Review

Involvement of Oxidative Stress in Mitochondrial Abnormalities During the Development of Heart Disease

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Involvement of Oxidative Stress in Mitochondrial Abnormalities During the Development of Heart Disease

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Abstract: Background: Several mitochondrial abnormalities such as defective energy production, depletion of energy stores, Ca^{2+}-accumulation, generation of reactive oxygen species and impaired intracellular signaling are associated with cardiac dysfunction during the development of different heart diseases. **Methods:** A narrative review was compiled by a search for applicable literature in MEDLINE via PubMed. **Results:** Mitochondria generate ATP through the processes of electron transport and oxidative phosphorylation, which is used as energy for cardiac contractile function. Mitochondria in fact are the key subcellular organelle for the regulation of intracellular Ca^{2+} concentration and are considered to serve as a buffer to maintain Ca^{2+} homeostasis in cardiomyocytes. However, during the development of heart disease, excessive accumulation of intracellular Ca^{2+} results in mitochondria Ca^{2+}-overload, which, in turn, impairs mitochondrial energy production and induces cardiac dysfunction. Mitochondria also generate reactive oxygen species (ROS), including superoxide and hydroxyl radicals as well as H_2O_2, a well-known oxidant that promotes lipid peroxidation and subsequent disturbance of Ca^{2+} homeostasis, cellular damage and death. **Conclusion:** Oxidative stress plays a critical role in mitochondrial disruption during the pathogenesis of different cardiac pathologies.

Keywords: mitochondria; oxidative stress; Ca^{2+}-handling defects; cell death; cardiac dysfunction; heart disease

1. Introduction

Oxidative stress and intracellular Ca^{2+}-overload are intimately involved in different cardiac pathologies including heart failure, diabetic cardiomyopathy and ischemia-reperfusion injury [1–7]. Such defects in cardiomyocyte Ca^{2+}-handling have been attributed to subcellular remodeling during the development of heart disease [8–14]. Mitochondria are the major source of ATP production through oxidative phosphorylation and electron transport required for cardiac function [15–18] and are regarded as multifunctional organelles involved in cardiomyocyte function and integrity. Indeed, mitochondria regulate key processes including mitophagy, apoptosis, redox balance and Ca^{2+}-homeostasis [19–24]. There is unequivocal evidence that mitochondria are a high source of reactive oxygen species (ROS), such as superoxide and hydroxyl radicals as well as the oxidant, H_2O_2, which promote lipid peroxidation leading to a dysregulation of cation homeostasis, cellular damage and cell death [25,26]. In addition, the excessive production of ROS can lead to an accumulation of 4-

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hydroxynonenal inside the mitochondria, which is a product of lipid peroxidation and reactive aldehyde, and is far more harmful than ROS [27]. Furthermore, the occurrence of oxidative stress that is accompanied by a depletion of antioxidant enzymes and other redox-regulating molecules, which exacerbates the imbalance between ROS generation and detoxification, contributing to the acceleration of myocardial abnormalities in structure and function.

Mitochondria are known to accumulate a considerable amount of Ca^{2+} and thus are considered as a Ca^{2+} reservoir/sink designed to maintain the intracellular concentration of free Ca^{2+} ($[Ca^{2+}]i$) within optimal range [15,16,28]. However, during the development of different cardiac diseases, an excessive amount of intracellular Ca^{2+} results in mitochondria Ca^{2+}-overload that subsequently harms mitochondrial energy production [15,16,29]. Taken together, it can be seen from Figure 1 that mitochondrial dysfunction in different types of heart disease and pathophysiological conditions is a key component in the pathogenesis of cardiac dysfunction. This functional decline of the heart is strongly associated with excessive ROS generation, which originates from multiple sources, including NADPH oxidase, monoamine oxidase, and mitochondrial respiratory complexes I, II, and III. Notably, NADPH oxidase 4 (NOX4) has been identified to be present in mitochondria mainly and serves as a principal driver of oxidative stress in heart failure [30]. Figure 2 summarizes the alterations in mitochondrial ROS generating systems.

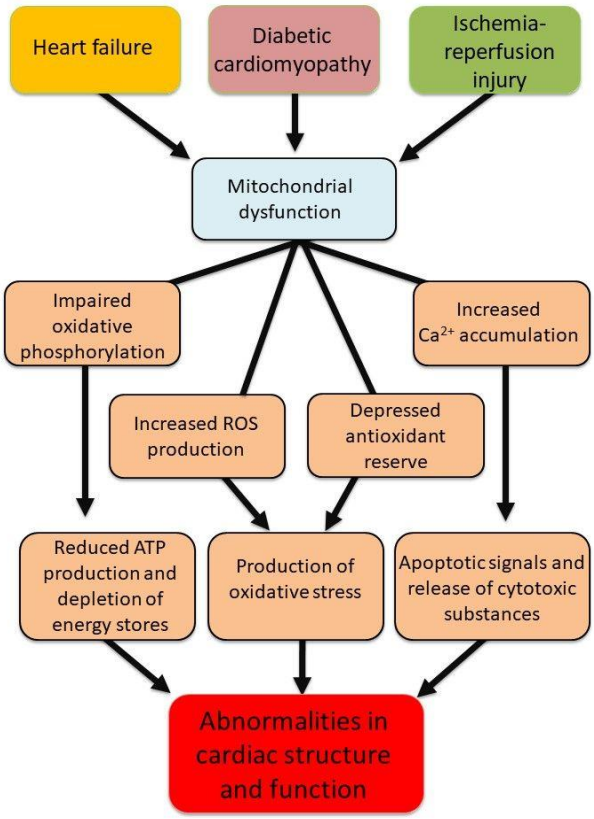

Figure 1. Abnormalities associated with mitochondrial dysfunction leading to changes in cardiomyocyte structure and function in different cardiac pathologies.

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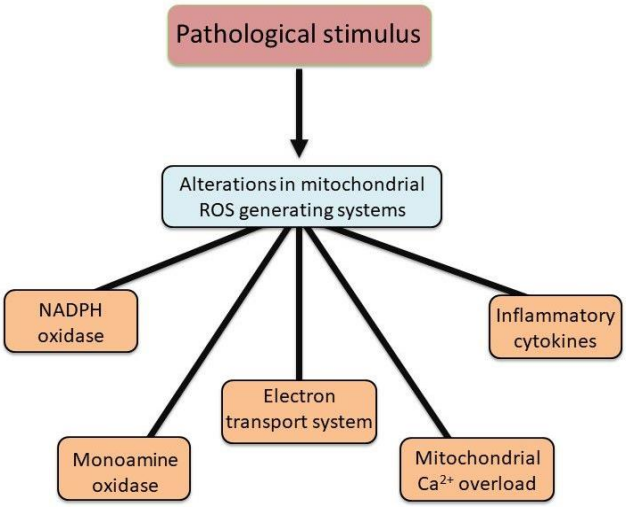

Figure 2. Alterations in different ROS generating systems in mitochondria due to pathological stimulus. Abbreviation: ROS = reactive oxygen species.

It should be mentioned that endothelium-associated xanthine oxidase (XO), which is known to generate superoxide anion, is activated by angiotensin II and NADPH oxidase activity in endothelial cells [31]. This surplus of superoxide anions generated through these pathways induces widespread damage to cellular macromolecules, including DNA, proteins, lipids, and carbohydrates, ultimately resulting in mitochondrial dysfunction and irreversible cytotoxicity [32]. Indeed, the interplay between ROS and mitochondrial components creates a self-amplifying cycle of oxidative damage, further exacerbating mitochondrial dysfunction, contractile impairment and overall progression of heart failure [33,34].

Oxidative stress represents a state of redox disequilibrium characterized by the excessive generation of ROS, including superoxide radicals, hydrogen peroxide, and hydroxyl radicals, alongside a concurrent reduction in endogenous antioxidant capacity [35]. The accumulation of mitochondrial ROS accompanied with a variety of different factors including heightened inflammatory response, formation of advanced glycation end-products and lipid peroxidation all collectively exacerbate oxidative stress (Figure 3).

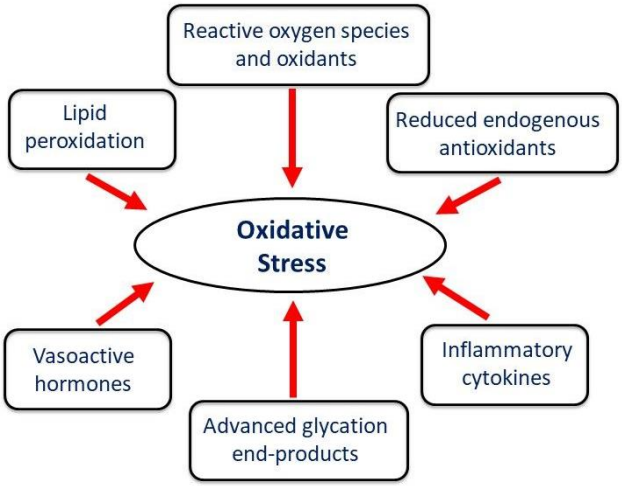

Figure 3. Different factors involved in the development of oxidative stress in diseased heart.

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The deleterious effects of oxidative stress are not limited to mitochondrial impairment, but also contribute to the pathological remodeling of the myocardium through the upregulation of pro-inflammatory cytokines and activation of fibroblasts in the extracellular matrix [36,37]. These mechanisms collectively drive interstitial fibrosis and increased myocardial stiffness, which are hallmarks of heart failure progression. It should be mentioned that Nrf2 (nuclear factor erythroid 2-related factor 2) is a transcription factor that regulates antioxidant responses and plays a critical role in cellular defense against oxidative stress, inflammation, and apoptosis. It is a key regulator of several genes for several endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase, which are involved in protecting against the development of oxidative damage and mitochondrial dysfunction, making it a promising therapeutic target in cardiovascular diseases [38]. In fact, numerous studies suggest that Nrf2 activation is a crucial cardioprotective mechanism against the adverse effects of ischemic myocardial injury.

The prolonged exposure of the heart to high levels of circulating vasoactive hormones including angiotensin II and catecholamines in chronic myocardial infarction have been shown to induce Ca^{2+}-handling abnormalities that have been linked to the occurrence of mitochondrial Ca^{2+}-overload, mitochondrial dysfunction, and the generation of oxidative stress all leading to an impairment of cardiovascular function [39–41]. Both Ca^{2+} and oxidative stress are considered to induce conformational alterations in the mitochondrial cristae embedded F1/F0, ATP synthase and permit the formation of membrane permeability transition pores (MPTP) for releasing solutes and proteins, including cytochrome C, apoptosis-inducing factor and Smac/DIABLO, from the mitochondrial matrix [42–44]. If the MPTP remains in the open state, the cardiomyocyte is unable to sustain its ATP levels ultimately leading to mitochondrial stress, cell death and cardiac dysfunction [45].

Taken together, Figure 4 demonstrates the critical role of mitochondria in alterations in cardiomyocyte structure and function through modulating energy metabolism, formation of the MPTP and inducing apoptotic signals. In view of the importance of mitochondria in normal cell function, this narrative review is intended to describe the role of defects in mitochondrial energy generation, increased ROS production, dysregulation of Ca^{2+}-handling and mitochondrial Ca^{2+}overlaod as well as cell apoptosis in cardiac dysfunction in different pathophysiological conditions such as heart failure, diabetic cardiomyopathy and ischemia-reperfusion injury. Accordingly, appropriate literature searched on MEDLINE via PubMed by using the search terms: mitochondrial dysfunction, cardiac ischemia-reperfusion injury, diabetic cardiomyopathy, heart failure, reactive oxygen species, oxidative stress, Ca^{2+}-handling, intracellular Ca^{2+}-overload and combination thereof was conducted and the articles cited in this review were those selected to provide support of our hypothesis.

2. Evidence of Involvement of ROS and Ca^{2+}-Overload in Cardiac Mitochondria

Mitochondria play a pivotal role in cellular redox signaling by generating ROS as by-products of oxidative phosphorylation [46–48]. However, not all oxidants have a role in signal transduction as it appears that this is dependent upon the cell type and animal species. Furthermore, low concentrations of oxidants or exposure for a transient period stimulate the signal transduction mechanisms for both cardiomyocyte function and gene expression for cell survival, while high concentrations of oxidants and/or exposure for a prolonged period of time produces oxidative stress and subsequent harmful outcomes [8,49].

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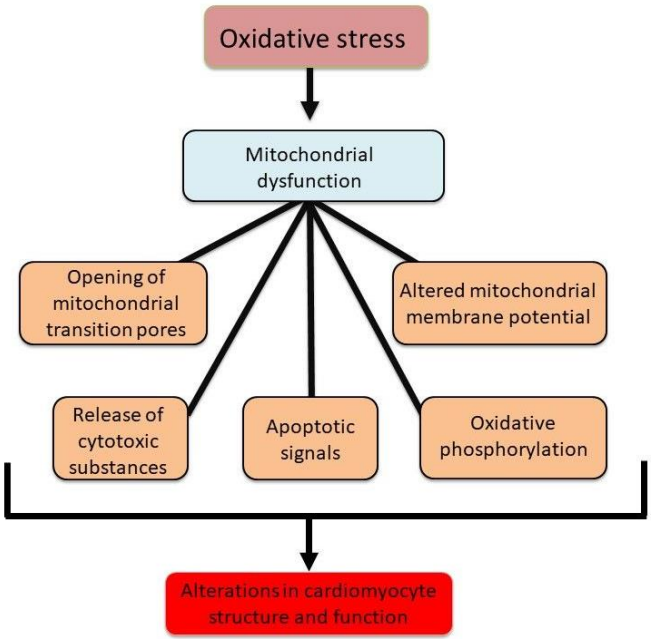

Figure 4. Mitochondrial abnormalities due to oxidative stress leading changes in cardiac function and structure.

The impairment of mitochondrial function by ROS generating systems and oxidants has been reported [50,51]. In this regard, normal rat hearts perfused with an ROS generating system, xanthine (X) plus xanthine oxidase (XO) has been shown to decrease mitochondrial state 3, uncoupled respiration and ADP-to-O ratio without any changes in the state 4 respiration (Figure 5). On the other hand, perfusion with a well known oxidant, H_2O_2 increased mitochondrial state 4 respiration and decreased the ADP-to-O ratio as well as mitochondrial state 3 and uncoupled respiration (Figure 5) [50]. The role of ROS and oxidants in mitochondrial impairment of oxidative stress was further demonstrated by the observations that the changes in mitochondrial function due to X plus XO were attenuated or prevented by the presence of SOD plus CAT, whereas those by H_2O_2 were attenuated by the presence of CAT plus mannitol, but not by CAT alone (Table 1) [50]. The impact of oxidant effect on $[Ca^{2+}]i$ is demonstrated by the data presented in Table 2. It was observed that the H2O2-induced increase in $[Ca^{2+}]i$ is concentration dependent (Table 2A) [51]. In contrast, incubation of cardiomyocytes with CAT before exposure to H_2O_2 attenuated the H_2O_2-induced increase in $[Ca^{2+}]i$. It should be noted that mannitol did not exert any effect on the H_2O_2-induced increase in $[Ca^{2+}]i$ (Table 2B) [51]. Taken together, it can be inferred that the formation of H_2O_2 in different cardiac pathologies can induce changes in Ca^{2+}-homeostasis in cardiomyocytes and induce cardiac contractile dysfunction.

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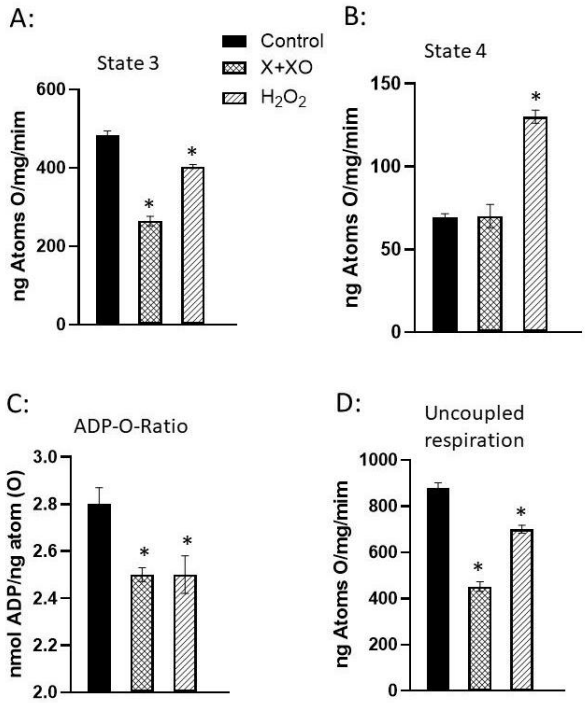

Figure 5. Mitochondrial respiration and oxidative phosphorylation activities of rat hearts perfused with xanthine +xanthine oxidase or H_2O_2. Hearts were perfused with 2 mM X and 60 mU/ml XO or with 100 μM H_2O_2 for 30 min. Data are taken from our paper [50]. Values are mean ± SE of 3 experiments. * = $p < 0.05$. Abbreviations: X = xanthine; XO = xanthine oxidase; H_2O_2 = hydrogen peroxide.

Table 1. Modification of ROS-induced mitochondrial oxidative phosphorylation by antioxidants.

	ADP-to-O Ratio (nmol ADP/ ng atom O)	Uncoupled Respiration (ng atoms O/min/mg protein)
A. X+XO Effects		
Control	3.0.6±0.15	575±9
X+XO	2.55±0.07*	196±7*
X+XO+SOD+CAT	2.81±2.0.04#	426±30*#
B. H_2O_2 Effects		
Control	3.13±0.09	543±29
H_2O_2	2.37±0.03*	153±5*
H_2O_2+CAT	2.52±0.04*	170±7*
H_2O_2+CAT+MAN	2.84±0.11#	195±12*#

Mitochondria isolated from unperfused hearts were incubated with 0.3 mM xanthine (X) and 11 mU xanthine oxidase (XO) for 3 min at 37°C. For antioxidant treatment, mitochondria were exposed for 2 min in the presence of 50 U/ml SOD and 50 U/ml CAT before exposing to X plus XO for 2 min. To study the effects of H_2O_2, mitochondria were incubated with 30 μM concentration of H_2O_2 for 3 min. The effect of CAT (8 mU/ml) or mannitol (20 mM) was examined by pretreatment of mitochondria for 2 min before exposing to 20 μM H_2O_2 for 3 min. All these preparations were washed twice and resuspended in a buffer to measure respiratory activities. *$p < 0.05$ vs. control; # $p < 0.05$ vs. respective value in the presence of X+XO or H_2O_2 alone. Values are means ± SE of 8 experiments. Data are from our paper Makazan et al [50].

Table 2. Modification of H_2O_2-induced increase in intracellular Ca^{2+} concentration by antioxidants.

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	Increase in $[Ca^{2+}]_i$ in cardiomyocytes (% of control)
A. H_2O_2-induced $[Ca^{2+}]i$	
Control	100
0.25 mM	141±11*
0.5 mM	168±17*
0.75 mM	216±12*
1.0 mM	240±23*
B. Antioxidants on H_2O_2-induced $[Ca^{2+}]i$	
Control	52.8±4.7
CAT	14.6±2.0*
MAN	48.9±5.6
CAT+MAN	8.7±2.5*

Concentration-dependent effects of H_2O_2 on rat cardiomyocyte $[Ca^{2+}]_i$. Data shown in (A) were recorded 10 minutes after incubation of Fura-2-loaded cells (10~/mL) with different concentrations of H_2O_2. Fura-2-loaded cardiomyocytes (10~ cells/mL) were treated with 10 μg/ml catalase (CAT), 20 mM mannitol (MAN) or both and blank buffer (control) for 10 minutes before exposure to 0.5 mM H_2O_2. The concentration of Ca2+ in the incubation medium was 1 mM. Fluorescent signals were recorded 10 minutes after the addition of H_2O_2 (B). Control value for $[Ca^{2+}]_i$ is 120.9±8.1 mM. Data are expressed as means ±SEM of 6-8 experiments. *$p < 0.05$ vs. control. The data are taken from our paper Wang et al [51].

3. Development of Mitochondrial Ca^{2+}-Overload due to Oxidative Stress

Mitochondria participate in I/R-injury due to oxidative stress and dysregulation of Ca^{2+}-homeostasis [52]. In addition, following I/R, cardiomyocytes accumulate high levels of peroxides, leading to mitochondrial dysfunction and induction of ferroptosis and exacerbation of ROS production and oxidative stress [53]. The oxidative stress induced abnormalities in Ca^{2+}-handling are known to lead to mitochondrial Ca^{2+}-overload resulting in impaired mitochondrial production of energy [54–56]. Interestingly, mitochondrial ATPase inhibitory factor-1, which is increased under conditions of oxidative stress has been reported to disturb mitochondrial Ca^{2+}-handling; however, the loss of mitochondrial Ca^{2+} uniporter (mCUP) has been reported to trigger arrhythmias attributed to a probable effect on SR Ca^{2+}-handling [57,58]. Interestingly, in I/R-injury, Ca^{2+}-influx in to mitochondria is considered to occur through the mCUP; however, deletion of the mCUP has been reported to result in an increase in mitochondrial Ca^{2+}, suggesting that some other mechanism may also be involved in Ca^{2+}-influx [59]. It should also be mentioned that a defect in the cross talk between mitochondrial function and control of ryanodine-receptor-mediated SR Ca^{2+}-release has been linked to an increase in the risk of arrhythmia in heart disease [60]. Clearly, targeting Ca^{2+}-homeostasis in cardiomyocytes and mitochondrial Ca^{2+}-overload due to oxidative stress would be seen as beneficial in attenuating calcium dysregulation in heart disease, including myocardial infarction, heart failure and cardiomyopathies [61].

The sensitivity of mitochondria to Ca^{2+} concentrations is critical, as both excessive and deficient Ca^{2+} levels can impair mitochondrial oxidative phosphorylation. High-glucose conditions in cardiomyocytes have been shown to reduce mCUP expression, decrease mitochondrial Ca^{2+} levels, and alter glucose and lipid metabolic profiles, further compromising cardiac function [62]. Mitochondrial Ca^{2+}-overload, in turn, contributes to oxidative stress, which exacerbates mitochondrial dysfunction and creates a vicious cycle of cellular injury. This cascade ultimately leads to apoptosis or necrosis, further impairing both systolic and diastolic heart function [63]. In the context of diabetic cardiomyopathy, the role of mCUP and its regulatory subunit, mitochondrial

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calcium uptake protein 1 (MICU1), has emerged as a critical factor in Ca^{2+}-transport. It has been shown that in diabetic mice, there is an upregulation of MICU1 expression in the heart, accompanied by a downregulation of MCU and associated regulatory proteins, such as EMRE, a key mCUP subunit. This imbalance leads to compromised mitochondrial Ca^{2+}- uptake, diminished mitochondrial function, and consequently reduced cardiac performance.

Mitochondria Ca^{2+} accumulation serves as a key trigger of mitochondrial dysfunction, especially when it occurs in the presence of additional stressors such as oxidative or nitrosative stress [64]. Ca^{2+} signaling has emerged as a critical modulator of mitochondrial function, with evidence indicating that Ca^{2+} contributes to the initiation of mitochondrion-dependent apoptosis [65]. Mitochondria serve as both ATP producers and crucial intracellular Ca^{2+} buffers. The mCUP, located on the inner mitochondrial membrane, plays a pivotal role in mediating Ca^{2+} influx into the mitochondrial matrix. Under normal physiological conditions, even modest fluctuations in Ca^{2+} levels are sufficient to activate dehydrogenases like FoF1-ATP, promoting ATP synthesis. However, under pathophysiological conditions, these processes are disrupted. Inositol trisphosphate receptors (IP_3Rs) are essential for maintaining intracellular calcium homeostasis. The release of Ca^{2+} from IP_3Rs functions as a second messenger, orchestrating various intracellular processes and inter-organellar communication in both physiological and pathological contexts. Over activation of IP_3Rs has been linked to the pathogenesis of several cardiac disorders, including ischemia, diabetes-induced arrhythmias, and cardiac hypertrophy. Dysregulated Ca^{2+} signaling within cytosolic, mitochondrial, and nucleoplasmic compartments contributes to the progression of these diseases [66]. Interestingly, an adaptive mechanism through which mitochondria mitigate ROS-induced damage is apoptosis induction. This process involves an increase in mitochondrial outer membrane permeability, leading to solute and water influx into the matrix, loss of membrane potential, cessation of ATP synthesis, and excessive mitochondrial calcium uptake—culminating in complete mitochondrial failure [67].

4. Mitochondrial Metabolic Alterations and Mitochondrial Dynamics

It is well established that mitochondrial dysfunction is a hallmark of cardiovascular diseases (CVDs), manifesting as impaired oxidative phosphorylation, excessive ROS production, altered calcium signaling, and disrupted metabolic homeostasis. Conditions such as ischemia-reperfusion injury, hypertension and diabetic cardiomyopathy are associated with compromised mitochondrial energetics and structural integrity, culminating in cardiac contractile dysfunction [68–74]. The heart is an energetically demanding organ, necessitating a continuous and substantial supply of adenosine triphosphate (ATP) to sustain contractile function. Despite its limited ATP reserves, the heart maintains an exceptionally efficient bioenergetic system, predominantly driven by mitochondria, which constitute nearly 30% of cardiomyocyte volume. These organelles facilitate ATP generation through oxidative phosphorylation, orchestrating substrate oxidation, electron transport, and ATP synthesis to meet the heart's metabolic demands [75–79]. Mitochondrial function is intricately linked to substrate availability, with ATP synthesis reliant on the oxidation of fatty acids, glucose, ketone bodies, and amino acids. It should be mentioned that biologically active amines such as spermine and agmatine have distinct roles in mitochondrial function that differentiate them from other amines; notably, spermine undergoes oxidative deamination by amine oxidases, producing ROS, which may further exacerbate the opening of the MPTP and contribute to apoptosis [44].

In the healthy myocardium, fatty acid oxidation contributes approximately 60–70% of ATP production, while glucose metabolism accounts for 20–30% [80–83]. The efficiency of ATP synthesis per unit of oxygen is higher for glucose than for fatty acids, a critical factor under hypoxic or ischemic conditions [84–87]. The intrinsic compensatory mechanisms that regulate intracellular calcium and the antioxidant defense system, which typically maintain mitochondrial substrate oxidation and ATP generation, become insufficient in the context of chronic cardiac dysfunction [88–90]. It should be mentioned that insulin resistance in diabetes reduces glucose transporter expression and pyruvate dehydrogenase activity, shifting myocardial energy reliance towards fatty acid β-oxidation. This metabolic shift increases oxygen consumption while decreasing ATP yield efficiency, predisposing

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mitochondria to oxidative stress and lipotoxic damage [91–94]. Prolonged metabolic perturbations, including excessive fatty acid uptake and β-oxidation inefficiencies, promote lipid accumulation, mitochondrial dysfunction, and cardiomyocyte apoptosis. These maladaptive changes contribute to myocardial energy deficits, compromised contractility, and heightened susceptibility to heart failure [95–100]. In addition, the chronic dysregulation of glycolipid metabolism in diabetes leads to both excessive ROS production and impaired ROS clearance. Mitochondria serve as the primary source of ROS in diabetic cardiomyocytes, and their dysfunction perpetuates a vicious cycle of oxidative damage. This process severely compromises cardiomyocyte function and survival by exacerbating metabolic disturbances, energy depletion, and oxidative stress-driven apoptosis [101]. However, it should be noted that chronic hyperglycemia during diabetes, further increases mitochondrial ROS production and impairs endogenous antioxidant defense mechanisms thus leading to excessive apoptosis and myocardial dysfunction [102,103]. A schematic diagram indicating the role of oxidative stress in inducing mitochondrial metabolic changes associated with depression in energy stores, cellular death and lipid deposits in cardiomyocytes and subsequent cardiac dysfunction in diseased hearts is shoen in Figure 6.

It should be pointed out that mitochondrial generated oxidative stress, signal transduction, metabolic reprogramming, regulation of iron and cell death depend on the mitochondrial quality control (MQC) system that includes mitochondrial dynamics (fission and fusion cycles) and mitochondrial biogenesis to maintain structural integrity and cardiac function [104–106]. Thus, targeting mitochondrial bioenergetics and metabolic flexibility represents a promising therapeutic strategy for mitigating CVD progression and preserving cardiac function. The mitochondrial ribosomal protein S5 (MRPS5/uS5m) is essential for maintaining mitochondrial protein translation and oxidative phosphorylation. Loss of MRPS5 in the developing heart leads to embryonic lethality, while postnatal loss impairs oxidative phosphorylation and mitochondrial protein synthesis, contributing to cardiac hypertrophy and heart failure [107]. Mitochondrial dynamics, including fusion and fission processes, are integral to maintaining mitochondrial function and integrity [108]. Fusion proteins such as Mfn-1, Mfn-2, and OPA-1 are essential for mitochondrial stability, as their inhibition leads to dilated cardiomyopathy, contractile dysfunction, increased apoptosis, and mitochondrial fragmentation [109]. Deletion of these fusion proteins results in abnormal mitochondrial morphology, ventricular wall thickening, and eccentric hypertrophy. Conversely, excessive mitochondrial fission disrupts mitochondrial mass, impairs oxidative phosphorylation, and results in ATP deficits, mitochondrial permeabilization, cytochrome C release, and apoptosis. The absence of dynamin-related protein 1 (Drp1), a key fission protein, results in lethal dilated cardiomyopathy [110], further highlighting the critical balance between fusion and fission in maintaining cardiac mitochondrial function.

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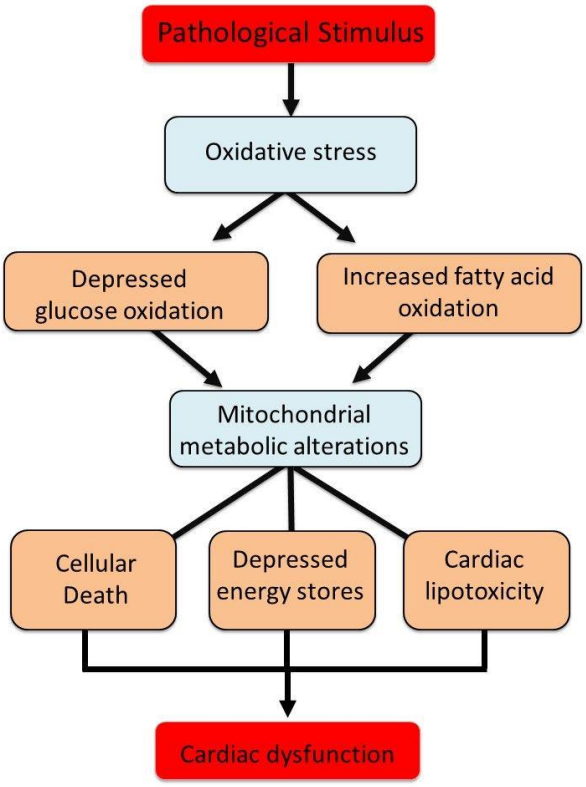

Figure 6. Involvement of oxidative stress in inducing changes in mitochondrial metabolism for the occurrence of cardiac dysfunction due to pathological stimulus. .

Alterations in mitochondrial ultrastructure and bioenergetics are widely observed in heart failure patients, particularly in the later disease stages. These changes include reductions in the activities of respiratory chain complexes (I–IV) and impaired oxidative phosphorylation capacity. Interestingly, in cases of chronic hypertrophy without systolic dysfunction, mitochondrial function appears to be preserved or even enhanced in both animal models and human studies [111–122]. Initial stages of cardiac hypertrophy are often characterized by an increase in oxidative phosphorylation activity, which gradually declines as the disease progresses toward heart failure [16,123]. A reduction in the expression of key oxidative phosphorylation components has been linked to mitochondrial respiratory deficits in heart failure and cardiomyopathies [124,125]. It is pointed out that in ventricular fibrillation, mitochondrial damage activates the mitochondrial apoptotic pathway, characterized by the release of cytochrome C into the cytosol, a reduction in caspase-9 levels, and the subsequent activation of caspase-3. This cascade coincides with significant impairments in LV function. Notably, cytochrome C "leaks" into the bloodstream, and its concentration is inversely proportional to survival outcomes [126]. Taken together, these findings underline the critical role of mitochondria during cardiac resuscitation by modulating both energy metabolism and apoptotic signaling pathways, positioning mitochondrial-targeted therapies as promising strategies for enhancing outcomes during cardiac resuscitation [126].

5. Novel Interventions Targeting Mitochondria in Different Cardiac Pathologies

In view of the fact that mitochondria constitute 30-40% of the cardiomyocyte [127], and that mitochondria are considered as the key determinants of cardiac injury and dysfunction [128–132], mitochondria represent a viable target for therapeutic intervention. For example, the regulation of mitochondrial Ca^{2+}- uptake and the interplay between various molecules and pathways offer promising avenues for therapeutic intervention. Mitochondria have been suggested to exhibit a cardioprotective role due to the presence of KATP channels [133]. In this regard, cardioprotection via

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hypoxic preconditioning or exposure to the ATP-dependent K^+- channel opener diazoxide increases mitochondrial resistance to oxidative damage. Thus, targeting the MPTP, either by direct inhibition or modulation of mitochondrial stressors, represents a promising therapeutic approach for conditions such as I/R-injury [65,134]. Interestingly, the reperfusion injury salvage kinase (RISK) pathway for cardioprotection involves the prevention of the opening of the membrane permeability transition pore and subsequent attenuation of cell death [135]. Thus, this pathway has emerged as an important cardioprotective target in I/R-injury.

In reperfusion-induced injury is a significant challenge during cardiac surgery, coronary thrombosis treatment, and stroke management. Preventing MPTP opening, either directly with agents like cyclosporine A or indirectly by reducing oxidative stress or Ca^{2+}- overload, represents a potential therapeutic strategy to mitigate reperfusion injury. Additionally, mice deficient in Cyclophilin D (CyP-D), a critical component of the MPTP, are protected from ischemia/reperfusion injury in the heart, further substantiating the role of MPTP in mediating cellular injury [136]. In less severe cellular insults, the MPTP may open transiently, leading to mitochondrial swelling sufficient to trigger cytochrome C release and activation of the apoptotic pathway, rather than necrosis. However, CyP-D knockout mice do not exhibit enhanced protection against a broad range of apoptotic stimuli, suggesting that the MPTP is not universally involved in apoptosis [136]. Recently, it has been suggested that circadian rhythm may play an important role in the control of I/R-injury [137]. MiRNAs regulate mitochondrial apoptosis through an effect on mitochondrial fission and fusion, generation of ROS and dysregulating Ca^{2+}-homeostasis [138]. Interestingly, it has been suggested that mitochondrial transplantation has the potential to exert beneficial actions in I/R-injury; however, clinical application is limited [139]. On the other hand, mitochondrial regeneration can be seen to increase oxidative phosphorylation and decrease oxidative stress and thus may be of clinical value under conditions of ischemic insult of the heart [140]. In fact, targeting MQC has emerged as a promising target in mitigating hypoxia-related cardiac dysfunction [141].

In the context of diabetic cardiomyopathy, the role of mCUP and its regulatory subunit, mitochondrial calcium uptake protein 1 (MICU1), has emerged as a critical factor in Ca^{2+}- transport. Studies have shown that in diabetic mice, there is an upregulation of MICU1 expression in the heart, accompanied by a downregulation of MCU and associated regulatory proteins, such as EMRE, a key mCUP subunit. This imbalance leads to compromised mitochondrial Ca^{2+}- uptake, diminished mitochondrial function, and, consequently, reduced cardiac performance. Importantly, restoring MCU expression has been shown to ameliorate both mitochondrial and cardiac dysfunction, highlighting the therapeutic potential of restoring mitochondrial Ca^{2+} homeostasis in diabetic cardiomyopathy treatment [142,143]. Distinct isoforms of IP_3Rs, IP_3R1 and IP_3R2, exhibit different roles in cardiac pathology. IP_3R1 is particularly involved in cardiac ischemia and arrhythmias associated with diabetes, while IP_3R2 is implicated in sepsis-induced cardiomyopathy and hypertrophy [66]. Thus, IP_3Rs have been shown to play pivotal roles in various forms of cell death, such as apoptosis, pyroptosis, and ferroptosis, underlining their multifaceted involvement in cardiac disease. Targeting IP_3Rs, either through genetic manipulation or pharmacological inhibition using IP_3R antagonists, has emerged as a promising therapeutic strategy to mitigate IP_3R-related pathologies, offering potential for therapeutic intervention in CVD [66].

6. Conclusion

From the foregoing discussion, it is evident that mitochondria are not only involved in energy production, but are also the major source of oxidative stress production as well as intracellular Ca^{2+}-accumulation. In fact, the development of oxidative stress and the occurrence of mitochondrial Ca^{2+}-overload are the main mechanisms for induction of energy store depletion and cardiac dysfunction. It is noteworthy that ROS generated by the activation of sarcolemmal NADPH oxidase 2 as well as extra-mitochondrial (endothelial cells, serum, cytosol) xanthine oxidase are also considered to promote the generation of mitochondrial oxidative stress during the development of heart disease [7,8,144–147]. It should be mentioned that mitochondria are known to contain different components

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for the production of ROS such as the electron transport chain, NADPH oxidase 4 and monoamine oxidase, in addition to endogenous antioxidants such as SOD, CAT and glutathione peroxidase. Particularly, it may also be noted that there occurs accumulation of different vasoactive hormones such as angiotensin II and endothelin (activators of NADPH oxidase 4) as well as catecholamines and serotonin (substrates for oxidation by MAO) in cardiomyocytes of diseased hearts. Furthermore, the occurrence of mitochondrial Ca^{2+}- overload has been associated with the activation of MAO and the induction of defects in electron transport system in mitochondria in ischemic heart disease.

There is now a wealth of information that has demonstrated that mitochondrial Ca^{2+}- overload and increased generation of ROS are central features in cardiac dysfunction in different cardiac pathologies including heart failure, diabetic cardiomyopathy and ischemia-reperfusion injury. Although mitochondria accumulate high amounts of Ca^{2+} and thus serve as an intracellular Ca^{2+} reservoir, abnormalities in the processes involved in energy production through oxidative phosphorylation produce an oxidative stress that impacts the structural and functional integrity of the cell. Indeed, ROS-induced ROS production by mitochondria exacerbates ROS generation and severity of oxidative stress. The mitochondria-generated ROS as a consequence of mitochondrial Ca^{2+}-overload leads to further deterioration of mitochondrial function. Accordingly, the mitochondria present viable therapeutic target for the prevention of cardiac dysfunction in at-risk population. Therefore, the development of specific interventions that are effective in attenuating mitochondrial metabolic alterations as well as the development of novel antioxidants that target mitochondrial ROS generating systems could be highly beneficial.

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References

1. Bolli, R.; Marban, E. Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* **1999**, *79*, 609-634.
2. Bolli, R.; Jeroudi, M.O.; Patel, B.S.; Aruoma, O.I.; Halliwell, B.; Lai, E.K.; McCay, P.B. Marked reduction of free radical generation and contractile dysfunction by antioxidant therapy begun at the time of reperfusion. Evidence that myocardial "stunning" is a manifestation of reperfusion injury. *Circ Res* **1989**, *65*, 607-622.
3. Dhalla, N.S.; Elmoselhi, A.B.; Hata, T.; Makino, N. Status of myocardial antioxidants in ischemia-reperfusion injury. *Cardiovasc Res* **2000**; *47*, 446-456.
4. Jennings, R.B.; Reimer, K.A. The cell biology of acute myocardial ischemia. Annu Rev Med 1991, 42, 225–246.
5. Piper, H.M.; Meuter, K.; Schafer, C. Cellular mechanisms of ischemia reperfusion injury. *Ann Thorac Surg* **2003**, *75*, S644-S648.
6. Dridi, H.; Santulli, G.; Bahlouli, L.; Miotto, M.C.; Weninger, G.; Marks, A.R. Mitochondrial calcium overload plays a causal role in oxidative stress in the failing heart. *Biomolecules* **2023**, *13*, 1409.
7. Dhalla, N.S.; Ostadal, P.; Tappia, P.S. Involvement of oxidative stress and antioxidants in modification of cardiac dysfunction due to ischemia-reperfusion injury. *Antioxidants* **2025**, *14*,340.
8. Dhalla, N.S.; Elimban, V.; Bartekova, M.; Adameova, A. Involvement of oxidative stress in the development of subcellular defects and heart disease. Biomedicines 2022, *10*, 393.
9. Kukreja, R.C.; Weaver, A.B.; Hess, M.L. Sarcolemmal Na-K-ATPase: inactivation by neutrophil-derived free radicals and oxidants. *Am J Physiol Heart Circ Physiol* **1990**, *259*, H1330-H1336.

 doi:10.20944/preprints202504.0012.v1

10. Ostadal, P.; Elmoselhi, A.B.; Zdobnicka, I.; Lukas, A.; Elimban, V.; Dhalla, N.S. Role of oxidative stress in ischemia-reperfusion-induced changes in Na-K ATPase isoform expression in rat heart. *Antioxid Redox Signal* **2004**, *6*, 914-923.
11. Saini, H.K.; Dhalla, N.S. Defective calcium handling in cardiomyocytes isolated from hearts subjected to ischemia-reperfusion. *Am J Physiol Heart Circ Physiol* **2005**, *288*, H2260-H2270.
12. Saini, H.K.; Elimban, V.; Dhalla, N.S. Attenuation of extracellular ATP response in cardiomyocytes isolated from hearts subjected to ischemia reperfusion. *Am J Physiol Heart Circ Physiol* **2005**, *289*, H614-H623.
13. Temsah, R.M.; Netticadan, T.; Chapman, D.; Takeda, S.; Mochizuki, S.; Dhalla, N.S. Alterations in sarcoplasmic reticulum function and gene expression in ischemic reperfused rat heart. *Am J Physiol Heart Circ Physiol* **1999**, *277*, H584-H594.
14. Zucchi, R.; Ronca-Testoni, S.; Yu, G.; Galbani, P.; Ronca, G.; Mariani, M. Effect of ischemia and reperfusion on cardiac ryanodine receptors sarcoplasmic reticulum Ca^{2+} channels. *Circ Res* **1994**, *74*, 271-280.
15. Ferrari R. The role of mitochondria in ischemic heart disease. *J Cardiovasc Pharmacol* **1996**, *28 Suppl 1*, S1–S10.
16. Lesnefsky, E.J.; Moghaddas, S.; Tandler, B.; Kerner, J.; Hoppel, C.L. Mitochondrial dysfunction in cardiac disease: ischemia-reperfusion, aging, and heart failure. *J Mol Cell Cardiol* **2001**, *33*, 1065–1089.
17. Williamson, J.R. Mitochondrial function in the heart. *Annu Rev Physiol* **1979**, *41*, 485-506.
18. Bhullar, S.K.; Dhalla, N.S. Status of mitochondrial oxidative phosphorylation during the development of heart failure. *Antioxidants* **2023**, *12*, 1941.
19. Giorgi, C.; Marchi, S.; Pinton, P. The machineries, regulation and cellular functions of mitochondrial calcium. Nat. Rev. *Mol Cell Biol* **2018**, *19*, 713-730.
20. Lopez-Crisosto, C.; Pennanen, C.; Vasquez-Trincado, C.; Morales, P.E.; Bravo-Sagua, R.; Quest, A.F.G.; Chiong, M.; Lavandero, S. Sarcoplasmic reticulum–mitochondria communication in cardiovascular pathophysiology. *Nat Rev Cardiol* **2017**, *14*, 342-360.
21. Bravo-San Pedro, J.M.; Kroemer, G.; Galluzzi, L. Autophagy and mitophagy in cardiovascular disease. *Circ Res* **2017**, *120*, 1812-1824.
22. Budde, H.; Hassoun, R.; Tangos, M.; Zhazykbayeva, S.; Herwig, M.; Varatnitskaya, M.; Sieme, M.; Delalat, S.; Sultana, I.; Kolijn,D.; et al. The interplay between S-glutathionylation and phosphorylation of cardiac troponin I and myosin binding protein C in end-stage human failing hearts. *Antioxidants* **2021**, *10*, 1134.
23. Leichert, L.I.; Gehrke, F.; Gudiseva, H.V.; Blackwell, T.; Ilbert, M.; Walker, A.K.; Strahler, J.R.; Andrews, P.C.; Jakob, U. Quantifying changes in the thiol redox proteome upon oxidative stress in vivo. *Proc Natl Acad Sci* **2008**, *105*, 8197-8202.
24. Delbridge, L.M.D.; Mellor, K.M.; Taylor, D.J.; Gottlieb, R.A. Myocardial stress and autophagy: Mechanisms and potential therapies. *Nat Rev Cardiol* **2017**, *14*, 412-425.
25. Ballinger, S.W. Mitochondrial dysfunction in cardiovascular disease. *Free Radic Biol Med* **2005**, *38*, 1278-1295.
26. Tani, M. Effects of anti-free radical agents on Na, Ca^{2+}, and function in reperfused rat hearts. *Am J Physiol Heart Circ Physiol* **1990**, *259*, H137-H143.
27. Mialet-Perez, J.; Parini, A. Cardiac monoamine oxidases: at the heart of mitochondrial dysfunction. *Cell Death Dis* **2020**, *11*, 54.
28. Ferrari, R.; Pedersini, P.; Bongrazio, M.; Gaia, G.; Bernocchi, P.; Di Lisa, F.; Visioli, O. Mitochondrial energy production and cation control in myocardial ischaemia and reperfusion. *Basic Res Cardiol* **1993**, *88*, 495-512.
29. Long, X.; Goldenthal, M.J.; Wu, G.M.; Marin-Garcia, J. Mitochondrial Ca^{2+}-flux and respiratory enzyme activity decline are early events in cardiomyocyte response to H_2O_2. *J Mol Cell Cardiol* **2004**, *37*, 63-70.
30. Stevenson, M.D.; Canugovi, C.; Vendrov, A.E.; Hayami, T.; Bowles, D.E.; Krause, K.H.; Madamanchi, N.R.; Runge, M.S. NADPH oxidase 4 regulates inflammation in ischemic heart failure: Role of soluble epoxide hydrolase. *Antioxid Redox Signal* **2019**, *31*, 39-58.
31. Landmesser, U.; Spiekermann, S.; Preuss, C.; Sorrentino, S.; Fischer, D.; Manes, C.; Mueller, M.; Drexler, H. Angiotensin II induces endothelial xanthine oxidase activation: role for endothelial dysfunction in patients with coronary disease. *Arterioscler Thromb Vasc Biol* **2007**, *27*, 943-948.

 doi:10.20944/preprints202504.0012.v1

32. Dia, M.; Gomez, L.; Thibault, H.; Tessier, N.; Leon, C.; Chouabe, C.; Ducreux, S.; Gallo-Bona, N.; et al. Reduced reticulum-mitochondria Ca^{2+} transfer is an early and reversible trigger of mitochondrial dysfunctions in diabetic cardiomyopathy. *Basic Res Cardiol* **2020**, *115*, 74.
33. Stowe, D.F.; Camara, A.K.S. Mitochondrial reactive oxygen species production in excitable cells: Modulators of mitochondrial and cell function. *Antioxid Redox Signal* **2009**, *11*, 1373-1414.
34. Marí, M.; Morales, A.; Colell, A.; García-Ruiz, C.; Fernández-Checa, J.C. Mitochondrial glutathione, a key survival anti-oxidant. *Antioxid Redox Signal* **2009**, *11*, 2685-2700.
35. Scialò, F.; Sriram, A.; Stefanatos, R.; Spriggs, R.V.; Loh, S.H.Y.; Martins, L.M.; Sanz, A. Mitochondrial complex I derived ROS regulate stress adaptation in Drosophila melanogaster. *Redox Biology*, **2020**, *32*, 101450.
36. Zhang, H.; Dhalla, N.S. The Role of pro-inflammatory cytokines in the pathogenesis of cardiovascular disease. *Int J Mol Sci* **2024**, *25*, 1082.
37. Hori, M.; Nishida, K. Oxidative stress and left ventricular remodelling after myocardial infarction. *Cardiovasc Res* **2009**, *81*, 457-464.
38. Wang, H.; Han, J.; Dmitrii, G.; Zhang, X.A. Potential targets of natural products for improving cardiac ischemic injury: The role of Nrf2 signaling transduction. *Molecules* **2024**, *29*, 2005.
39. Qin, F.; Yan, C.; Patel, R.; Liu, W.; Dong, E. Vitamins C and E attenuates apoptosis, β-adrenergic receptor desensitization, and sarcoplasmic reticular Ca^{2+} ATPase downregulation after myocardial infarction. *Free Radic Biol Med* **2006**, *40*, 1827-1842.
40. Shao, Q.; Ren, B.; Elimban, V.; Tappia, P.S.; Takeda, N.; Dhalla, N.S. Modification of sarcolemmal Na^+-K^+-ATPase and Na^+/Ca^{2+} exchanger in heart failure by blockade of renin-angiotensin system. *Am J Heart Physiol Circ Physiol* **2005**, *288*, H2637-H2646.
41. Li, Q.; Pogwizd, S.M.; Prabhu, S.D.; Zhou, L. Inhibiting Na^+-K^+ ATPase can impair mitochondrial energetics and induce abnormal Ca^{2+} cycling and automaticity in guinea pig cardiomyocytes. *PLoS ONE* **2014**, *9*, e93928.
42. Crow, M.T.; Mani, K.; Nam, Y.J.; Kitsis, R.N. The mitochondrial death pathway and cardiac myocyte apoptosis. *Circ Res* **2004**, *95*, 957-970.
43. Bertero, E.; Popoiu, T.A.; Maack, C. Mitochondrial calcium in cardiac ischemia/reperfusion injury and cardioprotection. *Basic Res Cardiol* **2024**, *119*, 569-585.
44. Grancara, S.; Ohkubo, S.; Artico, M.; Ciccariello, M.; Manente, S.; Bragadin, M.; Toninello, A.; Agostinelli, E. Milestones and recent discoveries on cell death mediated by mitochondria and their interactions with biologically active amines. *Amino Acids* **2016**, *48*, 2313-2326.
45. Morciano, G.; Pinton, P. Modulation of mitochondrial permeability transition pores in reperfusion injury: Mechanisms and therapeutic approaches. *Eur J Clin Invest* **2025**, *55*, e14331.
46. Circu, M.L.; Aw, T.Y. Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med* **2010**, *48*, 749-762.
47. Ferko, M.; Alanova, P.; Janko, D.; Opletalova, B.; Andelova, N. Mitochondrial peroxiredoxins and monoamine oxidase-A: Dynamic regulators of ROS signaling in cardioprotection. *Physiol Res* **2024**, *73*, 887-900.
48. Popoiu TA, Maack C, Bertero E. Mitochondrial calcium signaling and redox homeostasis in cardiac health and disease. *Front Mol Med* **2023**, *3*, 1235188.
49. Tappia, P.S.; Dent, M.R.; Dhalla, N.S. Oxidative stress and redox regulation of phospholipase D in myocardial disease. *Free Radic Biol Med* **2006**, *41*, 349-361.
50. Makazan, Z.; Saini, H.K.; Dhalla, N.S. Role of oxidative stress in alterations of mitochondrial function in ischemic-reperfused hearts. *Am J Physiol Heart Circ Physiol* **2007**, *292*, H1986-H1994.
51. Wang, X.; Takeda, S.; Mochizuki, S.; Jindal, R.; Dhalla, N.S. Mechanisms of hydrogen peroxide-induced increase in intracellular calcium in cardiomyocytes. *J Cardiovasc Pharmacol Ther* **1999**, *4*, 41-48.
52. Zhang, C.; Chang, X.; Zhao, D.; He, Y.; Dong, G.; Gao, L. Mitochondria and myocardial ischemia/reperfusion injury: Effects of Chinese herbal medicine and the underlying mechanisms. *J Pharm Anal* **2025**, *15*, 101051.

 doi:10.20944/preprints202504.0012.v1

53. Wang, R.; Chen, X.; Li, X.; Wang, K. Molecular therapy of cardiac ischemia-reperfusion injury based on mitochondria and ferroptosis. *J Mol Med* **2023**, *101*, 1059-1071.
54. Cortassa, S.; Juhaszova, M.; Aon, M.A.; Zorov, D.B.; Sollott, S.J. Mitochondrial Ca^{2+}, redox environment and ROS emission in heart failure: Two sides of the same coin? *J Mol Cell Cardiol* **2021**, *151*, 113-125.
55. Pavez-Giani, M.G.; Sanchez-Aguilera, P.I.; Bomer, n.; Miyamota, S.; Booij, H.G.; Giraldo, P.; Oberdorf-Maass, S.U.; Nijholt, K.T.; Yurista, S.R.; Milting, H.; et al. ATPase inhibitory factor-1 disrupts mitochondrial Ca^{2+} handling and promotes pathological cardiac hypertrophy through CaMKIIδ. *Int J Mol Sci* **2021**, *22*, 4427.
56. Bertero, E.; Nickel, A.; Kohlhaas, M.; Hohl, M.; Sequeira, V.; Brune, C.; Schwemmlein, J.; Abeber, M.; Schuh, K.; Kutschka, I.; et al. Loss of mitochondrial Ca^{2+} uniporter limits inotropic reserve and provides trigger and substrate for arrhythmias in Barth syndrome cardiomyopathy. *Circulation* **2021**, *144*, 1694-1713.
57. Donoso, P.; Sanchez, G.; Bull, R.; Hidalgo, C. Modulation of cardiac ryanodine receptor activity by ROS and RNS. *Front Biosci* **2011**, *16*, 553-567.
58. Tokuhisa, T.; Yano, M.; Obayashi, M.; Noma, T.; Mochizuki, M.; Oda, T.; Okuda, S.; Doi, M.; Liu, J.; Ikeda, Y.; et al. AT1 receptor antagonist restores cardiac ryanodine receptor function, rendering isoproterenol-induced failing heart less susceptible to Ca^{2+}-leak induced by oxidative stress. *Circ J* **2006**, *70*, 777-786.
59. Murphy, E.; Eisner, D.A. How does mitochondrial Ca^{2+} change during ischemia and reperfusion? Implications for activation of the permeability transition pore. *J Gen Physiol* **2025**, *157*, e202313520.
60. Hamilton S.; Terentyeva, R.; Clements, R.T.; Belevych, A.E.; Terentyev, D. Sarcoplasmic reticulum-mitochondria communication; implications for cardiac arrhythmia. *J Mol Cell Cardiol* **2021**, *156*, 105-113.
61. Morciano, G.; Rimessi, A.; Patergnani, S.; Vitto, V.A.M.; Danese, A.; Kahsay, A.; Palumbo, L.; Bonora, M.; et al. Calcium dysregulation in heart diseases: Targeting calcium channels to achieve a correct calcium homeostasis. *Pharmacol Res* **2022**, *177*, 106119.
62. Diaz-Juarez, J.; Suarez, J.; Cividini, F.; Scott, B.T.; Diemer, T.; Dai, A.; Dillmann, W.H. Expression of the mitochondrial calcium uniporter in cardiac myocytes improves impaired mitochondrial calcium handling and metabolism in simulated hyperglycemia. *Am J Physiol Cell Physiol* **2016**, *311*, C1005-C1013.
63. O-Uchi, J.; Ryu, S.Y.; Jhun, B.S.; Hurst, S.; Sheu, S.S. Mitochondrial ion channels/transporters as sensors and regulators of cellular redox signaling. *Antiox Redox Signal* **2014**, *21*, 987-1006.
64. Duchen, M.R. Roles of mitochondria in health and disease. *Diabetes* **2004**, *53*, S96-S102.
65. Pacher, P.; Csordás, G.; Hajnóczky, G. Mitochondrial Ca^{2+} signaling and cardiac apoptosis. *Biol Signals Recept* **2001**, *10*, 200-223.
66. Piamsiri, C.; Fefelova, N.; Pamarthi, S.H.; Gwathmey, J.K.; Chattipakorn, S.C.; Chattipakorn, N.; Xie, L.H. Potential roles of IP_3 receptors and calcium in programmed cell death and implications in cardiovascular diseases. *Biomolecules* **2024**, *14*, 1334.
67. Dhalla, N.S.; Temsah, R.M.; Netticadan, T. Role of oxidative stress in cardiovascular diseases. *J Hypertens* **2000**, *18*, 655-673.
68. Bell, E.L.; Klimova, T.A.; Eisenbart, J.; Moraes, C.T.; Murphy, M.P.; Budinger, G.S.; Chandel, N.S. The Qo site of the mitochondrial complex III is required for the transduction of hypoxic signaling via reactive oxygen species production. *J Cell Biol* **2007**, *177*, 1029-1036.
69. Stein, L.R.; Imai, S.-I. The dynamic regulation of NAD metabolism in mitochondria. Trends Endocrinol. *Metab* **2012**, *23*, 420-428.
70. Chen, W.W.; Birsoy, K.; Mihaylova, M.M.; Snitkin, H.; Stasinski, I.; Yucel, B.; Bayraktar, E.C.; Carette, J.E.; Clish, C.B.; Brum-melkamp, T.R.; et al. Inhibition of ATPIF1 ameliorates severe mitochondrial respiratory chain dysfunction in mammalian cells. *Cell Rep* **2014**, *7*, 27-34.
71. Wrogemann, K.; Nylen, E.G. Mitochondrial calcium overloading in cardiomyopathic hamsters. *J Mol Cell Cardiol* **1978**, *10*, 185-195.
72. Dhalla, N.S.; Lee, S.-L.; Shah, K.R.; Elimban, V.; Suzuki, S.; Jasmin, G. Behaviour of subcellular organelles during the development of congestive heart failure in cardiomyopathic hamsters (UM-X7. 1). In Cardiomyopathic Heart; New York Raven Press Ltd.: New York, NY, USA, 1994; pp. 1–14.

 doi:10.20944/preprints202504.0012.v1

73. Siasos, G.; Tsigkou, V.; Kosmopoulos, M.; Theodosiadis, D.; Simantiris, S.; Tagkou, N.M.; Tsimpiktsioglou, A.; Stampouloglou, P.K.; Oikonomou, E.; Mourouzis, K.; et al. Mitochondria and cardiovascular diseases—From pathophysiology to treatment. *Ann Transl Med* **2018**, *6*, 256.
74. Zhang, Y.; Marcillat, O.; Giulivi, C.; Ernster, L.; Davies, K.J. The oxidative inactivation of mitochondrial electron transport chain components and ATPase. *J Biol Chem* **1990**, *265*, 16330-16336.
75. Davis, R.E.; Williams, M. Mitochondrial function and dysfunction: An update. *J Pharmacol Exp Ther* **2012**, *342*, 598–607.
76. Van Der Bliek, A.M.; Sedensky, M.M.; Morgan, P.G. Cell biology of the mitochondrion. *Genetics* **2017**, *207*, 843–871.
77. Murphy, E.; Ardehali, H.; Balaban, R.S.; DiLisa, F.; Dorn, G.W.; Kitsis, R.N.; Otsu, K.; Ping, P.; Rizzuto, R.; Sack, M.N.; et al. Mitochondrial function, biology, and role in disease: A scientific statement from the American Heart Association. *Circ Res* **2016**, *118*, 1960-1991.
78. Galluzzi, L.; Kepp, O.; Trojel-Hansen, C.; Kroemer, G. Mitochondrial control of cellular life, stress, and death. *Circ Res* **2012**, *111*, 1198-1207.
79. Williamson, J.R. Mitochondrial heart function. *Ann Rev Physiol* **1979**, *41*, 485-506.
80. Mootha, V.K.; Bunkenborg, J.; Olsen, J.V.; Hjerrild, M.; Wisniewski, J.R.; Stahl, E.; Bolouri, M.S.; Ray, H.N.; Sihag, S.; Kamal, M.; et al. Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell* **2003**, *115*, 629-640.
81. Collins, T.J.; Berridge, M.J.; Lipp, P.; Bootman, M.D. Mitochondria are morphologically and functionally heterogeneous within cells. *EMBO J* **2002**, *21*, 1616-1627.
82. Glatz, J.F.; Nabben, M.; Young, M.E.; Schulze, P.C.; Taegtmeyer, H.; Luiken, J.J. Re-balancing cellular energy substrate metabolism to mend the failing heart. *Biochim Biophys Acta Mol Basis Dis* **2020**, *1866*, 165579.
83. Stanley, W.C.; Recchia, F.A.; Lopaschuk, G.D. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev* **2005**, *85*, 1093-1129.
84. Taylor, S.W.; Fahy, E.; Zhang, B.; Glenn, G.M.; Warnock, D.E.; Wiley, S.; Murphy, A.N.; Gaucher, S.P.; Capaldi, R.A.; Gibson, B.W.; et al. Characterization of the human heart mitochondrial proteome. *Nat Biotechnol* **2003**, *21*, 281-286.
85. Zhao, Q.; Sun, Q.; Zhou, L.; Liu, K.; Jiao, K. Complex Regulation of mitochondrial function during cardiac development. *J Am Heart Assoc* **2019**, *8*, e012731.
86. Pagliarini, D.J.; Rutter, J. Hallmarks of a new era in mitochondrial biochemistry. *Genes Dev* **2013**, *27*, 2615-2627.
87. Chandel, N.S. Mitochondria as signaling organelles. *BMC Biol* **2014**, *12*, 34.
88. Zhou, B.; Tian, R. Mitochondrial dysfunction in pathophysiology of heart failure. *J Clin Investig* **2018**, *128*, 3716-3726.
89. Neubauer, S. The failing heart-an engine out of fuel. *N Engl J Med* **2007**, *356*, 1140-1151.
90. Bertero, E.; Maack, C. Metabolic remodelling in heart failure. *Nat Rev Cardiol* **2018**, *15*, 457-470.
91. Ballinger, S.W. Mitochondrial dysfunction in cardiovascular disease. *Free Radic Biol Med* **2005**, *38*, 1278-1295.
92. Yang, J.; Guo, Q.; Feng, X.; Liu, Y.; Zhou, Y. Mitochondrial dysfunction in cardiovascular diseases: Potential targets for treatment. *Front Cell Dev Biol* **2022**, *10*, 841523.
93. Chistiakov, D.A.; Shkurat, T.P.; Melnichenko, A.A.; Grechko, A.V.; Orekhov, A.N. The role of mitochondrial dysfunction in cardiovascular disease: A brief review. *Ann Med* **2018**, *50*, 121-127.
94. Calbet, J.A.L.; Martín-Rodríguez, S.; Martin-Rincon, M.; Morales-Alamo, D. An integrative approach to the regulation of mitochondrial respiration during exercise: Focus on high-intensity exercise. *Redox Biol* **2020**, *35*, 101478.
95. Rosca, M.G.; Hoppel, C.L. Mitochondrial dysfunction in heart failure. *Heart Fail Rev* **2013**, *18*, 607-622.
96. Schwarzer, M.; Rohrbach, S.; Niemann, B. Heart and mitochondria: Pathophysiology and implications for cardiac surgeons. *Thorac Cardiovasc Surg* **2018**, *66*, 11-19.
97. Bonora, M.; Wieckowski, M.R.; Sinclair, D.A.; Kroemer, G.; Pinton, P.; Galluzzi, L. Targeting mitochondria for cardiovascular disorders: Therapeutic potential and obstacles. *Nat Rev Cardiol* **2019**, *16*, 33-55.
98. Borghetti, G.; von Lewinski, D.; Eaton, D.M.; Sourij, H.; Houser, S.R.; Wallner, M. Diabetic cardiomyopathy: Current and future therapies. Beyond glycemic control. *Front Physiol* **2018**, *9*, 1514.

 doi:10.20944/preprints202504.0012.v1

99. Ferrara, D.; Montecucco, F.; Dallegri, F.; Carbone, F. Impact of different ectopic fat depots on cardiovascular and metabolic diseases. *J Cell Physiol* **2019**, *234*, 21630-21641.
100. Paolisso, P.; Bergamaschi, L.; Saturi, G.; D'Angelo, E.C.; Magnani, I.; Toniolo, S.; Stefanizzi, A.; Rinaldi, A.; Bartoli, L.; Angeli, F.; et al. Secondary prevention medical therapy and outcomes in patients with myocardial infarction with non-obstructive coronary artery disease. *Front Pharmacol* **2020**, *10*, 1606.
101. Wang, X.L.; Gao, P.; Zou, Y.Z. The relationship between mitochondrial oxidative stress and diabetic cardiomyopathy. *Chin Clin. Pharmacol Therap* **2021**, *33*, 1080-1085.
102. Kaludercic, N.; Di Lisa, F. Mitochondrial ROS formation in the pathogenesis of diabetic cardiomyopathy. *Front Cardiovasc Med* **2020**, *7*, 12.
103. Evangelista, I.; Nuti, R.; Picchioni, T.; Dotta, F.; Palazzuoli, A. Molecular dysfunction and phenotypic derangement in diabetic cardiomyopathy. *Int J Mol Sci* **2019**, *20*, 3264.
104. Biala, A.K.; Dhingra, R.; Kirshenbaum, L.A. Mitochondrial dynamics: Orchestrating the journey to advanced age. *J Mol Cell Cardiol* **2015**, *83*, 37-43.
105. Chang, X.; Li, Y.; Cai, C.; Wu, F.; He, J.; Zhang, Y.; Zhong, J.; Tan, Y.; Liu, R.; Zhu, H.; Zhou, H. Mitochondrial quality control mechanisms as molecular targets in diabetic heart. *Metabolism* **2022**, *137*, 155313.
106. Tokuyama, T.; Yanagi, S. Role of mitochondrial dynamics in heart diseases. *Genes* **2023**, *14*, 1876.
107. Gao, F.; Liang, T.; Lu, Y.W.; Fu, X.; Dong, X.; Pu, L.; Hong, T.; et al. A defect in mitochondrial protein translation influences mitonuclear communication in the heart. *Nat Commun* **2023**, *14*, 1595.
108. Gabillard-Lefort, C.; Thibault, T.; Lenaers, G.; Wiesner, R.J.; Mialet-Perez, J.; Baris, O.R. Heart of the matter: Mitochondrial dynamics and genome alterations in cardiac aging. *Mech Ageing Dev* **2025**, *224*, 112044.
109. Chen, Y.; Liu, Y.; Dorn, G.W. 2nd. Mitochondrial fusion is essential for organelle function and cardiac homeostasis. *Circ Res* **2011**, *109*, 1327-1331.
110. Qin, Y.; Li, A.; Liu, B.; Jiang, W.; Gao, M.; Tian, X.; Gong, G. Mitochondrial fusion mediated by fusion promotion and fission inhibition directs adult mouse heart function toward a different direction. *FASEB J* **2020**, *34*, 663-675.
111. Jarreta, D.; Orús, J.; Barrientos, A.; Miró, O.; Roig, E.; Heras, M.; Moraes, C.T.; Cardellach, F.; Casademont, J. Mitochondrial function in heart muscle from patients with idiopathic dilated cardiomyopathy. *Cardiovasc Res* **2000**, *45*, 860-865.
112. Buchwald, A.; Till, H.; Unterberg, C.; Oberschmidt, R.; Figulla, H.R.; Wiegand, V. Alterations of the mitochondrial respiratory chain in human dilated cardiomyopathy. *Eur Heart J* **1990**, *11*, 509-516.
113. Abel, E.D.; Doenst, T. Mitochondrial adaptations to physiological vs. pathological cardiac hypertrophy. *Cardiovasc Res* **2011**, *90*, 234-242.
114. Bragoszewski, P.; Turek, M.; Chacinska, A. Control of mitochondrial biogenesis and function by the ubiquitin-proteasome system. *Open Biol* **2017**, *7*, 170007.
115. Berthiaume, J.M.; Kurdys, J.G.; Muntean, D.M.; Rosca, M.G. Mitochondrial NAD^+/NADH redox state and diabetic cardiomyopathy. *Antioxid Redox Signal* **2019**, *30*, 375-398.
116. Mongirdien, A.; Liuiz, A.; Karˇciauskait, D.; Mazgelyt, E.; Liekis, A.; Sadauskien, I. Relationship between oxidative stress and left ventricle markers in patients with chronic heart failure. *Cells* **2023**, *12*, 803.
117. Shah, A.K.; Bhullar, S.K.; Elimban, V.; Dhalla, N.S. Oxidative Stress as a mechanism for functional alterations in cardiac hypertrophy and heart failure. *Antioxidants* **2021**, *10*, 931.
118. Li, Y.Y.; Chen, D.; Watkins, S.C.; Feldman, A.M. Mitochondrial abnormalities in tumor necrosis factor-α–induced heart failure are associated with impaired DNA repair activity. *Circulation* **2001**, 104, 2492-2497.
119. Ozcan, C.; Bienengraeber, M.; Hodgson, D.M.; Mann, D.L.; Terzic, A. Mitochondrial tolerance to stress impaired in failing heart. *J Mol Cell Cardiol* **2003**, *35*, 1161-1166.
120. Sabbah, H.N.; Sharov, V.; Riddle, J.M.; Kono, T.; Lesch, M.; Goldstein, S. Mitochondrial abnormalities in myocardium of dogs with chronic heart failure. *J Mol Cell Cardiol* **1992**, *24*, 1333-1347.
121. Ide, T.; Tsutsui, H.; Hayashidani, S.; Kang, D.; Suematsu, N.; Nakamura, K.-I.; Utsumi, H.; Hamasaki, N.; Takeshita, A. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. *Circ Res* **2001**, *88*, 529-535.
122. Sobel, B.E.; Spann, J.F., Jr.; Pool, P.E.; Sonnenblick, E.H.; Braunwald, E. Normal oxidative phosphorylation in mitochondria from the failing heart. *Circ Res* **1967**, *21*, 355-364.

 doi:10.20944/preprints202504.0012.v1

123. Sordahl, L.; McCollum, W.; Wood, W.; Schwartz, A.; Peterzan, M.A.; Lygate, C.A.; Neubauer, S.; Rider, O.J.; Gong, G.; Liu, J.; et al. Mitochondria and sarcoplasmic reticulum function in cardiac hypertrophy and failure. *Am J Physiol Content* **1973**, *224*, 497-502.
124. Sack, M.N.; Rader, T.A.; Park, S.; Bastin, J.; McCune, S.A.; Kelly, D.P. Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. *Circulation* **1996**, *94*, 2837-2842.
125. Sabbah, H.N.; Gupta, R.C.; Kohli, S.; Wang, M.; Hachem, S.; Zhang, K. Chronic therapy with elamipretide (MTP-131), a novel mitochondria-targeting peptide, improves left ventricular and mitochondrial function in dogs with advanced heart failure. *Circ Heart Fail* **2016**, *9*, e002206.
126. Ayoub, I.M.; Radhakrishnan, J.; Gazmuri, R.J. Targeting mitochondria for resuscitation from cardiac arrest. *Crit Care Med* **2008**, *36*, S440-S446.
127. Rudokas, M.W.; McKay, M.; Toksoy, Z.; Eisen, J.N.; Bögner, M.; Young, L.H.; Akar, F.G. Mitochondrial network remodeling of the diabetic heart: implications to ischemia related cardiac dysfunction. *Cardiovasc Diabetol* **2024**, *23*, 261.
128. Ramachandra, C.J.A.; Hernandez-Resendiz, S.; Crespo-Avilan, G.E.; Lin, Y.H.; Hausenloy, D.J. Mitochondria in acute myocardial infarction and cardioprotection. *EBioMedicine* **2020**, *57*, 102884.
129. Bugger, H.; Pfeil, K. Mitochondrial ROS in myocardial ischemia reperfusion and remodeling. *Biochim Biophys Acta Mol Basis Dis* **2020**, *1866*, 165768.
130. Dongworth, R.K.; Hall, A.R.; Burke, N.; Hausenloy, D.J. Targeting mitochondria for cardioprotection: examining the benefit for patients. *Future Cardiol* **2014**, *10*, 255-272.
131. Yang, Y.; Owusu, F.B.; Wu, H.; Zhang, X.; Li, R.; Liu, Z.; Zhang, S.; Leng, L.; Wang, Q. Mitochondria as therapeutic targets for natural products in the treatment of cardiovascular diseases. *J Ethnopharmacol* **2025**, *6*, 119588.
132. Moe, G.W.; Marín-García, J. Role of cell death in the progression of heart failure. *Heart Fail Rev* **2016**, *21*, 157-167.
133. Peart, J.N.; Gross, G.J. Sarcolemmal and mitochondrial KATP channels and myocardial ischemic preconditioning. *J Cell Mol Med* **2002**, *6*, 453-464.
134. Halestrap, A.P.; Clarke, S.J.; Javadov, S.A. Mitochondrial permeability transition pore opening during myocardial reperfusion--a target for cardioprotection. *Cardiovasc Res* **2004**, *61*, 372-385.
135. Yellon, D.M.; Beikoghli Kalkhoran, S.; Davidson, S.M. The RISK pathway leading to mitochondria and cardioprotection: how everything started. *Basic Res Cardiol* **2023**, *118*, 22.
136. Halestrap, A.P. Calcium, mitochondria and reperfusion injury: a pore way to die. *Biochem Soc Trans* **2006**, *34*, 232-237.
137. Rabinovich-Nikitin, I.; Kirshenbaum, L.A. Circadian regulated control of myocardial ischemia-reperfusion injury. *Trends Cardiovasc Med* **2024**, *34*, 1-7.
138. Zhao, Y.; Ponnusamy, M.; Dong, Y.; Zhang, L.; Wang, K.; Li, P. Effects of miRNAs on myocardial apoptosis by modulating mitochondria related proteins. *Clin Exp Pharmacol Physiol* **2017**, *44*, 431-440.
139. Hayashida, K.; Takegawa, R.; Shoaib, M.; Aoki, T.; Choudhary, R.C.; Kuschner, C.E.; Nishikimi, M.; Miyara, S.J.; et al. Mitochondrial transplantation therapy for ischemia reperfusion injury: a systematic review of animal and human studies. *J Transl Med* **2021**, *19*, 214.
140. Lotz, C.; Herrmann, J.; Notz, Q.; Meybohm, P.; Kehl, F. Mitochondria and pharmacologic cardiac conditioning-at the heart of ischemic injury. *Int J Mol Sci* **2021**, *22*, 3224.
141. Guo, Z.; Tian, Y.; Liu, N.; Chen, Y.; Chen, X.; Yuan, G.; Chang, A.; Chang, X.; et al. Mitochondrial stress as a central player in the pathogenesis of hypoxia-related myocardial dysfunction: New insights. *Int J Med Sci* 2024, *21*, 2502-2509.
142. Suarez, J.; Cividini, F.; Scott, B.T.; Lehmann, K.; Diaz-Juarez, J.; Diemer, T.; Dai, A.; Suarez, J.A.; et al. Restoring mitochondrial calcium uniporter expression in diabetic mouse heart improves mitochondrial calcium handling and cardiac function. *J Biol Chem* **2018**, *293*, 8182-8195.
143. Dillmann, W.H. Diabetic cardiomyopathy. *Circ Res* **2019**, *124*, 1160-1162.
144. Dhalla, N.S.; Mota, K.O.; Elimban, V.; Shah, A.K.; de Vasconcelos, C.M.L.; Bhullar, S.K. Role of vasoactive hormone-induced signal transduction in cardiac hypertrophy and heart failure. *Cells* **2024**, *13*, 856.

 doi:10.20944/preprints202504.0012.v1

145. Li, T.; Wang, N.; Yi, D.; Xiao, Y.; Li, X.; Shao, B.; Wu, Z.; Bai, J.; Shi, X.; Wu, C.; et al. ROS-mediated ferroptosis and pyroptosis in cardiomyocytes: An update. *Life Sci* **2025**, 123565. doi: 10.1016/j.lfs.2025.123565.
146. Daiber, A.; Hahad, O.; Andreadou, I.; Steven, S.; Daub, S.; Münzel, T. Redox-related biomarkers in human cardiovascular disease - classical footprints and beyond. *Redox Biol* **2021**, *42*, 101875.
147. Bhullar, S.K.; Dhalla, N.S. Angiotensin II-induced signal transduction mechanisms for cardiac hypertrophy. *Cells* **2022**, *11*, 3336.