDH, and α-ketoglutarate [136–138]. This calcium-dependent stimulation accelerates the regeneration of NAD^+ to NADH, and FAD to $FADH_2$, and therefore, maintains their redox states

avoiding deprivation of reducing equivalents for ATP production.

In HF, defects in ion handling and pathological workload impose an elevated energetic demand, which can cause a mismatch between reducing equivalents production by the Krebs cycle (due to impaired mitochondrial calcium accumulation) and oxidation at the ETC. In turn, this imposed a pro-oxidative redox state that can compromise mitochondrial energetics, but also antioxidative capacity [92]. In this regard, experiments using C57BL/6 N mice demonstrated that in conditions of pathological metabolic demand (e.g. pathological elevation of afterload), impaired NADH regeneration by the Krebs cycle can be compensated by the reverse mode of the Nnt. The reversal mode of this enzyme consumes NAD^+ and NADPH to restore NADH and therefore, support ATP production. However, although this partially compensates for the elevated energetic demand, depletion of NADPH pool compromised NADPH-dependent antioxidative capacity [139]. Since the heart has a constant high ATP demand and high rate of ROS production, the concept of "redox-optimized ROS balance" by Aon and colleagues (2010) integrates, within a single framework, that the cellular redox environment is the main intermediary between mitochondrial energetics and ROS balance [92].

Using isolated cardiomyocytes and mitochondria from guinea pigs the authors suggested that in highly pro-oxidative conditions, such as pathological cardiac workload, NADH/NAD^+ redox couple pool becomes significantly oxidized and thereby compromises the mitochondrial antioxidative system, in agreement with the concept of reverse mode of the Nnt [139]. In highly pro-reductive conditions, such as cardiac ischemia, electron flux along the ETC becomes minimal, and the concentration of reduced NADH/NAD^+ pool increases. In this scenario, mitochondrial ROS production can outweigh the scavenging capacity, even at high level of NADPH, and therefore can cause ROS imbalance [92,140]. The authors postulate that "mitochondria have been evolutionarily optimized to maximize energy output while keeping ROS overflow to a minimum by operating in an intermediate redox state".

The importance of mitochondrial redox state was underscored in a different study using a guinea pig model of HF and sudden cardiac death. In this study, the authors demonstrated that preserving mitochondrial ROS balance by improving calcium-induced stimulation of the Krebs cycle and thereby, NAD(P)H/$NAD(P)^+$ redox state, abrogated oxidative stress during increased energy demand (e.g. workload transition). Besides, improving mitochondrial calcium accumulation could prevent pathological heart remodeling, and sudden cardiac death [141].

Understanding the fine-tuning regulation of mitochondrial energetics and redox state under oxidative stress has critical importance because of the increasing evidence demonstrating that age-related pathologies such as cardiovascular disease associate with the progressive deterioration of the redox state of cells and therefore, the organism [69,142,143]. The concept of redox-optimized ROS balance supports also the important role of mitochondrial calcium uptake and calcium-induced stimulation of Krebs cycle dehydrogenases to match energy supply, but also to sustain the regeneration of NADPH pool, and hence the main antioxidative system of the cell [26,136].

4.3. ROS and AGEs: the glycooxidative damage

The deleterious effects of oxidative stress on the development and progression of many cardiovascular diseases, including atherosclerosis and diabetic cardiovascular complications, have often been linked to the simultaneous accumulation of glycative post-translational modifications [144]. Glycation occurs when a sugar non-enzymatically reacts with an amine group of a protein to yield advanced glycation end products (AGEs), irreversible compounds that compromise the structure and function of intra- and extracellular proteins, in addition to binding their signal-transducing receptor (receptor for advanced glycation end products, RAGE) and causing a set of deleterious down-stream effects [145]. Accumulation of glycated and oxidized proteins is increased in the aging heart, and the generation of both AGEs and ROS are intertwined processes since many AGEs, including carboxymethyl lysine (CML) and pentosidine, are generated by a combination of oxidation and glycation [38,123,146]. It has been described that the production of ROS can lead to the generation of AGEs which, in turn, increases ROS production leading to a harmful positive feedback loop of malignant AGE and ROS accumulation known as glycoxidative stress [144,147]. Of note, during aging the heart decreases its metabolic capacity to oxidize fatty acids and becomes more reliant on glucose oxidation as a source of energy [1]. This metabolic shift leads to an increased generation of chemical intermediates (such as methylglyoxal) that favor protein glycation and exacerbate the oxidative stress present in aging.

In cultured neonatal rat cardiomyocytes and in H9c2 cardiomyocyte cell line, *in vitro* treatment with glycated albumin (AGE-BSA) led to the activation of a Nox2-containing NAPDH oxidase secondary to a protein kinase C-dependent translocation of RAC1 to the membrane, therefore stimulating ROS production in a manner independent of RAGE binding [147,148]. Other studies evidence the activation of NADPH through the binding of AGEs to their receptor RAGE; AGE-RAGE binding activates NOX and upregulates redox-sensitive transcription factor NF-κB, which consequently increases transcription and expression of RAGE and feeds the vicious cycle [144,146]. The use of a respiratory complex III inhibitor decreased ROS levels while AGE treatment increased ROS production in the mitochondrial compartment, which indicates a contribution of the mitochondria to AGE-induced ROS production [146,149].

AGEs may also increase ROS by overloading antioxidant systems, particularly GSH (Fig. 2). GSH plays several critical roles in cardiomyocytes: 1) it is the major intracellular ROS scavenger, 2) it is a cofactor of GPX, which is the most important enzyme for H_2O_2 detoxification, and 3) it is a cofactor of glyoxalase-1 (GLO1), the rate-limiting enzyme of the glyoxalase system that detoxifies major AGE precursors methylglyoxal and glyoxal. Therefore, depleting GSH stores will also trigger the vicious cycle of ROS and AGE generation [146]. It is important to remark that the myocardial concentration of GSH is reduced in the aged heart in several species, including humans [26,123]. GLO1 overexpression decreased both ROS and methylglyoxal-modified protein levels significantly in glucose treated cells, while GLO1 knockout increased RAGE expression as well as ROS and methylglyoxal modified proteins, which confirms again the relationship between AGEs and ROS [150]. Importantly, during aging glycative and oxidative damage is exacerbated by deficient GLO1 enzyme activity [123]. Antioxidant enzymes also include a group that can bind redox-active transition metals such as iron (Fe^{2+}) and cupper (Cu^+), which may otherwise lead to highly reactive hydroxyl radical production and accelerated AGE formation [146]. In addition, an early *in vitro* study disclosed that the incubation of anti-oxidants superoxide dismutase (SOD) and catalase with different sugars leads to the loss of antigenicity and their inactivation, pointing to a direct effect of AGEs on increased ROS accumulation [151].

Finally, accumulation of copious amount of oxidatively damaged proteins can overwhelm the proteasomal and lysosomal systems which are already deficient in the aged heart, therefore allowing for increased AGEs accumulation by saturating the apparatus accountable for elimination of damaged and dysfunctional proteins, and vice versa [13,146]. Although ROS have a short lifetime and can be removed by endogenous anti-oxidant enzymes, their harmful action is augmented by their role in increasing AGEs whose irreversible nature favors their long term accumulation and toxic consequences [144].

5. Contribution of ROS to IR injury

During acute myocardial infarction, cardiomyocyte death resulting from interrupted circulation and oxygen starvation can only be halted by restoring the blood flow to the area at risk, however, and paradoxically, this precise therapeutic maneuver produces additional cell death denominated as IR injury by a series of interrelated and/or independent

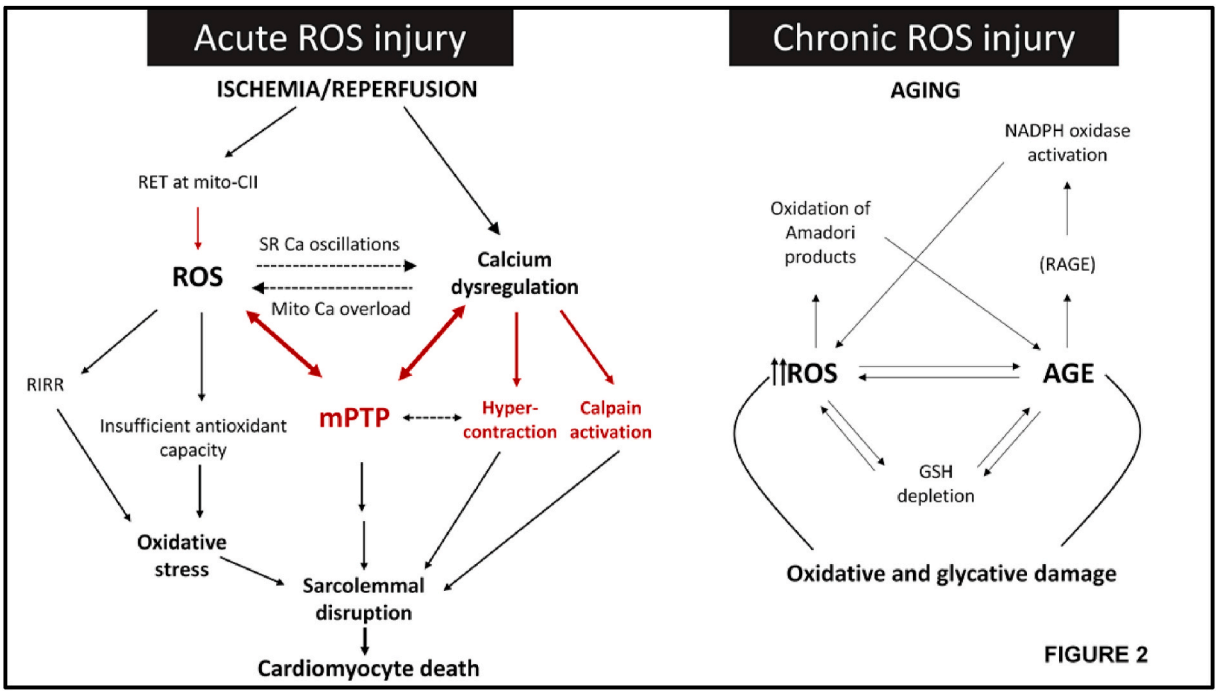

Fig. 2. Pathophysiological pathways involved in acute (myocardial IR injury) and chronic (aging) outcomes of altered mitochondrial ROS production. AGE: advanced glycation end products; GSH: glutathione; mPTP: mitochondrial permeability transition pore; RAGE: receptor for AGE; RET: reverse electron transport; RIRR: ROS-induced ROS release; SR: sarcoplasmic reticulum.

mechanisms that have been extensively reviewed elsewhere [152]. Solid evidence suggests that cytosolic calcium overload and concomitant calcium-dependent calpain activation are important triggers of cell fragility and hypercontraction that eventually lead to sarcolemmal rupture and necrosis during the first minutes of reperfusion [152]. Importantly, the occurrence of an abrupt mitochondrial failure due to sudden membrane permeabilization (mPTP opening) upon oxygen restoration further contributes to myocardial necrosis. Opening of mPTP uncouples mitochondrial respiration and causes drastic depolarization that culminates in an energetic collapse and cell death [16] (Fig. 2). Nevertheless, the relative contribution of hypercontraction, calpain activation and mPTP to the final extent of myocardial necrosis is a matter of debate and seems to depend on several factors, including the duration of the previous ischemic period [153], the degree of mitochondrial calcium concentration [154] and the rate of pH normalization [155] among others. ROS play a fundamental regulatory role in most of these events by several mechanisms.

Tissue reoxygenation refuels mitochondrial oxidative phosphorylation leading to massive ROS production which places mitochondria, specifically the ETC, as the major source of ROS during reperfusion in the highly metabolic heart [156]. Mitochondrial ROS produced by complexes I and III contribute to oxidative damage in IR injury [157], however an important role for ROS production has also been attributed to complex II both in forward mode and reverse mode through the reverse electron transport (RET) from complex II to I [158]. During the ischemic phase, SDH functions in reverse mode, producing succinate from fumarate thus leading to succinate accumulation, which upon reperfusion is rapidly re-oxidized by SDH initiating massive ROS generation by RET, where electrons are transported backward to complex I instead of forward to complex III [22,159]. Reversible inhibition of SDH by malonate has been shown to prevent succinate accumulation and ROS production, significantly reducing myocardial infarct size in both mouse and pig models subjected to IR injury [22,160]. Besides mitochondria-derived ROS, increased expression of NOX following reperfusion of the heart and decreased cell death in the presence of NOX inhibitors suggest a role for this enzyme in reperfusion injury as well [156]. Finally, ROS can itself activate other ROS producing enzymes or pathways which exacerbate oxidative damage. For instance, although hypoxia inhibitory factor-1α (HIF-1α) exerts multiple cardioprotective effects, like regulation of angiogenesis and redox homeostasis [161,162], in certain conditions (including aging) its role is more ambiguous, and a study demonstrated that the stimulation of HIF-1α production by ROS can activate NOX, leading to a positive feedback loop [163]; also tetrahydrobiopterin (BH4), the nitric oxide synthase (NOS) cofactor, can become oxidized leading to reduced BH4 levels and the consequent uncoupling of NOS which switches to the production of $O_2^{\bullet-}$ instead of nitric oxide (NO) [164].

ROS have also been found to participate in cell death during IR injury by mechanisms other than direct oxidative damage on proteins. Accumulating evidence indicates that ROS are one of the main triggers of mPTP during IR; in fact, part of the protection offered upon the reversible inhibition of SDH by malonate is due to decreased mPTP opening upon reduced ROS production [160]. The diminished $\Delta\Psi m$ secondary to mPTP further increases ROS production by the ETC, which are released into the cytosol where they trigger RIRR in adjacent mitochondria [50]. It is important to mention however, that beyond their role as drivers of mPTP opening, ROS can increase secondary to mPTP, as suggested by studies using Langendorff-perfused rat hearts subjected to transient IR [165], in which reperfusion-induced ROS production could only be detected after the occurrence of mPTP. ROS can also exacerbate calcium deregulation during IR by altering intracellular calcium handling properties. In fact, a mutual interplay between calcium and ROS signaling pathways exists such that on the one hand ROS can modulate redox-sensitive calcium channels and ATPases activities leading to further increase in intracellular calcium levels and aggravation of calcium deregulation, while on the other hand calcium can induce the activity of ROS-producing enzymes and increase mitochondrial ROS generation, therefore initiating a self-amplifying loop [128]. Heterogeneously damaged mitochondria within the same cell may contribute to damage through this calcium-ROS amplification loop. This hypothesis was tested in rat cardiomyocytes exposed to controlled ROS damage (laser irradiation), in which ROS-mediated opening of mPTP in

severely damaged mitochondria coexisted with energy production in intact viable mitochondria within the same cell [166]. In this experiment, mPTP opening in locally-restricted areas induced cell hypercontraction through SR-driven ATP-dependent calcium oscillations, in a way that closely resembles IR-induced cardiomyocyte death, as observed in the histopathological pattern of reperfused myocardial infarctions [17].

Although there is little data in cardiomyocytes, studies in other cell types have suggested a possible role for ROS in modifying the activity of calpains; whereas *S*-nitrosylation of calpains seems to inhibit their activity [167], the oxidative modification of their substrates seems to increase the susceptibility of the latter to calpain proteolysis [168]. Calpains, a family of calcium-dependent cysteine proteases are implicated in IR injury by degrading a wide array of proteins including sarcolemmal, myofibrillar, mitochondrial and other regulatory proteins [167]. Besides, oxidative stress-induced increase in intracellular calcium levels in the heart was proposed to be an indirect stimulus for calcium-dependent calpain activation [168].

It is well known that ROS are increased in aging as evidenced by increased levels of carbonyl formation on proteins, lipofuscin, and oxidatively damaged DNA among others [169], leading to impaired structure and function of the targeted molecules. In addition, antioxidant defenses in the aged heart decrease with a parallel increase in the resistance of highly oxidized proteins to proteolytic elimination and a less efficient autophagy, all factors contributing to reduced clearance of oxidatively damaged proteins [13]. The increase in ROS production/accumulation, mitochondrial calcium load and AGEs formation are likely to contribute to the reduced IR threshold consistently observed in the aged hearts. Whether these factors are related to the reduced efficiency of some cardioprotective interventions observed during aging remains unknown and deserves further investigation.

6. ROS in heart failure: a vicious cycle

HF is a leading cause of morbidity and mortality in developed countries [170], and despite advances in therapy, it still carries a threatening prognosis and a significant socioeconomic burden, mainly because of the aging of the population, and the constant increase in the prevalence of HF among elderly people [171,172]. Extensive research in humans and animal models has provided substantial evidence of increased ROS production in HF, which contributes to disease progression [82]. Moreover, ROS imbalance was confirmed in human ventricular myocardium explanted from patients with dilated cardiomyopathy. Using electro-paramagnetic resonance (EPR), the authors reported augmented mitochondrial ROS production and increased myocardial oxidative stress. Besides, enzyme activity assays revealed decreased activity of MnSOD, the main antioxidant enzyme that scavenges $O_2^{\bullet-}$ in the mitochondrial matrix [173]. The importance of the MnSOD-linked antioxidative capacity was also confirmed using transgenic mouse models lacking MnSOD (*Sod 2*). In this study, the authors showed that MnSOD mutant mice exhibited fatal dilated cardiomyopathy and HF, accompanied by severe DNA and mitochondrial oxidative damage, as well as decreased energy production. Moreover, mutant mice were significantly sensitive to hyperoxia. The authors demonstrated that the addition of exogenous ROS scavengers suppressed mitochondrial defects and attenuated cardiac remodeling. The phenotype of the MnSOD knockout mouse underscores the importance of mitochondria as a source of $O_2^{\bullet-}$ in the heart. Furthermore, these studies support the causal role of ROS imbalance in the pathogenesis of HF, and provides mechanistic insight into the potentially beneficial effects of antioxidants [174,175]. Further evidence was recently provided using a guinea pig model of nonischemic HF [176]. In this study, a mitochondria-targeted ROS sensor revealed increased mitochondrial ROS production in resting and contracting left ventricular myocytes from failing hearts. The authors suggested that ROS imbalance disrupt optimal redox signaling that drives gene expression of genes involved in mitochondrial function, ion handling, and mitochondrial function.

Cardiac excitation-contraction coupling (EC coupling) is the process in which an electrical impulse (i.e. action potential, AP) traveling along the sarcolemma of the cardiomyocyte is transduced into a mechanical response (cell contraction) [177]. During this process, the depolarization of the sarcolemmal membrane triggers the opening of voltage-gated calcium (Ca^{2+}) channels (L-type Ca^{2+} channels), producing an influx of Ca^{2+} into the cytosol of the cardiomyocyte. Here, Ca^{2+} triggers further Ca^{2+} release from the SR via Ca^{2+} release channels (RyR2), a process termed "Ca^{2+}-induced Ca^{2+} release" [178]. Subsequently, Ca^{2+} can interact with troponin C at myofilaments and causes the shortening of the cell during systole. During diastole, Ca^{2+} is removed from the cytosol by different mechanism including the SR Ca^{2+}-ATPase (SERCA), sarcolemmal sodium/Ca^{2+} exchanger (NCX) and sarcolemmal Ca^{2+} ATPase [179]. During each cytosolic Ca^{2+} transient, Ca^{2+} is taken up by mitochondria via the mitochondrial Ca^{2+} uniporter (MCU), however, this uptake is considered rather inconsequential for cytosolic Ca^{2+} transients, though this view is still to some extent controversial [47,180,181].

ROS can affect EC coupling by direct oxidative modification of molecules involved in ion handling or indirectly by post-translational oxidative modification of redox-sensitive kinases [182,183]. For example, oxidative activation of protein kinase A (PKA) can produce hyper-phosphorylation of L-type calcium channels and SR calcium release channels (RyR2), resulting in disturbed cytosolic calcium handling. In turn, this can favor arrhythmias and contributes to myocardial disease progression [184,185]. Another redox-sensitive kinase involved in EC coupling and HF is calcium/calmodulin-dependent protein kinase II (CaMKII). CaMKII can undergo oxidation, resulting in permanent activation even after dissociation of calcium/calmodulin [186]. CaMKII is an important regulator of proteins involved in sodium and calcium handling. For instance, CaMKII can phosphorylate voltage-gated sodium-channels [187], producing an increase in the late sodium current (I_{Na}) and intracellular sodium levels in cardiac myocytes. Together with ROS-induced inhibition of the sodium-potassium ATPase (NKA), the primary sodium-extrusion mechanism of the cell, both account for increased intracellular sodium observed in the human failing heart [188]. On one hand, cytosolic sodium accumulation can activate the reverse-mode of the sodium/calcium exchanger (NCX) during the action potential [189], increasing cytosolic calcium concentrations and blunting efficient mitochondrial calcium uptake [137]. On the other hand, elevated cytosolic sodium can also reduce mitochondrial calcium content by accelerating calcium efflux trough the mitochondrial sodium/calcium exchanger [81].

The role of NCX-mediated calcium influx in failing hearts was confirmed using guinea-pig cardiomyocytes. In this study, the authors demonstrated that increased contribution of the NCX to cytosolic calcium transients impairs mitochondrial calcium uptake and the bioenergetic feedback response. Increased NCX-mediated calcium influx poses an energetic demand on the cell that may not be matched efficiently by mitochondrial calcium uptake and subsequent stimulation of Krebs cycle dehydrogenases [137]. Because the redox state of NADH is closely linked to NADPH, which is required for the antioxidative capacity of the matrix, reduced mitochondrial calcium uptake in failing myocytes increases ROS formation. In turn, oxidative stress promotes an increase in intracellular sodium, resulting in further NCX-mediated calcium influx [190] and therefore, can initiate a vicious cycle of defective EC coupling, reduced mitochondrial calcium uptake, energetic deficit, and further oxidative stress.

Elevated ROS can also activate hypertrophic signaling through direct oxidation of histone deacetylase 4 (HDAC4) [191], resulting in nuclear export and de-suppression of gene transcription factors involved in cardiac hypertrophy such as the myocyte enhancer factor 2 (MEF2) and calcineurin-NFAT [191,192]. Chronic exposure to different agonists including angiotensin II (Ang-II) and endothelin-1 can also activate the hypertrophic program through activation NADPH-mediated ROS

production [193].

Taken together, in HF, ROS imbalance can affect EC coupling and mitochondrial bioenergetics, leading to a vicious cycle that includes oxidative modification of redox-sensitive kinases, cytosolic sodium accumulation, and further mitochondrial ROS emission. In turn, this can trigger apoptotic signaling, arrhythmias and contributes to cardiac remodeling by post-translational oxidative modification of HDAC4 and disinhibition of pro-hypertrophic gene transcription [194,195].

7. Mitochondria-directed antioxidant therapies in aging

The physiological process of aging begins in early adulthood. Although most of the studies pointed out the contribution of intrinsic thermodynamic instability of most complex biological molecules as the fundamental cause of aging, the precise mechanisms are not known yet. Experimental studies indicate an increased production of mitochondrial ROS in the aged rat heart in parallel with more disorganized and fragmented mitochondria and a concomitant decrease in ATP levels, mitochondrial membrane depolarization and alteration of mitochondrial dynamics, along with mitochondrial and cytosolic calcium overload [196]. These and other similar observations have provided strong evidence that age-associated increase in mitochondrial ROS may underlie mitochondrial defects in aged cardiomyocytes and be translated into organ functional insufficiencies. Therefore, the crosstalk between mitochondrial ROS, redox balance, and other cellular signaling pathways plays an important role in aging-associated functional deficiencies [197,198]. Consequently, a specific and selective inhibition of unregulated mitochondrial ROS production seems a promising therapeutic strategy for a healthier aging.

The most common approach for reducing mitochondrial ROS is based on the delivery of known redox agents including MitoQ and SkQ1 to the mitochondrial matrix by their conjugation to delocalized cations [71]. The Szeto-Schiller peptides represent a different chemical approach to reduce mitochondrial ROS via their targeting to cardiolipin on the inner mitochondrial membrane [199,200]. It has been shown that mitochondria-targeted redox agents may also modulate electron flux in the ETC, increase ATP production and preserve mitochondrial structure while reducing electron-leak and ROS production [71,199,200]. Such mitochondria-targeted antioxidants have been developed by conjugating the lipophilic triphenylphosphonium (TPP) cation to an antioxidant moiety, such as ubiquinol or alpha-tocopherol [201]. The positive charge of the TPP component enables the antioxidant to enter the mitochondria following the electrochemical gradient. However, in certain conditions, this type of chemical agents can induce unexpected side effects due to their TPP moiety, which otherwise is essential to facilitate their accumulation inside the mitochondria. Thus, both MitoQ and SkQ1 were reported to impair mitochondrial bioenergetics [202] because their TPP moiety reduced oxidative phosphorylation and ATP production [203]. However, and overall, the effects of these antioxidants shift more towards their beneficial outcomes, and their protective effects have been well documented in several contexts. During heart preservation before cardiac transplant, the addition of MitoQ to the storage solution decreased oxidative damage associate with IR injury in the donor mouse hearts [204], which in turn resulted in an attenuated innate immune response in the recipient mice [204].

Among mitochondria-targeted antioxidants, MitoSOD, MitoTEMPO, MitoPeroxidase, and MitoE2 are scavengers of $O_2^{\bullet -}$ and H_2O_2, and prevent lipid peroxidation, respectively [201]. These compounds cross easily through all biological membranes and thus can accumulate within mitochondria [201]. In agreement with this, MitoTEMPO treatment of heart tissue from aged rats induced significant structural and functional improvements, including attenuated cardiac contractile dysfunction, mostly due to reduced ROS production in the left ventricular cardiomyocytes [205]. In an experimental context of prolonged limb ischemia, infusion with MitoTEMPO significantly improved the recovery of regional blood flow in aged mice subjected to femoral artery ligation [206]. In this study, scavenging of mitochondrial ROS attenuated mtDNA damage, improved the growth of collateral vessels and preserved respiration in the mitochondria of skeletal muscles. Through its mitochondrial ROS scavenging properties, MitoTEMPO treatment has also been reported to promote autophagy and to prevent arrhythmic disturbances by improving aberrant SR-dependent calcium handling in cardiomyocytes from aged rabbits [207]. Another recent study has provided evidence supporting the concept that attenuation of mitochondrial ROS production in aged cardiomyocytes by MitoTEMPO treatment may have beneficial antiarrhythmic effects through the preservation of calcium homeostasis [208]. MitoTEMPO has also been shown to be protective against doxorubicin-induced cardiotoxicity and the complications of diabetic cardiomyopathy [209]. Moreover, attenuation of excessive mitochondrial ROS production with MitoTEMPO has proven to reduce aging-associated vascular dysfunction and atherosclerosis secondary to hyperlipidemia [210].

A different approach is based on targeting inducible proteins whose activation produces cytoprotective effects. Thus, activation of several oxidant sensors and their associated pathways, such as the Nrf2 pathway and the NF-κB/REL family of transcription factors, has been proposed as an indirect antioxidant therapy [211]. Indeed, Nrf2 can accumulate rapidly in the nucleus where it *trans*-activates the antioxidant response element in the promoter region of many antioxidant genes [212]. NF-κB targets genes include ferritin heavy chain and SOD2 [213,214]. Other innovative therapeutic strategies to reduce the endogenous oxidative damage are based on targeting long non-coding RNAs (lncRNAs), which play important roles in molecular processes responsible for the aging phenotype, like deficient proteostasis and autophagy [215]. In a mouse model of HF, MitoQ treatment preserved Mfn-2 expression through its effect on lncRNAs and alleviated the adverse remodeling of mitochondrial ultrastructure/network usually present in failing cardiomyocytes, while reducing oxidative stress [216]. Another recently tested strategy reported to promote cytoprotection was based on the forced co-expression of the anti-senescent protein telomerase reverse transcriptase and the anti-apoptotic transcription factor myocardin in aged murine adipose-derived mesenchymal stromal cells [217,218]. This intervention improved the persistence of the transplanted cells and enhanced tissue repair after stem cell therapy [217,218].

The effects of antioxidant supplementation on the context of aging, either by preventing the molecular damage, alleviating its consequences or both is a matter of debate [219–221]. An important limitation for an adequate interpretation of the results in this field is that a great number of experimental models testing the benefits of exogenous antioxidants were focused on their potential effects on lifespan extension rather than on their efficiency to prevent aging-related diseases [222]. Moreover, and although substantial evidence supports the central role of mitochondrial oxidative stress in aging, ROS also have important signaling properties involved in the control of cellular homeostasis, and therefore caution must be taken when supplementing with antioxidants. There are various issues that should be addressed before translating these therapeutic strategies to clinical trials, including a better understanding of the most relevant oxidation pathways in the aging process, the selection of reliable markers of the oxidative and antioxidative status both in the organism and cells, the identification of the possible pro-oxidant effect of certain antioxidants and the discrimination of the source of intracellular ROS (cytosolic or mitochondrial), among others. For the latter, the use of specific, sensitive and reproducible methods is imperative. A number of different methodologies have been developed for the detection of total and mitochondrial ROS production. However, these methods tend to be unspecific, are subject to artifacts and are not infallible in determining the exact subcellular localization [223]. Early methods included cytochrome *c* reduction and spin trapping, however, those were not sensitive to low levels of ROS and required the use of highly toxic detection chemicals [224]. More recently, several safer and more selective methods based on fluorescent probes, electron-spin resonance, immunoassays, HPLC superoxide detection and

immune-spin trapping have been developed [223]. Nevertheless, the translation of these methods into biomedical applications is still missing, thus limiting their widespread use in the clinical setting, which contributes to the relatively low number of clinical studies in the field [225,226].

8. Concluding remarks

In cardiomyocytes, adequate mitochondrial ROS balance is involved in several protective signaling pathways and contributes to the maintenance of tissue homeostasis through the regulation of cell quality control processes (i.e., autophagy). When the redox balance of the cells is compromised, increased ROS production can cause oxidative damage to biological molecules and have detrimental functional consequences. During aging, unregulated mitochondrial ROS production (either by an excessive generation or by an insufficient antioxidant capacity) has been consistently documented in different tissues and organs, including the heart, in which oxidative stress has been shown to contribute to the pathophysiology of several aging-related cardiac diseases, particularly, HF, IR injury and arrhythmias. The rates of mitochondrial ROS production and elimination vary depending on the localization and the biochemical properties of the specific mitochondrial subcellular population, their adequate interaction with other organelles (i.e., SR) and the interrelation of mitochondrial ROS with other cellular chemical reactions, signaling molecules and metabolic state. Treatment of cardiovascular diseases with general ROS scavengers has largely failed, both in preclinical and clinical settings. The reasons for such failure may be multiple, including the fact that this type of approach does not necessarily target pathological ROS, therefore interfering with otherwise essential physiological processes. Targeting ROS imbalance at a particular cell source, like mitochondria, may help overcome some of these limitations. Still, for a therapeutic benefit, the development of mitochondria-targeted antioxidants must be based on a thorough understanding of the complex and dynamic mitochondrial biology throughout life.

Declaration of competing interest

The authors declare no conflict of interest.

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References

[1] E.J. Lesnefsky, Q. Chen, C.L. Hoppel, Mitochondrial metabolism in aging heart, Circ. Res. 118 (2016) 1593–1611, https://doi.org/10.1161/CIRCRESAHA.116.307505.

[2] H. D, The biologic clock: the mitochondria? J. Am. Geriatr. Soc. 20 (1972) 145–147, https://doi.org/10.1111/j.1532-5415.1972.tb00787.x.

[3] A. Trifunovic, A. Wredenberg, M. Falkenberg, J.N. Spelbrink, A.T. Rovio, C. E. Bruder, M. Bohlooly-Y, S. Gidlöf, A. Oldfors, R. Wibom, J. Törnell, H.T. Jacobs, N.-G. Larsson, Premature ageing in mice expressing defective mitochondrial DNA polymerase, Nature 429 (2004) 417–423, https://doi.org/10.1038/nature02517.

[4] G.C. Kujoth, A. Hiona, T.D. Pugh, S. Someya, K. Panzer, S.E. Wohlgemuth, T. Hofer, A.Y. Seo, R. Sullivan, W.A. Jobling, J.D. Morrow, H. Van Remmen, J. M. Sedivy, T. Yamasoba, M. Tanokura, R. Weindruch, C. Leeuwenburgh, T. A. Prolla, Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging, Science 309 (2005) 481–484, https://doi.org/10.1126/science.1112125.

[5] D.-F. Dai, L.F. Santana, M. Vermulst, D.M. Tomazela, M.J. Emond, M.J. MacCoss, K. Gollahon, G.M. Martin, L.A. Loeb, W.C. Ladiges, P.S. Rabinovitch, Overexpression of catalase targeted to mitochondria attenuates murine cardiac aging, Circulation 119 (2009) 2789–2797, https://doi.org/10.1161/CIRCULATIONAHA.108.822403.

[6] E. Migliaccio, M. Giorgio, S. Mele, G. Pelicci, P. Reboldi, P.P. Pandolfi, L. Lanfrancone, P.G. Pelicci, The p66shc adaptor protein controls oxidative stress response and life span in mammals, Nature 402 (1999) 309–313, https://doi.org/10.1038/46311.

[7] M. Ristow, Unraveling the truth about antioxidants: mitohormesis explains ROS-induced health benefits, Nat. Med. 20 (2014) 709–711, https://doi.org/10.1038/nm.3624.

[8] A. Sanz, Mitochondrial reactive oxygen species: do they extend or shorten animal lifespan? Biochim. Biophys. Acta 1857 (2016) 1116–1126, https://doi.org/10.1016/j.bbabio.2016.03.018.

[9] D.J. Hausenloy, J.A. Barrabes, H.E. Bøtker, S.M. Davidson, F. Di Lisa, J. Downey, T. Engstrom, P. Ferdinandy, H.A. Carbrera-Fuentes, G. Heusch, B. Ibanez, E. K. Iliodromitis, J. Inserte, R. Jennings, N. Kalia, R. Kharbanda, S. Lecour, M. Marber, T. Miura, M. Ovize, M.A. Perez-Pinzon, H.M. Piper, K. Przyklenk, M. R. Schmidt, A. Redington, M. Ruiz-Meana, G. Vilahur, J. Vinten-Johansen, D. M. Yellon, D. Garcia-Dorado, Ischaemic conditioning and targeting reperfusion injury: a 30 year voyage of discovery, Basic Res. Cardiol. 111 (2016) 70, https://doi.org/10.1007/s00395-016-0588-8.

[10] M. Tanaka, H. Fujiwara, K. Yamasaki, S. Sasayama, Superoxide dismutase and N-2-mercaptopropionyl glycine attenuate infarct size limitation effect of ischaemic preconditioning in the rabbit, Cardiovasc. Res. 28 (1994) 980–986, https://doi.org/10.1093/cvr/28.7.980.

[11] S.P. Fortmann, B.U. Burda, C.A. Senger, J.S. Lin, E.P. Whitlock, Vitamin and mineral supplements in the primary prevention of cardiovascular disease and cancer: an updated systematic evidence review for the U.S. Preventive Services Task Force, Ann. Intern. Med. 159 (2013) 824–834, https://doi.org/10.7326/0003-4819-159-12-201312170-00729.

[12] C. López-Otín, M.A. Blasco, L. Partridge, M. Serrano, G. Kroemer, The hallmarks of aging, Cell 153 (2013) 1194–1217, https://doi.org/10.1016/j.cell.2013.05.039.

[13] M. Ruiz-Meana, D. Bou-Teen, P. Ferdinandy, M. Gyongyosi, M. Pesce, C. Perrino, R. Schulz, J.P.G. Sluijter, C.G. Tocchetti, T. Thum, R. Madonna, Cardiomyocyte ageing and cardioprotection: consensus document from the ESC working groups cell biology of the heart and myocardial function, Cardiovasc. Res. 116 (2020) 1835–1849, https://doi.org/10.1093/cvr/cvaa132.

[14] J.B. Strait, E.G. Lakatta, Aging-associated cardiovascular changes and their relationship to heart failure, Heart Fail. Clin. 8 (2012) 143–164, https://doi.org/10.1016/j.hfc.2011.08.011.

[15] H. Morita, I. Komuro, Heart failure as an aging-related phenotype, Int. Heart J. 59 (2018) 6–13, https://doi.org/10.1536/ihj.17-519.

[16] C. Fernandez-Sanz, M. Ruiz-Meana, J. Castellano, E. Miro-Casas, E. Nuñez, J. Inserte, J. Vázquez, D. Garcia-Dorado, Altered FoF1 ATP synthase and susceptibility to mitochondrial permeability transition pore during ischaemia and reperfusion in aging cardiomyocytes, Thromb. Haemostasis 113 (2015) 441–451, https://doi.org/10.1160/TH14-10-0901.

[17] H.M. Piper, D. García-Dorado, M. Ovize, A fresh look at reperfusion injury, Cardiovasc. Res. 38 (1998) 291–300, https://doi.org/10.1016/s0008-6363(98)00033-9.

[18] M. Ruiz-Meana, K. Boengler, D. Garcia-Dorado, D.J. Hausenloy, T. Kaambre, G. Kararigas, C. Perrino, R. Schulz, K. Ytrehus, Ageing, sex, and cardioprotection, Br. J. Pharmacol. 177 (2019) 5270–5286, https://doi.org/10.1111/bph.14951.

[19] E.J. Lesnefsky, C.L. Hoppel, Ischemia-reperfusion injury in the aged heart: role of mitochondria, Arch. Biochem. Biophys. 420 (2003) 287–297, https://doi.org/10.1016/j.abb.2003.09.046.

[20] E. Murphy, Primary and secondary signaling pathways in early preconditioning that converge on the mitochondria to produce cardioprotection, Circ. Res. 94 (2004) 7–16, https://doi.org/10.1161/01.RES.0000108082.76667.F4.

[21] S.M. Davidson, A. Adameová, L. Barile, H.A. Cabrera-Fuentes, A. Lazou, P. Pagliaro, K.-O. Stensløkken, D. Garcia-Dorado, EU-CARDIOPROTECTION COST Action (CA16225), Mitochondrial and mitochondrial-independent pathways of myocardial cell death during ischaemia and reperfusion injury, J. Cell Mol. Med. 24 (2020) 3795–3806, https://doi.org/10.1111/jcmm.15127.

[22] L. Valls-Lacalle, I. Barba, E. Miró-Casas, M. Ruiz-Meana, A. Rodríguez-Sinovas, D. García-Dorado, Selective inhibition of succinate dehydrogenase in reperfused myocardium with intracoronary malonate reduces infarct size, Sci. Rep. 8 (2018) 2442–2452, https://doi.org/10.1038/s41598-018-20866-4.

[23] J. Zhang, D. Ren, J. Fedorova, Z. He, J. Li, SIRT1/SIRT3 modulates redox homeostasis during ischemia/reperfusion in the aging heart, Antioxidants 9 (2020) 858–874, https://doi.org/10.3390/antiox9090858.

[24] I. Andreadou, R. Schulz, A. Papapetropoulos, B. Turan, K. Ytrehus, P. Ferdinandy, A. Daiber, F. Di Lisa, The role of mitochondrial reactive oxygen species, NO and H2 S in ischaemia/reperfusion injury and cardioprotection, J. Cell Mol. Med. 24 (2020) 6510–6522, https://doi.org/10.1111/jcmm.15279.

[25] E.L. Holmuhamedov, A. Oberlin, K. Short, A. Terzic, A. Jahangir, Cardiac subsarcolemmal and interfibrillar mitochondria display distinct responsiveness to protection by diazoxide, PloS One 7 (2012), e44667, https://doi.org/10.1371/journal.pone.0044667.

[26] C. Fernandez-Sanz, M. Ruiz-Meana, E. Miro-Casas, E. Nuñez, J. Castellano, M. Loureiro, I. Barba, M. Poncelas, A. Rodriguez-Sinovas, J. Vázquez, D. Garcia-Dorado, Defective sarcoplasmic reticulum-mitochondria calcium exchange in aged mouse myocardium, Cell Death Dis. 5 (2014) e1573, https://doi.org/10.1038/cddis.2014.526.

[27] H.E. Bøtker, H.A. Cabrera-Fuentes, M. Ruiz-Meana, G. Heusch, M. Ovize, Translational issues for mitoprotective agents as adjunct to reperfusion therapy in

patients with ST-segment elevation myocardial infarction, J. Cell Mol. Med. 24 (2020) 2717–2729, https://doi.org/10.1111/jcmm.14953.
[28] A. Szewczyk, L. Wojtczak, Mitochondria as a pharmacological target, Pharmacol. Rev. 54 (2002) 101–127, https://doi.org/10.1124/pr.54.1.101.
[29] M. Zhang, A. Perino, A. Ghigo, E. Hirsch, A.M. Shah, NADPH oxidases in heart failure: poachers or gamekeepers? Antioxidants Redox Signal. 18 (2013) 1024–1041, https://doi.org/10.1089/ars.2012.4550.
[30] N.D. Roe, J. Ren, Nitric oxide synthase uncoupling: a therapeutic target in cardiovascular diseases, Vasc. Pharmacol. 57 (2012) 168–172, https://doi.org/10.1016/j.vph.2012.02.004.
[31] U.E. Ekelund, R.W. Harrison, O. Shokek, R.N. Thakkar, R.S. Tunin, H. Senzaki, D. A. Kass, E. Marbán, J.M. Hare, Intravenous allopurinol decreases myocardial oxygen consumption and increases mechanical efficiency in dogs with pacing-induced heart failure, Circ. Res. 85 (1999) 437–445, https://doi.org/10.1161/01.res.85.5.437.
[32] N. Kaludercic, A. Carpi, T. Nagayama, V. Sivakumaran, G. Zhu, E.W. Lai, D. Bedja, A. De Mario, K. Chen, K.L. Gabrielson, M.L. Lindsey, K. Pacak, E. Takimoto, J.C. Shih, D.A. Kass, F. Di Lisa, N. Paolocci, Monoamine oxidase B prompts mitochondrial and cardiac dysfunction in pressure overloaded hearts, Antioxidants Redox Signal. 20 (2014) 267–280, https://doi.org/10.1089/ars.2012.4616.
[33] N. Kaludercic, J. Mialet-Perez, N. Paolocci, A. Parini, F. Di Lisa, Monoamine oxidases as sources of oxidants in the heart, J. Mol. Cell. Cardiol. 73 (2014) 34–42, https://doi.org/10.1016/j.yjmcc.2013.12.032.
[34] A. Maurel, C. Hernandez, O. Kunduzova, G. Bompart, C. Cambon, A. Parini, B. Francés, Age-dependent increase in hydrogen peroxide production by cardiac monoamine oxidase A in rats, Am. J. Physiol. Heart Circ. Physiol. 284 (2003) H1460–H1467, https://doi.org/10.1152/ajpheart.00700.2002.
[35] N. Manzella, Y. Santin, D. Maggiorani, H. Martini, V. Douin-Echinard, J.F. Passos, F. Lezoualc'h, C. Binda, A. Parini, J. Mialet-Perez, Monoamine oxidase-A is a novel driver of stress-induced premature senescence through inhibition of parkin-mediated mitophagy, Aging Cell 17 (2018), e12811, https://doi.org/10.1111/acel.12811.
[36] J.F. Turrens, Mitochondrial formation of reactive oxygen species, J. Physiol. 552 (2003) 335–344, https://doi.org/10.1113/jphysiol.2003.049478.
[37] A. Nickel, M. Kohlhaas, C. Maack, Mitochondrial reactive oxygen species production and elimination, J. Mol. Cell. Cardiol. 73 (2014) 26–33, https://doi.org/10.1016/j.yjmcc.2014.03.011.
[38] O.S. Kornfeld, S. Hwang, M.-H. Disatnik, C.-H. Chen, N. Qvit, D. Mochly-Rosen, Mitochondrial reactive oxygen species at the heart of the matter: new therapeutic approaches for cardiovascular diseases, Circ. Res. 116 (2015) 1783–1799, https://doi.org/10.1161/CIRCRESAHA.116.305432.
[39] G.C. Brown, V. Borutaite, There is no evidence that mitochondria are the main source of reactive oxygen species in mammalian cells, Mitochondrion 12 (2012) 1–4, https://doi.org/10.1016/j.mito.2011.02.001.
[40] S. Dröse, U. Brandt, The mechanism of mitochondrial superoxide production by the cytochrome bc1 complex, J. Biol. Chem. 283 (2008) 21649–21654, https://doi.org/10.1074/jbc.M803236200.
[41] L. Bleier, I. Wittig, H. Heide, M. Steger, U. Brandt, S. Dröse, Generator-specific targets of mitochondrial reactive oxygen species, Free Radic. Biol. Med. 78 (2015) 1–10, https://doi.org/10.1016/j.freeradbiomed.2014.10.511.
[42] M.P. Murphy, How mitochondria produce reactive oxygen species, Biochem. J. 417 (2009) 1–13, https://doi.org/10.1042/BJ20081386.
[43] R.S. Balaban, S. Nemoto, T. Finkel, Mitochondria, oxidants, and aging, Cell 120 (2005) 483–495, https://doi.org/10.1016/j.cell.2005.02.001.
[44] M.M. Sedensky, P.G. Morgan, Mitochondrial respiration and reactive oxygen species in mitochondrial aging mutants, Exp. Gerontol. 41 (2006) 237–245, https://doi.org/10.1016/j.exger.2006.01.004.
[45] J. Galino, M. Ruiz, S. Fourcade, A. Schlüter, J. López-Erauskin, C. Guilera, M. Jove, A. Naudi, E. García-Arumí, A.L. Andreu, A.A. Starkov, R. Pamplona, I. Ferrer, M. Portero-Otin, A. Pujol, Oxidative damage compromises energy metabolism in the axonal degeneration mouse model of X-adrenoleukodystrophy, Antioxidants Redox Signal. 15 (2011) 2095–2107, https://doi.org/10.1089/ars.2010.3877.
[46] M.D. Brand, The sites and topology of mitochondrial superoxide production, Exp. Gerontol. 45 (2010) 466–472, https://doi.org/10.1016/j.exger.2010.01.003.
[47] E. Bertero, C. Maack, Calcium signaling and reactive oxygen species in mitochondria, Circ. Res. 122 (2018) 1460–1478, https://doi.org/10.1161/CIRCRESAHA.118.310082.
[48] L. Zhou, M.A. Aon, T. Almas, S. Cortassa, R.L. Winslow, B. O'Rourke, A reaction-diffusion model of ROS-induced ROS release in a mitochondrial network, PLoS Comput. Biol. 6 (2010), e1000657, https://doi.org/10.1371/journal.pcbi.1000657.
[49] M.A. Aon, S. Cortassa, E. Marbán, B. O'Rourke, Synchronized whole cell oscillations in mitochondrial metabolism triggered by a local release of reactive oxygen species in cardiac myocytes, J. Biol. Chem. 278 (2003) 44735–44744, https://doi.org/10.1074/jbc.M302673200.
[50] D.B. Zorov, C.R. Filburn, L.O. Klotz, J.L. Zweier, S.J. Sollott, Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes, J. Exp. Med. 192 (2000) 1001–1014, https://doi.org/10.1084/jem.192.7.1001.
[51] H.T.F. Facundo, J.G. de Paula, A.J. Kowaltowski, Mitochondrial ATP-sensitive K+ channels prevent oxidative stress, permeability transition and cell death, J. Bioenerg. Biomembr. 37 (2005) 75–82, https://doi.org/10.1007/s10863-005-4130-1.
[52] H. Cui, Y. Kong, H. Zhang, Oxidative stress, mitochondrial dysfunction, and aging, J. Signal Transduct. (2012) 646354, https://doi.org/10.1155/2012/646354.
[53] A.-L. Bulteau, L.I. Szweda, B. Friguet, Mitochondrial protein oxidation and degradation in response to oxidative stress and aging, Exp. Gerontol. 41 (2006) 653–657, https://doi.org/10.1016/j.exger.2006.03.013.
[54] H.-S. Wong, P.A. Dighe, V. Mezera, P.-A. Monternier, M.D. Brand, Production of superoxide and hydrogen peroxide from specific mitochondrial sites under different bioenergetic conditions, J. Biol. Chem. 292 (2017) 16804–16809, https://doi.org/10.1074/jbc.R117.789271.
[55] P. Pasdois, J.E. Parker, E.J. Griffiths, A.P. Halestrap, The role of oxidized cytochrome c in regulating mitochondrial reactive oxygen species production and its perturbation in ischaemia, Biochem. J. 436 (2011) 493–505, https://doi.org/10.1042/BJ20101957.
[56] A. Okado-Matsumoto, I. Fridovich, Subcellular distribution of superoxide dismutases (SOD) in rat liver: Cu,Zn-SOD in mitochondria, J. Biol. Chem. 276 (2001) 38388–38393, https://doi.org/10.1074/jbc.M105395200.
[57] J. Vasquez-Vivar, B. Kalyanaraman, M.C. Kennedy, Mitochondrial aconitase is a source of hydroxyl radical. An electron spin resonance investigation, J. Biol. Chem. 275 (2000) 14064–14069, https://doi.org/10.1074/jbc.275.19.14064.
[58] T. Ago, S. Matsushima, J. Kuroda, D. Zablocki, T. Kitazono, J. Sadoshima, The NADPH oxidase Nox4 and aging in the heart, Aging (N Y) 2 (2010) 1012–1016, https://doi.org/10.18632/aging.100261.
[59] P.H.G.M. Willems, R. Rossignol, C.E.J. Dieteren, M.P. Murphy, W.J.H. Koopman, Redox homeostasis and mitochondrial dynamics, Cell Metabol. 22 (2015) 207–218, https://doi.org/10.1016/j.cmet.2015.06.006.
[60] I. Valle, A. Alvarez-Barrientos, E. Arza, S. Lamas, M. Monsalve, PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells, Cardiovasc. Res. 66 (2005) 562–573, https://doi.org/10.1016/j.cardiores.2005.01.026.
[61] H. Liang, W.F. Ward, PGC-1alpha: a key regulator of energy metabolism, Adv. Physiol. Educ. 30 (2006) 145–151, https://doi.org/10.1152/advan.00052.2006.
[62] S.J. Stohs, D. Bagchi, Oxidative mechanisms in the toxicity of metal ions, Free Radic. Biol. Med. 18 (1995) 321–336, https://doi.org/10.1016/0891-5849(94)00159-h.
[63] M. Valko, H. Morris, M.T.D. Cronin, Metals, toxicity and oxidative stress, Curr. Med. Chem. 12 (2005) 1161–1208, https://doi.org/10.2174/0929867053764635.
[64] F. Di Lisa, P. Bernardi, Modulation of mitochondrial permeability transition in ischemia-reperfusion injury of the heart. Advantages and limitations, Curr. Med. Chem. 22 (2015) 2480–2487, https://doi.org/10.2174/0929867322666150530210005.
[65] S.L. Sensi, H.Z. Yin, S.G. Carriedo, S.S. Rao, J.H. Weiss, Preferential Zn2+ influx through Ca2+-permeable AMPA/kainate channels triggers prolonged mitochondrial superoxide production, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 2414–2419, https://doi.org/10.1073/pnas.96.5.2414.
[66] E. Tuncay, C.V. Bitirim, Y. Olgar, A. Durak, G.A. Rutter, B. Turan, Zn2+-transporters ZIP7 and ZnT7 play important role in progression of cardiac dysfunction via affecting sarco(endo)plasmic reticulum-mitochondria coupling in hyperglycemic cardiomyocytes, Mitochondrion 44 (2019) 41–52, https://doi.org/10.1016/j.mito.2017.12.011.
[67] K. Ogawa, K. Suzuki, M. Okutsu, K. Yamazaki, S. Shinkai, The association of elevated reactive oxygen species levels from neutrophils with low-grade inflammation in the elderly, Immun. Ageing 5 (2008) 13, https://doi.org/10.1186/1742-4933-5-13.
[68] C. Vida, I.M. de Toda, J. Cruces, A. Garrido, M. Gonzalez-Sanchez, M. De la Fuente, Role of macrophages in age-related oxidative stress and lipofuscin accumulation in mice, Redox Biol 12 (2017) 423–437, https://doi.org/10.1016/j.redox.2017.03.005.
[69] I. Liguori, G. Russo, F. Curcio, G. Bulli, L. Aran, D. Della-Morte, G. Gargiulo, G. Testa, F. Cacciatore, D. Bonaduce, P. Abete, Oxidative stress, aging, and diseases, Clin. Interv. Aging 13 (2018) 757–772, https://doi.org/10.2147/CIA.S158513.
[70] Y.C. Jang, H. Van Remmen, The mitochondrial theory of aging: insight from transgenic and knockout mouse models, Exp. Gerontol. 44 (2009) 256–260, https://doi.org/10.1016/j.exger.2008.12.006.
[71] D.-F. Dai, Y.A. Chiao, D.J. Marcinek, H.H. Szeto, P.S. Rabinovitch, Mitochondrial oxidative stress in aging and healthspan, Longev. Heal. 3 (2014) 6, https://doi.org/10.1186/2046-2395-3-6.
[72] A. Trifunovic, N.-G. Larsson, Mitochondrial dysfunction as a cause of ageing, J. Intern. Med. 263 (2008) 167–178, https://doi.org/10.1111/j.1365-2796.2007.01905.x.
[73] S. Dey, A. Sidor, B. O'Rourke, Compartment-specific control of reactive oxygen species scavenging by antioxidant pathway enzymes, J. Biol. Chem. 291 (2016) 11185–11197, https://doi.org/10.1074/jbc.M116.726968.
[74] S. Boudina, Cardiac aging and insulin resistance: could insulin/insulin-like growth factor (IGF) signaling be used as a therapeutic target? Curr. Pharmaceut. Des. 19 (2013) 5684–5694, https://doi.org/10.2174/1381612811319320004.
[75] M.F. Beal, Oxidatively modified proteins in aging and disease, Free Radic. Biol. Med. 32 (2002) 797–803, https://doi.org/10.1016/s0891-5849(02)00780-3.
[76] V. Pesce, A. Cormio, F. Fracasso, J. Vecchiet, G. Felzani, A.M. Lezza, P. Cantatore, M.N. Gadaleta, Age-related mitochondrial genotypic and phenotypic alterations in human skeletal muscle, Free Radic. Biol. Med. 30 (2001) 1223–1233, https://doi.org/10.1016/s0891-5849(01)00517-2.
[77] T.A. Luhtala, E.B. Roecker, T. Pugh, R.J. Feuers, R. Weindruch, Dietary restriction attenuates age-related increases in rat skeletal muscle antioxidant enzyme

activities, J. Gerontol. 49 (1994) B231–B238, https://doi.org/10.1093/geronj/49.5.b231.
[78] E. Barreiro, C. Coronell, B. Laviña, A. Ramírez-Sarmiento, M. Orozco-Levi, J. Gea, PENAM Project, Aging, sex differences, and oxidative stress in human respiratory and limb muscles, Free Radic. Biol. Med. 41 (2006) 797–809, https://doi.org/10.1016/j.freeradbiomed.2006.05.027.
[79] M.A. Aon, B.A. Stanley, V. Sivakumaran, J.M. Kembro, B. O'Rourke, N. Paolocci, S. Cortassa, Glutathione/thioredoxin systems modulate mitochondrial H2O2 emission: an experimental-computational study, J. Gen. Physiol. 139 (2012) 479–491, https://doi.org/10.1085/jgp.201210772.
[80] W. Ying, NAD+/NADH and NADP+/NADPH in cellular functions and cell death: regulation and biological consequences, Antioxidants Redox Signal. 10 (2008) 179–206, https://doi.org/10.1089/ars.2007.1672.
[81] M. Kohlhaas, T. Liu, A. Knopp, T. Zeller, M.F. Ong, M. Böhm, B. O'Rourke, C. Maack, Elevated cytosolic Na+ increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes, Circulation 121 (2010) 1606–1613, https://doi.org/10.1161/CIRCULATIONAHA.109.914911.
[82] D.B. Sawyer, Oxidative stress in heart failure: what are we missing? Am. J. Med. Sci. 342 (2011) 120–124, https://doi.org/10.1097/MAJ.0b013e3182249fcd.
[83] M. Wang, J. Zhang, S.J. Walker, R. Dworakowski, E.G. Lakatta, A.M. Shah, Involvement of NADPH oxidase in age-associated cardiac remodeling, J. Mol. Cell. Cardiol. 48 (2010) 765–772, https://doi.org/10.1016/j.yjmcc.2010.01.006.
[84] M.D. Herrera, C. Mingorance, R. Rodríguez-Rodríguez, M. Alvarez de Sotomayor, Endothelial dysfunction and aging: an update, Ageing Res. Rev. 9 (2010) 142–152, https://doi.org/10.1016/j.arr.2009.07.002.
[85] A. Oudot, C. Martin, D. Busseuil, C. Vergely, L. Demaison, L. Rochette, NADPH oxidases are in part responsible for increased cardiovascular superoxide production during aging, Free Radic. Biol. Med. 40 (2006) 2214–2222, https://doi.org/10.1016/j.freeradbiomed.2006.02.020.
[86] D.A. Siwik, P.J. Pagano, W.S. Colucci, Oxidative stress regulates collagen synthesis and matrix metalloproteinase activity in cardiac fibroblasts, Am. J. Physiol. Cell Physiol. 280 (2001) C53–C60, https://doi.org/10.1152/ajpcell.2001.280.1.C53.
[87] W. Chen, N.G. Frangogiannis, The role of inflammatory and fibrogenic pathways in heart failure associated with aging, Heart Fail. Rev. 15 (2010) 415–422, https://doi.org/10.1007/s10741-010-9161-y.
[88] A. Orlandi, A. Francesconi, M. Marcellini, A. Ferlosio, L.G. Spagnoli, Role of ageing and coronary atherosclerosis in the development of cardiac fibrosis in the rabbit, Cardiovasc. Res. 64 (2004) 544–552, https://doi.org/10.1016/j.cardiores.2004.07.024.
[89] M.Y. Vyssokikh, S. Holtze, O.A. Averina, K.G. Lyamzaev, A.A. Panteleeva, M. V Marey, R.A. Zinovkin, F.F. Severin, M. V Skulachev, N. Fasel, T.B. Hildebrandt, V. P. Skulachev, Mild depolarization of the inner mitochondrial membrane is a crucial component of an anti-aging program, Proc. Natl. Acad. Sci. U.S.A. 117 (2020) 6491–6501, https://doi.org/10.1073/pnas.1916414117.
[90] W.S. da-Silva, A. Gómez-Puyou, M.T. de Gómez-Puyou, R. Moreno-Sanchez, F. G. De Felice, L. de Meis, M.F. Oliveira, A. Galina, Mitochondrial bound hexokinase activity as a preventive antioxidant defense: steady-state ADP formation as a regulatory mechanism of membrane potential and reactive oxygen species generation in mitochondria, J. Biol. Chem. 279 (2004) 39846, https://doi.org/10.1074/jbc.M403835200, 55.
[91] L.E. Meyer, L.B. Machado, A.P.S.A. Santiago, W.S. Da-Silva, F.G. De Felice, O. Holub, M.F. Oliveira, A. Galina, Mitochondrial creatine kinase activity prevents reactive oxygen species generation: antioxidant role of mitochondrial kinase-dependent ADP re-cycling activity, J. Biol. Chem. 281 (2006) 37361–37371, https://doi.org/10.1074/jbc.M604123200.
[92] M.A. Aon, S. Cortassa, B. O'Rourke, Redox-optimized ROS balance: a unifying hypothesis, Biochim. Biophys. Acta 1797 (2010) 865–877, https://doi.org/10.1016/j.bbabio.2010.02.016.
[93] Q. Chen, E.J. Vazquez, S. Moghaddas, C.L. Hoppel, E.J. Lesnefsky, Production of reactive oxygen species by mitochondria: central role of complex III, J. Biol. Chem. 278 (2003) 36027–36031, https://doi.org/10.1074/jbc.M304854200.
[94] L.K. Kwong, R.S. Sohal, Age-related changes in activities of mitochondrial electron transport complexes in various tissues of the mouse, Arch. Biochem. Biophys. 373 (2000) 16–22, https://doi.org/10.1006/abbi.1999.1495.
[95] S. Kumaran, M. Subathra, M. Balu, C. Panneerselvam, Age-associated decreased activities of mitochondrial electron transport chain complexes in heart and skeletal muscle: role of L-carnitine, Chem. Biol. Interact. 148 (2004) 11–18, https://doi.org/10.1016/j.cbi.2003.10.010.
[96] M.I. Rodríguez, M. Carretero, G. Escames, L.C. López, M.D. Maldonado, D.-X. Tan, R.J. Reiter, D. Acuña-Castroviejo, Chronic melatonin treatment prevents age-dependent cardiac mitochondrial dysfunction in senescence-accelerated mice, Free Radic. Res. 41 (2007) 15–24, https://doi.org/10.1080/10715760600936359.
[97] I. Rebrin, R.S. Sohal, Comparison of thiol redox state of mitochondria and homogenates of various tissues between two strains of mice with different longevities, Exp. Gerontol. 39 (2004) 1513–1519, https://doi.org/10.1016/j.exger.2004.08.014.
[98] S. Kumaran, M. Subathra, M. Balu, C. Panneerselvam, Supplementation of L-carnitine improves mitochondrial enzymes in heart and skeletal muscle of aged rats, Exp. Aging Res. 31 (2005) 55–67, https://doi.org/10.1080/03610730590882846.
[99] G. Lenaz, Quinone specificity of complex I, Biochim. Biophys. Acta 1364 (1998) 207–221, https://doi.org/10.1016/s0005-2728(98)00028-0.
[100] A. Rakovic, A. Grünewald, J. Kottwitz, N. Brüggemann, P.P. Pramstaller, K. Lohmann, C. Klein, Mutations in PINK1 and Parkin impair ubiquitination of Mitofusins in human fibroblasts, PloS One 6 (2011), e16746, https://doi.org/10.1371/journal.pone.0016746.
[101] A. Valerio, E. Nisoli, Nitric oxide, interorganelle communication, and energy flow: a novel route to slow aging, Front. Cell Dev. Biol. 3 (2015) 6, https://doi.org/10.3389/fcell.2015.00006.
[102] H. Otani, Reactive oxygen species as mediators of signal transduction in ischemic preconditioning, Antioxidants Redox Signal. 6 (2004) 449–469, https://doi.org/10.1089/152308604322899521.
[103] S. Antonucci, J.F. Mulvey, N. Burger, M. Di Sante, A.R. Hall, E.C. Hinchy, S. T. Caldwell, A. V Gruszczyk, S. Deshwal, R.C. Hartley, N. Kaludercic, M. P. Murphy, F. Di Lisa, T. Krieg, Selective mitochondrial superoxide generation in vivo is cardioprotective through hormesis, Free Radic. Biol. Med. 134 (2019) 678–687, https://doi.org/10.1016/j.freeradbiomed.2019.01.034.
[104] D.-F. Dai, S.C. Johnson, J.J. Villarin, M.T. Chin, M. Nieves-Cintrón, T. Chen, D. J. Marcinek, G.W. Dorn, Y.J. Kang, T.A. Prolla, L.F. Santana, P.S. Rabinovitch, Mitochondrial oxidative stress mediates angiotensin II-induced cardiac hypertrophy and Galphaq overexpression-induced heart failure, Circ. Res. 108 (2011) 837–846, https://doi.org/10.1161/CIRCRESAHA.110.232306.
[105] S.E. Schriner, N.J. Linford, G.M. Martin, P. Treuting, C.E. Ogburn, M. Emond, P. E. Coskun, W. Ladiges, N. Wolf, H. Van Remmen, D.C. Wallace, P.S. Rabinovitch, Extension of murine life span by overexpression of catalase targeted to mitochondria, Science 308 (2005) 1909, https://doi.org/10.1126/science.1106653, 11.
[106] N. Basisty, D.-F. Dai, A. Gagnidze, L. Gitari, J. Fredrickson, Y. Maina, R.P. Beyer, M.J. Emond, E.J. Hsieh, M.J. MacCoss, G.M. Martin, P.S. Rabinovitch, Mitochondrial-targeted catalase is good for the old mouse proteome, but not for the young: "reverse" antagonistic pleiotropy? Aging Cell 15 (2016) 634–645, https://doi.org/10.1111/acel.12472.
[107] R. Scherz-Shouval, E. Shvets, E. Fass, H. Shorer, L. Gil, Z. Elazar, Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4, EMBO J. 26 (2007) 1749–1760, https://doi.org/10.1038/sj.emboj.7601623.
[108] N. Kaludercic, M.C. Maiuri, S. Kaushik, Á.F. Fernández, J. de Bruijn, F. Castoldi, Y. Chen, J. Ito, R. Mukai, T. Murakawa, J. Nah, F. Pietrocola, T. Saito, S. Sebti, M. Semenzato, L. Tsansizi, S. Sciarretta, J. Madrigal-Matute, Comprehensive autophagy evaluation in cardiac disease models, Cardiovasc. Res. 116 (2020) 483–504, https://doi.org/10.1093/cvr/cvz233.
[109] Y. Li, Y. Ma, L. Song, L. Yu, L. Zhang, Y. Zhang, Y. Xing, Y. Yin, H. Ma, SIRT3 deficiency exacerbates p53/Parkin-mediated mitophagy inhibition and promotes mitochondrial dysfunction: implication for aged hearts, Int. J. Mol. Med. 41 (2018) 3517–3526, https://doi.org/10.3892/ijmm.2018.3555.
[110] A. Nakai, O. Yamaguchi, T. Takeda, Y. Higuchi, S. Hikoso, M. Taniike, S. Omiya, I. Mizote, Y. Matsumura, M. Asahi, K. Nishida, M. Hori, N. Mizushima, K. Otsu, The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress, Nat. Med. 13 (2007) 619–624, https://doi.org/10.1038/nm1574.
[111] J.-O. Pyo, S.-M. Yoo, H.-H. Ahn, J. Nah, S.-H. Hong, T.-I. Kam, S. Jung, Y.-K. Jung, Overexpression of Atg5 in mice activates autophagy and extends lifespan, Nat. Commun. 4 (2013) 2300, https://doi.org/10.1038/ncomms3300.
[112] A. Shirakabe, Y. Ikeda, S. Sciarretta, D.K. Zablocki, J. Sadoshima, Aging and autophagy in the heart, Circ. Res. 118 (2016) 1563–1576, https://doi.org/10.1161/CIRCRESAHA.116.307474.
[113] T. Eisenberg, M. Abdellatif, S. Schroeder, U. Primessnig, S. Stekovic, T. Pendl, A. Harger, J. Schipke, A. Zimmermann, A. Schmidt, M. Tong, C. Ruckenstuhl, C. Dammbrueck, A.S. Gross, V. Herbst, C. Magnes, G. Trausinger, S. Narath, A. Meinitzer, Z. Hu, A. Kirsch, K. Eller, D. Carmona-Gutierrez, S. Büttner, F. Pietrocola, O. Knittelfelder, E. Schrepfer, P. Rockenfeller, C. Simonini, A. Rahn, M. Horsch, K. Moreth, J. Beckers, H. Fuchs, V. Gailus-Durner, F. Neff, D. Janik, B. Rathkolb, J. Rozman, M.H. de Angelis, T. Moustafa, G. Haemmerle, M. Mayr, P. Willeit, M. von Frieling-Salewsky, B. Pieske, L. Scorrano, T. Pieber, R. Pechlaner, J. Willeit, S.J. Sigrist, W.A. Linke, C. Mühlfeld, J. Sadoshima, J. Dengjel, S. Kiechl, G. Kroemer, S. Sedej, F. Madeo, Cardioprotection and lifespan extension by the natural polyamine spermidine, Nat. Med. 22 (2016) 1428–1438, https://doi.org/10.1038/nm.4222.
[114] R. Kiffin, C. Christian, E. Knecht, A.M. Cuervo, Activation of chaperone-mediated autophagy during oxidative stress, Mol. Biol. Cell 15 (2004) 4829–4840, https://doi.org/10.1091/mbc.e04-06-0477.
[115] X. Zhang, X. Cheng, L. Yu, J. Yang, R. Calvo, S. Patnaik, X. Hu, Q. Gao, M. Yang, M. Lawas, M. Delling, J. Marugan, M. Ferrer, H. Xu, MCOLN1 is a ROS sensor in lysosomes that regulates autophagy, Nat. Commun. 7 (2016) 12109, https://doi.org/10.1038/ncomms12109.
[116] M.D. Bootman, T. Chehab, G. Bultynck, J.B. Parys, K. Rietdorf, The regulation of autophagy by calcium signals: do we have a consensus? Cell Calcium 70 (2018) 32–46, https://doi.org/10.1016/j.ceca.2017.08.005.
[117] J.W. Palmer, B. Tandler, C.L. Hoppel, Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle, J. Biol. Chem. 252 (1977) 8731, 9, http://www.ncbi.nlm.nih.gov/pubmed/925018.
[118] C. Crochemore, M. Mekki, C. Corbière, A. Karoui, R. Noël, C. Vendeville, J.-M. Vaugeois, C. Monteil, Subsarcolemmal and interfibrillar mitochondria display distinct superoxide production profiles, Free Radic. Res. 49 (2015) 331–337, https://doi.org/10.3109/10715762.2015.1006212.
[119] E.J. Lesnefsky, B. Tandler, J. Ye, T.J. Slabe, J. Turkaly, C.L. Hoppel, Myocardial ischemia decreases oxidative phosphorylation through cytochrome oxidase in subsarcolemmal mitochondria, Am. J. Physiol. 273 (1997) H1544–H1554, https://doi.org/10.1152/ajpheart.1997.273.3.H1544.
[120] A. Rodriguez-Sinovas, K. Boengler, A. Cabestrero, P. Gres, M. Morente, M. Ruiz-Meana, I. Konietzka, E. Miró, A. Totzeck, G. Heusch, R. Schulz, D. Garcia-Dorado,

Translocation of connexin 43 to the inner mitochondrial membrane of cardiomyocytes through the heat shock protein 90-dependent TOM pathway and its importance for cardioprotection, Circ. Res. 99 (2006) 93–101, https://doi.org/10.1161/01.RES.0000230315.56904.de.
[121] K. Boengler, S. Stahlhofen, A. van de Sand, P. Gres, M. Ruiz-Meana, D. Garcia-Dorado, G. Heusch, R. Schulz, Presence of connexin 43 in subsarcolemmal, but not in interfibrillar cardiomyocyte mitochondria, Basic Res. Cardiol. 104 (2009) 141–147, https://doi.org/10.1007/s00395-009-0007-5.
[122] S.W. Fannin, E.J. Lesnefsky, T.J. Slabe, M.O. Hassan, C.L. Hoppel, Aging selectively decreases oxidative capacity in rat heart interfibrillar mitochondria, Arch. Biochem. Biophys. 372 (1999) 399–407, https://doi.org/10.1006/abbi.1999.1508.
[123] M. Ruiz-Meana, M. Minguet, D. Bou-Teen, E. Miro-Casas, C. Castans, J. Castellano, E. Bonzon-Kulichenko, A. Igual, R. Rodriguez-Lecoq, J. Vázquez, D. Garcia-Dorado, Ryanodine receptor glycation favors mitochondrial damage in the senescent heart, Circulation 139 (2019) 949–964, https://doi.org/10.1161/CIRCULATIONAHA.118.035869.
[124] G. Csordás, C. Renken, P. Várnai, L. Walter, D. Weaver, K.F. Buttle, T. Balla, C. A. Mannella, G. Hajnóczky, Structural and functional features and significance of the physical linkage between ER and mitochondria, J. Cell Biol. 174 (2006) 915–921, https://doi.org/10.1083/jcb.200604016.
[125] M. Kohlhaas, A.G. Nickel, C. Maack, Mitochondrial energetics and calcium coupling in the heart, J. Physiol. 595 (2017) 3753–3763, https://doi.org/10.1113/JP273609.
[126] S.M. Davidson, M.R. Duchen, Calcium microdomains and oxidative stress, Cell Calcium 40 (2006) 561–574, https://doi.org/10.1016/j.ceca.2006.08.017.
[127] S. Judge, Y.M. Jang, A. Smith, T. Hagen, C. Leeuwenburgh, Age-associated increases in oxidative stress and antioxidant enzyme activities in cardiac interfibrillar mitochondria: implications for the mitochondrial theory of aging, Faseb. J. 19 (2005) 419–421, https://doi.org/10.1096/fj.04-2622fje.
[128] A. Görlach, K. Bertram, S. Hudecova, O. Krizanova, Calcium and ROS: a mutual interplay, Redox Biol 6 (2015) 260–271, https://doi.org/10.1016/j.redox.2015.08.010.
[129] L.L. Cooper, W. Li, Y. Lu, J. Centracchio, R. Terentyeva, G. Koren, D. Terentyev, Redox modification of ryanodine receptors by mitochondria-derived reactive oxygen species contributes to aberrant Ca2+ handling in ageing rabbit hearts, J. Physiol. 591 (2013) 5895–5911, https://doi.org/10.1113/jphysiol.2013.260521.
[130] W. Xie, G. Santulli, S.R. Reiken, Q. Yuan, B.W. Osborne, B.-X. Chen, A.R. Marks, Mitochondrial oxidative stress promotes atrial fibrillation, Sci. Rep. 5 (2015) 11427, https://doi.org/10.1038/srep11427.
[131] G. Santulli, W. Xie, S.R. Reiken, A.R. Marks, Mitochondrial calcium overload is a key determinant in heart failure, Proc. Natl. Acad. Sci. U.S.A. 112 (2015) 11389–11394, https://doi.org/10.1073/pnas.1513047112.
[132] M. Ruiz-Meana, A. Abellán, E. Miró-Casas, E. Agulló, D. Garcia-Dorado, Role of sarcoplasmic reticulum in mitochondrial permeability transition and cardiomyocyte death during reperfusion, Am. J. Physiol. Heart Circ. Physiol. 297 (2009) H1281–H1289, https://doi.org/10.1152/ajpheart.00435.2009.
[133] A.Y.H. Woo, R. Xiao, β-Adrenergic receptor subtype signaling in heart: from bench to bedside, Acta Pharmacol. Sin. 33 (2012) 335–341, https://doi.org/10.1038/aps.2011.201.
[134] B. Hegyi, T. Bányász, L.T. Izu, L. Belardinelli, D.M. Bers, Y. Chen-Izu, β-adrenergic regulation of late Na+ current during cardiac action potential is mediated by both PKA and CaMKII, J. Mol. Cell. Cardiol. 123 (2018) 168–179, https://doi.org/10.1016/j.yjmcc.2018.09.006.
[135] R. Brandes, D.M. Bers, Increased work in cardiac trabeculae causes decreased mitochondrial NADH fluorescence followed by slow recovery, Biophys. J. 71 (1996) 1024–1035, https://doi.org/10.1016/S0006-3495(96)79303-7.
[136] C. Maack, S. Cortassa, M.A. Aon, A.N. Ganesan, T. Liu, B. O'Rourke, Elevated cytosolic Na+ decreases mitochondrial Ca2+ uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes, Circ. Res. 99 (2006) 172–182, https://doi.org/10.1161/01.RES.0000232546.92777.05.
[137] M. Kohlhaas, C. Maack, Adverse bioenergetic consequences of Na+-Ca2+ exchanger-mediated Ca2+ influx in cardiac myocytes, Circulation 122 (2010) 2273–2280, https://doi.org/10.1161/CIRCULATIONAHA.110.968057.
[138] B. Glancy, R.S. Balaban, Role of mitochondrial Ca2+ in the regulation of cellular energetics, Biochemistry 51 (2012) 2959–2973, https://doi.org/10.1021/bi2018909.
[139] A.G. Nickel, A. von Hardenberg, M. Hohl, J.R. Löffler, M. Kohlhaas, J. Becker, J.-C. Reil, A. Kazakov, J. Bonnekoh, M. Stadelmaier, S.-L. Puhl, M. Wagner, I. Bogeski, S. Cortassa, R. Kappl, B. Pasieka, M. Lafontaine, C.R.D. Lancaster, T. S. Blacker, A.R. Hall, M.R. Duchen, L. Kästner, P. Lipp, T. Zeller, C. Müller, A. Knopp, U. Laufs, M. Böhm, M. Hoth, C. Maack, Reversal of mitochondrial transhydrogenase causes oxidative stress in heart failure, Cell Metabol. 22 (2015) 472–484, https://doi.org/10.1016/j.cmet.2015.07.008.
[140] S. Cortassa, B. O'Rourke, M.A. Aon, Redox-optimized ROS balance and the relationship between mitochondrial respiration and ROS, Biochim. Biophys. Acta 1837 (2014) 287–295, https://doi.org/10.1016/j.bbabio.2013.11.007.
[141] T. Liu, E. Takimoto, V.L. Dimaano, D. DeMazumder, S. Kettlewell, G. Smith, A. Sidor, T.P. Abraham, B. O'Rourke, Inhibiting mitochondrial Na+/Ca2+ exchange prevents sudden death in a Guinea pig model of heart failure, Circ. Res. 115 (2014) 44–54, https://doi.org/10.1161/CIRCRESAHA.115.303062.
[142] F.G. Akar, M.A. Aon, G.F. Tomaselli, B. O'Rourke, The mitochondrial origin of postischemic arrhythmias, J. Clin. Invest. 115 (2005) 3527–3535, https://doi.org/10.1172/JCI25371.
[143] C. Percy, B. Pat, P. Poronnik, G. Gobe, Role of oxidative stress in age-associated chronic kidney pathologies, Adv. Chron. Kidney Dis. 12 (2005) 78–83, https://doi.org/10.1053/j.ackd.2004.10.006.
[144] E. Schleicher, U. Friess, Oxidative stress, AGE, and atherosclerosis, Kidney Int. Suppl. 72 (2007) S17–S26, https://doi.org/10.1038/sj.ki.5002382.
[145] J. Chaudhuri, Y. Bains, S. Guha, A. Kahn, D. Hall, N. Bose, A. Gugliucci, P. Kapahi, The role of advanced glycation end products in aging and metabolic diseases: bridging association and causality, Cell Metabol. 28 (2018) 337–352, https://doi.org/10.1016/j.cmet.2018.08.014.
[146] K. Nowotny, T. Jung, A. Höhn, D. Weber, T. Grune, Advanced glycation end products and oxidative stress in type 2 diabetes mellitus, Biomolecules 5 (2015) 194–222, https://doi.org/10.3390/biom5010194.
[147] M. Zhang, A.L. Kho, N. Anilkumar, R. Chibber, P.J. Pagano, A.M. Shah, A.C. Cave, Glycated proteins stimulate reactive oxygen species production in cardiac myocytes: involvement of Nox2 (gp91phox)-containing NADPH oxidase, Circulation 113 (2006) 1235–1243, https://doi.org/10.1161/CIRCULATIONAHA.105.581397.
[148] Y.-C. Yang, C.-Y. Tsai, C.-L. Chen, C.-H. Kuo, C.-W. Hou, S.-Y. Cheng, R. Aneja, C.-Y. Huang, W.-W. Kuo, Pkcδ activation is involved in ROS-mediated mitochondrial dysfunction and apoptosis in cardiomyocytes exposed to advanced glycation end products (ages), Aging Dis 9 (2018) 647–663, https://doi.org/10.14336/AD.2017.0924.
[149] A. Dobi, S.B. Bravo, B. Veeren, B. Paradela-Dobarro, E. Álvarez, O. Meilhac, W. Viranaicken, P. Baret, A. Devin, P. Rondeau, Advanced glycation end-products disrupt human endothelial cells redox homeostasis: new insights into reactive oxygen species production, Free Radic. Res. 53 (2019) 150–169, https://doi.org/10.1080/10715762.2018.1529866.
[150] D. Yao, M. Brownlee, Hyperglycemia-induced reactive oxygen species increase expression of the receptor for advanced glycation end products (RAGE) and RAGE ligands, Diabetes 59 (2010) 249–255, https://doi.org/10.2337/db09-0801.
[151] H. Yan, J.J. Harding, Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase, Biochem. J. 328 (1997) 599–605, https://doi.org/10.1042/bj3280599.
[152] M. Ruiz-Meana, D. García-Dorado, Translational cardiovascular medicine (II). Pathophysiology of ischemia-reperfusion injury: new therapeutic options for acute myocardial infarction, Rev. Esp. Cardiol. 62 (2009) 199–209, https://doi.org/10.1016/s1885-5857(09)71538-5.
[153] M. Ruiz-Meana, J. Inserte, C. Fernandez-Sanz, V. Hernando, E. Miro-Casas, I. Barba, D. Garcia-Dorado, The role of mitochondrial permeability transition in reperfusion-induced cardiomyocyte death depends on the duration of ischemia, Basic Res. Cardiol. 106 (2011) 1259–1268, https://doi.org/10.1007/s00395-011-0225-5.
[154] D. Garcia-Dorado, M. Ruiz-Meana, J. Inserte, A. Rodriguez-Sinovas, H.M. Piper, Calcium-mediated cell death during myocardial reperfusion, Cardiovasc. Res. 94 (2012) 168–180, https://doi.org/10.1093/cvr/cvs116.
[155] J. Inserte, M. Ruiz-Meana, A. Rodríguez-Sinovas, I. Barba, D. Garcia-Dorado, Contribution of delayed intracellular pH recovery to ischemic postconditioning protection, Antioxidants Redox Signal. 14 (2011) 923–939, https://doi.org/10.1089/ars.2010.3312.
[156] D.N. Granger, P.R. Kvietys, Reperfusion injury and reactive oxygen species: the evolution of a concept, Redox Biol 6 (2015) 524–551, https://doi.org/10.1016/j.redox.2015.08.020.
[157] S. Stewart, E.J. Lesnefsky, Q. Chen, Reversible blockade of electron transport with amobarbital at the onset of reperfusion attenuates cardiac injury, Transl. Res. 153 (2009) 224–231, https://doi.org/10.1016/j.trsl.2009.02.003.
[158] C.L. Quinlan, A.L. Orr, I. V Perevoshchikova, J.R. Treberg, B.A. Ackrell, M. D. Brand, Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions, J. Biol. Chem. 287 (2012) 27255–27264, https://doi.org/10.1074/jbc.M112.374629.
[159] E.T. Chouchani, V.R. Pell, E. Gaude, D. Aksentijević, S.Y. Sundier, E.L. Robb, A. Logan, S.M. Nadtochiy, E.N.J. Ord, A.C. Smith, F. Eyassu, R. Shirley, C.-H. Hu, A.J. Dare, A.M. James, S. Rogatti, R.C. Hartley, S. Eaton, A.S.H. Costa, P. S. Brookes, S.M. Davidson, M.R. Duchen, K. Saeb-Parsy, M.J. Shattock, A. J. Robinson, L.M. Work, C. Frezza, T. Krieg, M.P. Murphy, Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS, Nature 515 (2014) 431–435, https://doi.org/10.1038/nature13909.
[160] L. Valls-Lacalle, I. Barba, E. Miró-Casas, J.J. Alburquerque-Béjar, M. Ruiz-Meana, M. Fuertes-Agudo, A. Rodríguez-Sinovas, D. García-Dorado, Succinate dehydrogenase inhibition with malonate during reperfusion reduces infarct size by preventing mitochondrial permeability transition, Cardiovasc. Res. 109 (2016) 374–384, https://doi.org/10.1093/cvr/cvv279.
[161] G.L. Semenza, Hypoxia-inducible factor 1 and cardiovascular disease, Annu. Rev. Physiol. 76 (2014) 39–56, https://doi.org/10.1146/annurev-physiol-021113-170322.
[162] V.L. Dengler, M. Galbraith, J.M. Espinosa, Transcriptional regulation by hypoxia inducible factors, Crit. Rev. Biochem. Mol. Biol. 49 (2014) 1–15, https://doi.org/10.3109/10409238.2013.838205.
[163] S. Bonello, C. Zähringer, R.S. BelAiba, T. Djordjevic, J. Hess, C. Michiels, T. Kietzmann, A. Görlach, Reactive oxygen species activate the HIF-1alpha promoter via a functional NFkappaB site, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 755–761, https://doi.org/10.1161/01.ATV.0000258979.92828.bc.
[164] C. Dumitrescu, R. Biondi, Y. Xia, A.J. Cardounel, L.J. Druhan, G. Ambrosio, J. L. Zweier, Myocardial ischemia results in tetrahydrobiopterin (BH4) oxidation with impaired endothelial function ameliorated by BH4, Proc. Natl. Acad. Sci. U. S.A. 104 (2007) 15081–15086, https://doi.org/10.1073/pnas.0702986104.

[165] T. Andrienko, P. Pasdois, A. Rossbach, A.P. Halestrap, Real-time fluorescence measurements of ROS and [Ca2+] in ischemic/reperfused rat hearts: detectable increases occur only after mitochondrial pore opening and are attenuated by ischemic preconditioning, PloS One 11 (2016), e0167300, https://doi.org/10.1371/journal.pone.0167300.
[166] M. Ruiz-Meana, A. Abellán, E. Miró-Casas, D. Garcia-Dorado, Opening of mitochondrial permeability transition pore induces hypercontracture in Ca2+ overloaded cardiac myocytes, Basic Res. Cardiol. 102 (2007) 542–552, https://doi.org/10.1007/s00395-007-0675-y.
[167] J. Inserte, V. Hernando, D. Garcia-Dorado, Contribution of calpains to myocardial ischaemia/reperfusion injury, Cardiovasc. Res. 96 (2012) 23–31, https://doi.org/10.1093/cvr/cvs232.
[168] K. Min, O.-S. Kwon, A.J. Smuder, M.P. Wiggs, K.J. Sollanek, D.D. Christou, J.-K. Yoo, M.-H. Hwang, H.H. Szeto, A.N. Kavazis, S.K. Powers, Increased mitochondrial emission of reactive oxygen species and calpain activation are required for doxorubicin-induced cardiac and skeletal muscle myopathy, J. Physiol. 593 (2015) 2017, https://doi.org/10.1113/jphysiol.2014.286518, 36.
[169] K. Venkataraman, S. Khurana, T.C. Tai, Oxidative stress in aging–matters of the heart and mind, Int. J. Mol. Sci. 14 (2013) 17897–17925, https://doi.org/10.3390/ijms140917897.
[170] K.K. Ho, J.L. Pinsky, W.B. Kannel, D. Levy, The epidemiology of heart failure: the Framingham Study, J. Am. Coll. Cardiol. 22 (1993) 6A–13A, https://doi.org/10.1016/0735-1097(93)90455-a.
[171] C. Cook, G. Cole, P. Asaria, R. Jabbour, D.P. Francis, The annual global economic burden of heart failure, Int. J. Cardiol. 171 (2014) 368–376, https://doi.org/10.1016/j.ijcard.2013.12.028.
[172] C. Maack, T. Eschenhagen, N. Hamdani, F.R. Heinzel, A.R. Lyon, D.J. Manstein, J. Metzger, Z. Papp, C.G. Tocchetti, M.B. Yilmaz, S.D. Anker, J.-L. Balligand, J. Bauersachs, D. Brutsaert, L. Carrier, S. Chlopicki, J.G. Cleland, R.A. de Boer, A. Dietl, R. Fischmeister, V.-P. Harjola, S. Heymans, D. Hilfiker-Kleiner, J. Holzmeister, G. de Keulenaer, G. Limongelli, W.A. Linke, L.H. Lund, J. Masip, M. Metra, C. Mueller, B. Pieske, P. Ponikowski, A. Ristić, F. Ruschitzka, P. M. Seferović, H. Skouri, W.H. Zimmermann, A. Mebazaa, Treatments targeting inotropy, Eur. Heart J. 40 (2019) 3626–3644, https://doi.org/10.1093/eurheartj/ehy600.
[173] F. Sam, D.L. Kerstetter, D.R. Pimental, S. Mulukutla, A. Tabaee, M.R. Bristow, W. S. Colucci, D.B. Sawyer, Increased reactive oxygen species production and functional alterations in antioxidant enzymes in human failing myocardium, J. Card. Fail. 11 (2005) 473–480, https://doi.org/10.1016/j.cardfail.2005.01.007.
[174] S. Melov, P. Coskun, M. Patel, R. Tuinstra, B. Cottrell, A.S. Jun, T.H. Zastawny, M. Dizdaroglu, S.I. Goodman, T.T. Huang, H. Miziorko, C.J. Epstein, D.C. Wallace, Mitochondrial disease in superoxide dismutase 2 mutant mice, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 846–851, https://doi.org/10.1073/pnas.96.3.846.
[175] S. Melov, S.R. Doctrow, J.A. Schneider, J. Haberson, M. Patel, P.E. Coskun, K. Huffman, D.C. Wallace, B. Malfroy, Lifespan extension and rescue of spongiform encephalopathy in superoxide dismutase 2 nullizygous mice treated with superoxide dismutase-catalase mimetics, J. Neurosci. 21 (2001) 8348–8353. http://www.ncbi.nlm.nih.gov/pubmed/11606622.
[176] S. Dey, D. DeMazumder, A. Sidor, D.B. Foster, B. O'Rourke, Mitochondrial ROS drive sudden cardiac death and chronic proteome remodeling in heart failure, Circ. Res. 123 (2018) 356–371, https://doi.org/10.1161/CIRCRESAHA.118.312708.
[177] D.A. Eisner, J.L. Caldwell, K. Kistamás, A.W. Trafford, Calcium and excitation-contraction coupling in the heart, Circ. Res. 121 (2017) 181–195, https://doi.org/10.1161/CIRCRESAHA.117.310230.
[178] M. Endo, M. Tanaka, Y. Ogawa, Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibres, Nature 228 (1970) 34–36, https://doi.org/10.1038/228034a0.
[179] D.M. Bers, Cardiac excitation-contraction coupling, Nature 415 (2002) 198–205, https://doi.org/10.1038/415198a.
[180] E.N. Dedkova, L.A. Blatter, Calcium signaling in cardiac mitochondria, J. Mol. Cell. Cardiol. 58 (2013) 125–133, https://doi.org/10.1016/j.yjmcc.2012.12.021.
[181] G.S.B. Williams, L. Boyman, A.C. Chikando, R.J. Khairallah, W.J. Lederer, Mitochondrial calcium uptake, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 10479–10486, https://doi.org/10.1073/pnas.1300410110.
[182] J.P. Brennan, S.C. Bardswell, J.R. Burgoyne, W. Fuller, E. Schröder, R. Wait, S. Begum, J.C. Kentish, P. Eaton, Oxidant-induced activation of type I protein kinase A is mediated by RI subunit interprotein disulfide bond formation, J. Biol. Chem. 281 (2006) 21827–21836, https://doi.org/10.1074/jbc.M603952200.
[183] K. Kiselyov, S. Muallem, ROS and intracellular ion channels, Cell Calcium 60 (2016) 108–114, https://doi.org/10.1016/j.ceca.2016.03.004.
[184] S. Wagner, A.G. Rokita, M.E. Anderson, L.S. Maier, Redox regulation of sodium and calcium handling, Antioxidants Redox Signal. 18 (2013) 1063–1077, https://doi.org/10.1089/ars.2012.4818.
[185] A.S. Johnston, S.E. Lehnart, J.R. Burgoyne, Ca(2+) signaling in the myocardium by (redox) regulation of PKA/CaMKII, Front. Pharmacol. 6 (2015) 166, https://doi.org/10.3389/fphar.2015.00166.
[186] J.R. Erickson, M.A. Joiner, X. Guan, W. Kutschke, J. Yang, C. V Oddis, R. K. Bartlett, J.S. Lowe, S.E. O'Donnell, N. Aykin-Burns, M.C. Zimmerman, K. Zimmerman, A.-J.L. Ham, R.M. Weiss, D.R. Spitz, M.A. Shea, R.J. Colbran, P. J. Mohler, M.E. Anderson, A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation, Cell 133 (2008) 462–474, https://doi.org/10.1016/j.cell.2008.02.048.
[187] S. Wagner, N. Dybkova, E.C.L. Rasenack, C. Jacobshagen, L. Fabritz, P. Kirchhof, S.K.G. Maier, T. Zhang, G. Hasenfuss, J.H. Brown, D.M. Bers, L.S. Maier, Ca2+/calmodulin-dependent protein kinase II regulates cardiac Na+ channels, J. Clin. Invest. 116 (2006) 3127–3138, https://doi.org/10.1172/JCI26620.
[188] C.R. Weber, V. Piacentino, S.R. Houser, D.M. Bers, Dynamic regulation of sodium/calcium exchange function in human heart failure, Circulation 108 (2003) 2224–2229, https://doi.org/10.1161/01.CIR.0000095274.72486.94.
[189] A.A. Armoundas, I.A. Hobai, G.F. Tomaselli, R.L. Winslow, B. O'Rourke, Role of sodium-calcium exchanger in modulating the action potential of ventricular myocytes from normal and failing hearts, Circ. Res. 93 (2003) 46–53, https://doi.org/10.1161/01.RES.0000080932.98903.D8.
[190] T. Liu, B. O'Rourke, Enhancing mitochondrial Ca2+ uptake in myocytes from failing hearts restores energy supply and demand matching, Circ. Res. 103 (2008) 279–288, https://doi.org/10.1161/CIRCRESAHA.108.175919.
[191] T. Ago, T. Liu, P. Zhai, W. Chen, H. Li, J.D. Molkentin, S.F. Vatner, J. Sadoshima, A redox-dependent pathway for regulating class II HDACs and cardiac hypertrophy, Cell 133 (2008) 978–993, https://doi.org/10.1016/j.cell.2008.04.041.
[192] J. Wei, S. Joshi, S. Speransky, C. Crowley, N. Jayathilaka, X. Lei, Y. Wu, D. Gai, S. Jain, M. Hoosien, Y. Gao, L. Chen, N.H. Bishopric, Reversal of pathological cardiac hypertrophy via the MEF2-coregulator interface, JCI Insight 2 (2017), e91068, https://doi.org/10.1172/jci.insight.91068.
[193] A. Oudot, C. Vergely, A. Ecarnot-Laubriet, L. Rochette, Angiotensin II activates NADPH oxidase in isolated rat hearts subjected to ischaemia-reperfusion, Eur. J. Pharmacol. 462 (2003) 145–154, https://doi.org/10.1016/s0014-2999(03)01315-3.
[194] S. Kohli, S. Ahuja, V. Rani, Transcription factors in heart: promising therapeutic targets in cardiac hypertrophy, Curr. Cardiol. Rev. 7 (2011) 262–271, https://doi.org/10.2174/157340311799960618.
[195] W.T. Pu, Q. Ma, S. Izumo, NFAT transcription factors are critical survival factors that inhibit cardiomyocyte apoptosis during phenylephrine stimulation in vitro, Circ. Res. 92 (2003) 725–731, https://doi.org/10.1161/01.RES.0000069211.82346.46.
[196] M.K. Shigenaga, T.M. Hagen, B.N. Ames, Oxidative damage and mitochondrial decay in aging, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 10771–10778, https://doi.org/10.1073/pnas.91.23.10771.
[197] B.A.I. Payne, P.F. Chinnery, Mitochondrial dysfunction in aging: much progress but many unresolved questions, Biochim. Biophys. Acta 1847 (2015) 1347–1353, https://doi.org/10.1016/j.bbabio.2015.05.022.
[198] M. Makrecka-Kuka, E. Liepinsh, A.J. Murray, H. Lemieux, M. Dambrova, K. Tepp, M. Puurand, T. Käämbre, W.H. Han, P. de Goede, K.A. O'Brien, B. Turan, E. Tuncay, Y. Olgar, A.P. Rolo, C.M. Palmeira, N.T. Boardman, R.C.I. Wüst, T. S. Larsen, Altered mitochondrial metabolism in the insulin-resistant heart, Acta Physiol. 228 (2020), e13430, https://doi.org/10.1111/apha.13430.
[199] V.P. Skulachev, V.N. Anisimov, Y.N. Antonenko, L.E. Bakeeva, B. V Chernyak, V. P. Erichev, O.F. Filenko, N.I. Kalinina, V.I. Kapelko, N.G. Kolosova, B.P. Kopnin, G.A. Korshunova, M.R. Lichinitser, L.A. Obukhova, E.G. Pasyukova, O. I. Pisarenko, V.A. Roginsky, E.K. Ruuge, I.I. Senin, I.I. Severina, M. V Skulachev, I. M. Spivak, V.N. Tashlitsky, V.A. Tkachuk, M.Y. Vyssokikh, L.S. Yaguzhinsky, D. B. Zorov, An attempt to prevent senescence: a mitochondrial approach, Biochim. Biophys. Acta 1787 (2009) 437–461, https://doi.org/10.1016/j.bbabio.2008.12.008.
[200] A. V Birk, S. Liu, Y. Soong, W. Mills, P. Singh, J.D. Warren, S. V Seshan, J. D. Pardee, H.H. Szeto, The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin, J. Am. Soc. Nephrol. 24 (2013) 1250–1261, https://doi.org/10.1681/ASN.2012121216.
[201] G.F. Kelso, C.M. Porteous, C. V Coulter, G. Hughes, W.K. Porteous, E. C. Ledgerwood, R.A. Smith, M.P. Murphy, Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties, J. Biol. Chem. 276 (2001) 4588–4596, https://doi.org/10.1074/jbc.M009093200.
[202] M.P. Murphy, R.A.J. Smith, Targeting antioxidants to mitochondria by conjugation to lipophilic cations, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 629–656, https://doi.org/10.1146/annurev.pharmtox.47.120505.105110.
[203] C. Reily, T. Mitchell, B.K. Chacko, G. Benavides, M.P. Murphy, V. Darley-Usmar, Mitochondrially targeted compounds and their impact on cellular bioenergetics, Redox Biol 1 (2013) 86–93, https://doi.org/10.1016/j.redox.2012.11.009.
[204] A.J. Dare, A. Logan, T.A. Prime, S. Rogatti, M. Goddard, E.M. Bolton, J.A. Bradley, G.J. Pettigrew, M.P. Murphy, K. Saeb-Parsy, The mitochondria-targeted anti-oxidant MitoQ decreases ischemia-reperfusion injury in a murine syngeneic heart transplant model, J. Heart Lung Transplant. 34 (2015) 1471–1480, https://doi.org/10.1016/j.healun.2015.05.007.
[205] Y. Olgar, S. Degirmenci, A. Durak, D. Billur, B. Can, G. Kayki-Mutlu, E. Arioglu-Inan, B. Turan, Aging related functional and structural changes in the heart and aorta: MitoTEMPO improves aged-cardiovascular performance, Exp. Gerontol. 110 (2018) 172–181, https://doi.org/10.1016/j.exger.2018.06.012.
[206] S. Miura, S.-I. Saitoh, T. Kokubun, T. Owada, H. Yamauchi, H. Machii, Y. Takeishi, Mitochondrial-targeted antioxidant maintains blood flow, mitochondrial function, and redox balance in old mice following prolonged limb ischemia, Int. J. Mol. Sci. 18 (2017) 1897, https://doi.org/10.3390/ijms18091897.
[207] K.R. Murphy, B. Baggett, L.L. Cooper, Y. Lu, J. O-Uchi, J.M. Sedivy, D. Terentyev, G. Koren, Enhancing autophagy diminishes aberrant Ca2+ homeostasis and arrhythmogenesis in aging rabbit hearts, Front. Physiol. 10 (2019) 1277, https://doi.org/10.3389/fphys.2019.01277.
[208] Y. Olgar, D. Billur, E. Tuncay, B. Turan, MitoTEMPO provides an antiarrhythmic effect in aged-rats through attenuation of mitochondrial reactive oxygen species, Exp. Gerontol. 136 (2020) 110961, https://doi.org/10.1016/j.exger.2020.110961.

[209] J. Ma, Y. Wang, D. Zheng, M. Wei, H. Xu, T. Peng, Rac1 signalling mediates doxorubicin-induced cardiotoxicity through both reactive oxygen species-dependent and -independent pathways, Cardiovasc. Res. 97 (2013) 77–87, https://doi.org/10.1093/cvr/cvs309.
[210] A.E. Vendrov, K.C. Vendrov, A. Smith, J. Yuan, A. Sumida, J. Robidoux, M. S. Runge, N.R. Madamanchi, NOX4 NADPH oxidase-dependent mitochondrial oxidative stress in aging-associated cardiovascular disease, Antioxidants Redox Signal. 23 (2015) 1389–1409, https://doi.org/10.1089/ars.2014.6221.
[211] L. Baird, A.T. Dinkova-Kostova, The cytoprotective role of the Keap1-Nrf2 pathway, Arch. Toxicol. 85 (2011) 241–272, https://doi.org/10.1007/s00204-011-0674-5.
[212] K.M. Holmström, R. V Kostov, A.T. Dinkova-Kostova, The multifaceted role of Nrf2 in mitochondrial function, Curr. Opin. Toxicol. 1 (2016) 80–91, https://doi.org/10.1016/j.cotox.2016.10.002.
[213] C.G. Pham, C. Bubici, F. Zazzeroni, S. Papa, J. Jones, K. Alvarez, S. Jayawardena, E. De Smaele, R. Cong, C. Beaumont, F.M. Torti, S. V Torti, G. Franzoso, Ferritin heavy chain upregulation by NF-kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species, Cell 119 (2004) 529–542, https://doi.org/10.1016/j.cell.2004.10.017.
[214] H. Kamata, S.-I. Honda, S. Maeda, L. Chang, H. Hirata, M. Karin, Reactive oxygen species promote TNFalpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases, Cell 120 (2005) 649–661, https://doi.org/10.1016/j.cell.2004.12.041.
[215] C.-S. Catana, C.-A. Crișan, D. Opre, I. Berindan-Neagoe, Implications of long non-coding RNAs in age-altered proteostasis, Aging Dis 11 (2020) 692–704, https://doi.org/10.14336/AD.2019.0814.
[216] S. Kim, J. Song, P. Ernst, M.N. Latimer, C.-M. Ha, K.Y. Goh, W. Ma, N.-S. Rajasekaran, J. Zhang, X. Liu, S.D. Prabhu, G. Qin, A.R. Wende, M.E. Young, L. Zhou, MitoQ regulates redox-related noncoding RNAs to preserve mitochondrial network integrity in pressure-overload heart failure, Am. J. Physiol. Heart Circ. Physiol. 318 (2020) H682–H695, https://doi.org/10.1152/ajpheart.00617.2019.
[217] R. Madonna, S. Angelucci, F. Di Giuseppe, V. Doria, Z. Giricz, A. Görbe, P. Ferdinandy, R. De Caterina, Proteomic analysis of the secretome of adipose tissue-derived murine mesenchymal cells overexpressing telomerase and myocardin, J. Mol. Cell. Cardiol. 131 (2019) 171–186, https://doi.org/10.1016/j.yjmcc.2019.04.019.
[218] R. Madonna, D. Pieragostino, C. Rossi, S. Guarnieri, C.T. Nagy, Z. Giricz, P. Ferdinandy, P. Del Boccio, M.A. Mariggiò, Y.-J. Geng, R. De Caterina, Transplantation of telomerase/myocardin-co-expressing mesenchymal cells in the mouse promotes myocardial revascularization and tissue repair, Vasc. Pharmacol. 135 (2020) 106807, https://doi.org/10.1016/j.vph.2020.106807.
[219] D. Fusco, G. Colloca, M.R. Lo Monaco, M. Cesari, Effects of antioxidant supplementation on the aging process, Clin. Interv. Aging 2 (2007) 377–387. http://www.ncbi.nlm.nih.gov/pubmed/18044188.
[220] M.E. Obrenovich, Y. Li, K. Parvathaneni, B.B. Yendluri, H.H. Palacios, J. Leszek, G. Aliev, Antioxidants in health, disease and aging, CNS Neurol. Disord. - Drug Targets 10 (2011) 192–207, https://doi.org/10.2174/187152711794480375.
[221] D.M. Monti, M.M. Rigano, S.M. Monti, H.S. Peixoto, Role of antioxidants in the protection from aging-related diseases, Oxid. Med. Cell. Longev. 8 (2019) 7450693, https://doi.org/10.1155/2019/7450693.
[222] I. Sadowska-Bartosz, G. Bartosz, Effect of antioxidants supplementation on aging and longevity, BioMed Res. Int. 2014 (2014) 404680, https://doi.org/10.1155/2014/404680.
[223] S.I. Dikalov, D.G. Harrison, Methods for detection of mitochondrial and cellular reactive oxygen species, Antioxidants Redox Signal. 20 (2014) 372–382, https://doi.org/10.1089/ars.2012.4886.
[224] S. Dikalov, K.K. Griendling, D.G. Harrison, Measurement of reactive oxygen species in cardiovascular studies, Hypertens. (Dallas, Tex. 49 (1979) 717–727, https://doi.org/10.1161/01.HYP.0000258594.87211.6b, 2007.
[225] V. Conti, V. Izzo, G. Corbi, G. Russomanno, V. Manzo, F. De Lise, A. Di Donato, A. Filippelli, Antioxidant supplementation in the treatment of aging-associated diseases, Front. Pharmacol. 7 (2016) 24, https://doi.org/10.3389/fphar.2016.00024.
[226] A.J. Braakhuis, R. Nagulan, V. Somerville, The effect of MitoQ on aging-related biomarkers: a systematic review and meta-analysis, Oxid. Med. Cell. Longev. 12 (2018) 8575263, https://doi.org/10.1155/2018/8575263.