Review

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Targeting Ferroptosis via Mitochondria Dynamics in Myocardial Ischemia/Reperfusion Injury

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Myocardial infarction remains a significant worldwide public health issue, primarily owing to its mortality and morbidity rates. This condition is due to myocardial ischemia, appearing once the heart's blood flow is obstructed or significantly reduced, causing the death of heart muscle cells. Reperfusion prevents further death of cardiomyocytes, restoring coronary flow. However, the initial lack of coronary blood flow and the subsequent restoration induce ischemia/reperfusion injury (IRI) due to abrupt metabolic and biochemical changes, such as calcium overload, activation of inflammatory cells, and oxidative stress (OS). OS is associated with damage to cellular biomolecules such as proteins, lipids, DNA, or carbohydrates and with organelles such as mitochondria, activating mitochondrial dynamics. These oxidative conditions may also trigger ferroptosis, cell death linked to cellular oxidation. While ferroptosis induction is desirable in certain diseases like cancer, it is not beneficial in situations such as myocardial IRI. Although considerable research has been conducted on ferroptosis in myocardial IRI, the potential impact of reducing ferroptosis via mitochondrial dynamics in IRI remains to be reviewed. Consequently, this review concentrates on mitochondrial dynamics during myocardial ferroptosis in IRI and explores the potential therapy to inhibit myocardial ferroptosis by targeting mitochondrial dynamics to mitigate IRI.

Keywords: ischemia; reperfusion; ferroptosis; treatments related to ferroptosis; oxidative stress; mitochondrial dynamics

Introduction

Myocardial infarction (MI) remains a public health problem worldwide with the highest levels of mortality and morbidity [1] and occurs when blood flow to the heart is blocked or severely reduced, a process known as ischemia. It is known that a plaque induces this blockage [2]. The process of plaque formation is called atherosclerosis, where there is a buildup of fat and cholesterol in the coronary arteries. The plaques block and can even rupture the arteries, forming a clot that interrupts blood flow, inducing a heart attack that damages and promotes cell death of cardiomyocytes due to lack of blood flow [3]. The coronary flow will be restored as a first intervention to avoid the further death of cardiomyocytes during the infarction, a process known as reperfusion. Note that MI is only one of the conditions of myocardial ischemia/reperfusion (I/R), as other conditions are susceptible to I/R, such as organ transplantation or sepsis [4]. However, ischemic heart disease (IHD) is still the leading cause of death globally. For instance, Khan *et al*. [5] showed that IHD affects around 126 million individuals of the world's population, representing approximately 1.72%, where nine million deaths were due to IHD worldwide. This group of researchers also found that men are more affected than women, starting with affection in the fourth decade and increasing with age. Although different strategies have been implemented to reduce this condition, such as primary percutaneous coronary intervention (PPCI) or thrombolytic drugs, it has been observed that even these techniques induce an increase in infarction due to a process of myocardial damage by reperfusion [6]. Related to this, new strategies have been developed, such as ischemic conditioning, which has emerged as the most promising method to mitigate ischemia/reperfusion injury (IRI) and is associated with intermittent cycles of ischemic and reperfusion [7]. This therapy has been shown to rescue or reduce infarct size (IS) by up to 68% in rabbits and up to 35% in patients [8]. However, the damage persists with collateral consequences. Therefore, it is projected that the global prevalence of IHD will continue to grow, being the first cause of death and disability worldwide [9].

It has been shown that IRI indicates two phases: the first without coronary blood flow supply (ischemia) and the second with it (reperfusion) [10]. During ischemia, the flow of oxygen (O_2) and nutrients decreases due to a general blockage of the coronary arteries. O_2 deprivation stops oxidative phosphorylation (OXPHOS), leading to depolarization of the mitochondrial membrane along with adenosine triphosphate (ATP) depletion and inhibition of cardiac contraction [11]. ATP depletion is exacerbated by the breakdown of any available ATP, as the F1Fo-ATPase works in reverse, hydrolyzing ATP to maintain the mitochondrial membrane potential ($\Delta\Psi m$) [12]. Without O_2,

cellular metabolism switches to anaerobic glycolysis, resulting in lactate accumulation, which lowers intracellular pH to below 7 [13]. The intracellular accumulation of protons (H^+) activates the sodium (Na^+)-H^+ ion exchanger, which moves H^+ out of the cell in exchange for Na^+ entry. This would activate the 3Na^+-2 potassium (K^+) ATPase, which would eject Na^+ to introduce K^+; however, since this enzyme is ATP-dependent, an intracellular accumulation of Na^+ is propagated. In this case, another ion exchanger comes into action, such as 2Na^+-calcium (Ca^{2+}), which is not ATP-dependent and whose function is to remove Na^+ and introduce Ca^{2+}, resulting in intracellular Ca^{2+} overload. Ca^{2+} overload is an inducer of cell death, such as apoptosis [13]. If acute ischemia is maintained for more than 20 minutes, a wavefront of cardiomyocyte death begins in the subendocardium that can extend to the epicardium, so rapid reperfusion is required to avoid the induction of cardiomyocyte death [11,14]. Thus, the reestablishment of blood flow is necessary to save the ischemic myocardium from infarction. Although reperfusion is the most efficacious therapy for minimizing IS in MI, the restoration of blood flow also introduces myocardial injury; both ischemia and reperfusion injuries are known as IRI, leading to cardiomyocyte death [13]. This is because reperfusion allows a rapid replenishment of ATP, reestablishing ionic homeostasis, but also reactivates the ATPase of the sarcoplasmic reticulum (SR), hyperactivating the Ca^{2+} release channel, resulting in an overload of Ca^{2+} in the cytosol, which leads to hypercontraction of cardiomyocytes [15]. This Ca^{2+} released from the SR can be captured by the mitochondria, leading to an overload of mitochondrial Ca^{2+} and the opening of the mitochondrial permeability transition pore (mPTP) [16]. Once the mPTP is open, the mitochondria cannot maintain the $\Delta\Psi m$; so ATP is quickly depleted again, ionic pumps stop working, and cells can die by different processes such as necrosis [16]. Ca^{2+} overload also induces the conversion of the enzyme xanthine oxidase dehydrogenase to xanthine oxidase, producing reactive oxygen species (ROS) such as superoxide ion radical ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), which induces oxidative stress (OS) [17]. Reperfusion also increases $O_2^{\bullet-}$ by complex I and III, where the electrons that reduce O_2 in the $O_2^{\bullet-}$ production are provided by mitochondrial succinate that has accumulated during ischemia, resulting in a synergistic increase of ROS and OS [11]. If OS is not reduced, ROS promotes oxidative damage, oxidizing lipids, proteins, carbohydrates, and DNA, damaging the myocardial cells, and promoting cell death, such as ferroptosis (Fig. 1) [18]. Ferroptosis, a cell death dependent on OS and cellular oxidative damage, is desired in some diseases, such as cancer, but not in others, such as IRI in the myocardium [19]. Some processes reduce ROS damage to mitochondria and alleviate cellular damage, such as mitochondrial dynamics, which refers to mitochondrial fusion and fission. Although considerable research has been conducted on ferroptosis in myocardial IRI, the possible effects of reducing ferroptosis via mitochondrial dynamics in IRI have not been reviewed. Thus, this review focuses on the possible deactivation of ferroptosis via mitochondrial dynamics in myocardial IRI.

Mitochondria and ROS in Myocardial IRI

Myocardial IRI directly affects mitochondria because this organelle is dependent on O_2 and nutrients that are restricted during myocardial ischemia and are damaged during myocardial reperfusion. Note that mitochondria coordinate bioenergetic processes such as ATP production through OXPHOS and the Krebs cycle [20], and are also critical for other functions, including metabolism, cell growth, survival, and cell death, as well as biosynthetic processes such as synthesizing amino acids, lipids, and nucleotides, calcium stabilization, and signaling, and even hormone production [21]. Mitochondria also play a crucial role in heme synthesis, ketone body production, regulation of the urea cycle balancing nitrogen, pyrimidine and purine biosynthesis, gluconeogenesis, and lipid degradation and elongation [22]. Besides inheritance, mitochondria are also essential for regulating cellular signaling by facilitating protein-protein interactions and managing intracellular messenger levels like Ca^{2+} [23].

For normal cardiac contraction, heart cells require enormous energy in ATP form, mainly obtained through mitochondrial OXPHOS [24]. It has been shown that OXPHOS is fed through the electron transport system (ETS) by different processes, like the tricarboxylic acid cycle (TCA) and fatty acid oxidation (FAO) [25]. In TCA, reducing agents such as nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) are synthesized as follows: during glycolysis, glucose is metabolized to pyruvate in the cytosol and internalized into the mitochondria, where it is transformed into acetyl-CoA [26]. Then, acetyl-CoA is incorporated into the TCA, producing NADH, $FADH_2$, guanosine triphosphate (GTP), and coA. NADH and $FADH_2$ enter the ETS, functioning as electron donors and promoting OXPHOS. Upon entering the ETS, both molecules generate a proton gradient between the inner and outer mitochondrial membrane [22]. This proton gradient is a driving force to produce ATP through ATP synthase in the OXPHOS, where the final electron acceptor is O_2. Although O_2 functions as the last acceptor of electrons in OXPHOS, this molecule is also the acceptor of electron leakage in OXPHOS [27]. It has been shown that when mitochondria have OXPHOS coupled under normal conditions, ETS can release up to 4% of electrons. This electron leak reduces O_2 to its most reactive radical, the $O_2^{\bullet-}$, as secondary products of ETS, triggering the production of ROS, such as H_2O_2 [28]. ROS production is essential since ROS levels are necessary for cellular signaling, acting as secondary messengers and activating var-

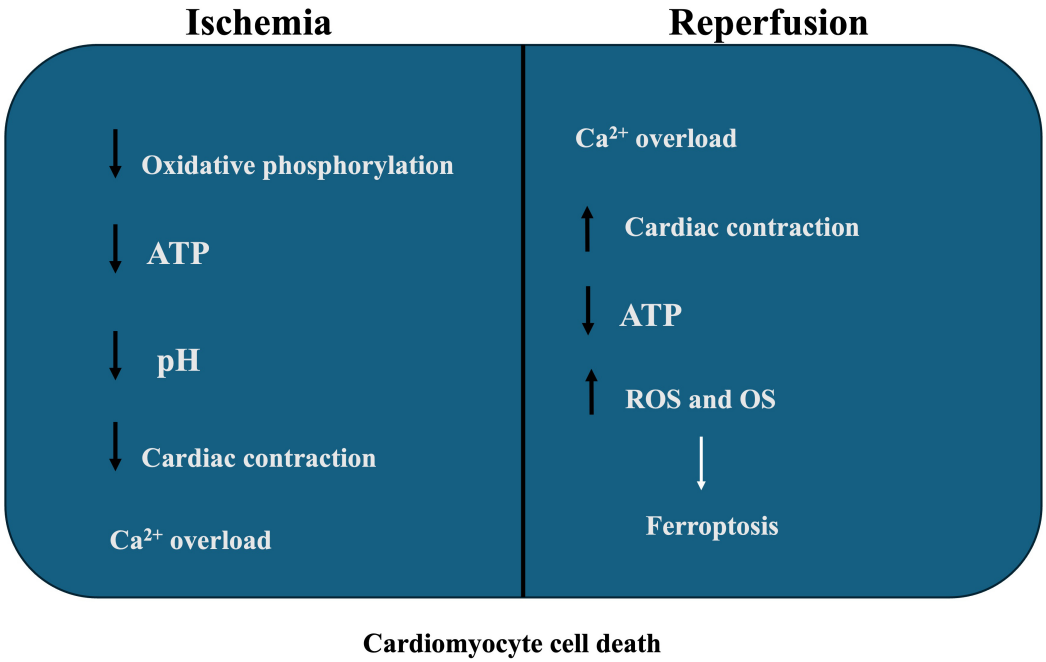

Fig. 1. Myocardial ischemia/reperfusion injury (IRI). During ischemia, oxygen deprivation stops oxidative phosphorylation, leading to adenosine triphosphate (ATP) depletion and inhibition of cardiac contraction. The absence of oxygen switches aerobic glycolysis to anaerobic glycolysis, resulting in lactate accumulation and a decrease in pH below 7. The intracellular accumulation of protons (H^+) activates the ion exchangers: H^+-sodium (Na^+) and $2Na^+$-calcium (Ca^{2+}), resulting in intracellular Ca^{2+} overload. Ca^{2+} overload is an inducer of cell death, such as apoptosis, so rapid reperfusion is required to avoid the induction of cardiomyocyte death. Although reperfusion allows for rapid ATP replenishment, it also reactivates the sarcoplasmic reticulum (SR) ATPase, hyperactivating the Ca^{2+} release channel, promoting Ca^{2+} leaks, resulting in Ca^{2+} overload in the cytosol, and leading to cardiomyocyte hypercontraction. This Ca^{2+} released from the SR induces the opening of the mitochondrial permeability transition pore (mPTP), depleting ATP. Ca^{2+} overload also causes the conversion of the enzyme xanthine oxidase dehydrogenase to xanthine oxidase, producing reactive oxygen species (ROS) and oxidative stress (OS) cell death, such as ferroptosis. pH, potential of hydrogen. Fig. 1 was created with Microsoft PowerPoint 2021 (Microsoft Corporation, Redmond, WA, USA).

ious signaling pathways and cellular enzymes [29]. However, in pathological conditions such as IRI, mitochondria can be decoupled, and the leakage of electrons can rise to 24% [30]. As mentioned in IRI, the lack of O_2 and respiratory substrates stops OXPHOS, increasing electron leakage and augmenting ROS production. This induces mitochondrial transmembrane potential collapse, mitochondrial swelling, Ca^{2+} overload, cytochrome c release, disruption of cell membranes, and eventually cell death, such as necrosis [31]. Although reestablishing blood flow and reoxygenating the tissue restores organ function, cardiac cells are damaged due to ROS production due to reperfusion [13]. This is because when O_2 supply is restored, mitochondrial ROS production increases in an inadequate attempt to restore ATP production by the mitochondria, promoting subsequent peroxidation of proteins, mitochondrial respiratory complexes, and phospholipids. For instance, the peroxidation of cardiolipin avoids the regular activities of OXPHOS complexes III and IV [32]. Moreover, ROS induces complex I sulfhydryl (SH) group oxidation, intensifying ROS production during IRI [33]. IRI also impairs Ca^{2+} homeostasis, inducing mPTP opening, $\Delta\Psi m$ loss, cytochrome c release, and apoptosis during IRI [34]. It has been shown that ROS production causes oxidative damage in DNA [28]. This is because during Fenton and Haber-Weiss reactions, $O_2^{\bullet -}$ and H_2O_2 may react together or with different metals, such as ferrous ion (Fe^{2+}), to produce hydroxyl radical ($^{\bullet}OH$), which is a highly reactive oxidant and oxidizes guanine in DNA producing 8-oxoguanine [35].

Furthermore, $O_2^{\bullet -}$ reacts with nitric oxide ($NO^{\bullet}$), producing peroxynitrite ($ONOO^-$); both $ONOO^-$ and $^{\bullet}OH$ are highly reactive, reacting with lipids, producing lipid radicals and chain lipid peroxidation [28]. Lipid peroxidation can produce aldehydes, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), which form adducts directly with proteins and DNA, inducing oxidative damage and cell death, such as ferroptosis [19]. To mitigate oxidative damage and ferroptosis, cells utilize antioxidants to maintain redox homeostasis by neutralizing ROS. Antioxidants encompass both enzymatic and non-enzymatic types [36]. For example, $O_2^{\bullet -}$ is converted to H_2O_2 by enzymes like superoxide dismutase (SOD) and dual oxidase

(DUOXs), while H_2O_2 is further reduced to water (H_2O) by catalase (cat) and glutathione peroxidase (GPX) [37]. Non-enzymatic antioxidants like glutathione (GSH) also support the activity of enzymatic antioxidants such as GPX. Transcription factors that respond to oxidative stress, including nuclear factor erythroid 2-related factor 2 (Nrf2) and forkhead box O (FoxO), regulate the expression of antioxidant enzymes and those related to GSH production [38]. However, oxidative damage may still occur if ROS levels exceed protective capabilities or if antioxidant defenses are depleted, promoting ferroptosis [19].

Mitochondria Dynamics in Myocardial IRI

Maintaining healthy mitochondria is essential for cardiac cell homeostasis during IRI, preventing cell death. In this sense, mitochondrial homeostasis related to mitochondrial dynamics, including fusion and fission processes, is critical to maintaining mitochondria in good condition [39]. The fusion process involves the union of two mitochondria to enhance the necessary ATP requirements. Conversely, the fission process involves mitochondrial division and occurs when mitochondria are damaged [39]. Fusion and fission processes induce mitochondria to be long filaments, spheres, or ovoids, where the morphology of mitochondria dictates many of their functions [40]. Mitochondria must balance fusion and fission events, allowing proper mitochondrial functioning in the cell. For instance, high fission levels correspond to extensive mitochondrial fragmentation associated with cellular metabolic dysfunction and cell death [41]. On the other hand, continuous fusion produces a hyperfused network of mitochondria, protecting metabolic insults and preserving cellular integrity [39]. Thus, the existence of a balance between mitochondrial fission and fusion processes shapes mitochondria to meet cellular metabolic demands and regulate mitochondrial damage. It should be noted that mitochondrial fission and fusion are involved in maintaining normal cellular functions, such as mitochondrial distribution during cell growth, cell division, and differentiation. Therefore, their deregulation is closely associated with health and disease states such as IRI [42]. Furthermore, mitochondrial fission is essential for growing and dividing cells to contain the appropriate number of mitochondria [43]. In non-proliferating cells such as cardiomyocytes, mitochondrial fusion is thought to induce complementation between damaged mitochondria, allowing the sharing of RNA, protein components, and lipids with other mitochondria, mitigating the effects of mtDNA mutations caused by cell damage [44].

In mitochondrial fusion, the tethering of two adjacent mitochondria is done via mitofusin (Mfn)1 and Mfn2, which mediate the fusion of the outer mitochondrial membranes (OMMs) of two mitochondria in a guanosine triphosphate (GTP)-dependent manner. The inner mitochondrial membrane (IMM) fusion is produced by optic atrophy 1 (OPA1), which mediates the formation of a single elongated mitochondrion [45]. Mfns have been shown to stabilize mitochondrial cristae shape, favoring ATP synthase oligomerization and protecting mitochondria from stress induced by respiratory chain inhibition [46,47]. The mitofusin synthesis is regulated by transcriptional mechanisms such as the transcriptional mitochondrial coactivators phosphatidylglycerol phospholipase C-1-alpha and beta (PGC-1-α and β), which regulate the expression of the respiratory complex machinery and mitochondrial biogenesis [48]. Different activators of the protein deacetylase sirtuin 1 (SIRT1), like resveratrol, decreased PGC-1α acetylation in mice, increasing its activity, leading to the induction of mitochondrial biogenesis and increased mitochondrial size in skeletal muscle, giving metabolic advantages like increased aerobic capacity [49]. PGC-1-β also induces mitochondrial fusion by increasing the transcription of Mfn2 through estrogen-related receptor alpha (ERRα) coactivation; conversely, mitofusins are ubiquitylated and degraded via proteasome during apoptosis [50]. Thus, mitofusins deletion or abrogation of GTPase activity avoids mitochondrial fusion, inducing the fragmentation of mitochondria. Mitochondrial phospholipids are essential for mitochondrial membrane fusion, and alterations of the local phospholipid composition on the OMM can induce membrane bilayer curvature or destabilization to promote fusion or fission. For instance, phospholipids that cause negative lipid membrane curvature, such as phosphatidic acid (PA), upregulate mitochondrial fusion. This was demonstrated when mitoPLD, a phospholipase D localized in the mitochondria that produces PA from cardiolipin, facilitated Mfn-mediated fusion [51]. However, the overexpression of phosphatidic acid–preferring phospholipase A1 (PA-PLA1) or lipin 1 phosphatase, an enzyme that degrades PA, induces mitochondrial fragmentation or mitochondrial fission [51]. It has been shown that during myocardial IRI, the oxidized phosphatidylcholines (OxPCs) are significantly elevated resulting in cell death, where OxPCs such as 1-palmitoyl-2-(5′-oxo-valeroyl)-sn-glycero-3-phosphocholine (POVPC) and 1-palmitoyl-2-(9′-oxo-nonanoyl)-sn-glycero-3-phosphocholine (PONPC) disrupt mitochondrial bioenergetics by reducing maximal respiratory rate and spare respiratory capacity, even these oxidized lipids dramatically reduce GPX4 levels resulting in cell death by ferroptosis [52]. Interestingly, suppressing OxPCs by EO6, an IgM antibody that binds and inactivates OxPCs, or using ferrostatin, an inhibitor of ferroptosis, inhibited cell death (Table 1, Ref. [52–65]) [52]. Although mitochondrial dynamics markers were not measured in the previous research work, it has been observed that mitochondrial fragmentation or mitochondrial fission is induced in mitochondrial dysfunction in IRI, as mitochondrial fission promotes the autophagic clearance of dysfunctional mitochondria, a process called mitophagy that allows mitochondrial adaptation to physiological demands [66].

Table 1. Different compounds that target ferroptosis or mitochondrial dynamics.

Compound	Ferroptosis	Mitochondrial fusion	Mitochondrial fission	Reference
EO6	Binds and inactivates oxidized phosphatidylcholines (OxPCs), reducing ferroptosis and avoiding cardyomicite cell death, during IRI.			[52]
Melatonin	Reduce oxidative stress and ferroptosis in IRI.	Induces the optic atrophy 1 (OPA1), mitofusin 1 (Mfn1), and mitofusin 2 (Mfn2) expression, reducing infarct area, and cardiac dysfunction in IRI.		[54,55]
Dynasore, and selective dynamin-related protein 1 (DRP1) Inhibitor Peptide (P110)			Inhibits DRP1, reducing mitochondrial fission, preserving mitochondrial morphology, depleting cytosolic Ca^{2+}, and inhibiting mPTP opening, preventing apoptosis in cardiomyocytes in IRI.	[53,56]
Mitochondrial division inhibitor 1 (Mdivi-1)			Inhibitis DRP1, decreasing mitochondrial fission, reducing IRI.	[57]
Epigallocatechin gallate			Inhibits OMA1 Zinc Metallopeptidase (OMA1), reducing mitochondrial fission, preventing apoptosis, and reducing cell death after IRI.	[58]
Ubiquitin-specific protease 7 (USP7) inhibition	Decreases iron content and increases glutathione peroxidase 4 (GPX4), preventing ferroptosis, and decreasing cell death in IRI.			[62]
Compound 968	Reduce ferroptosis, improving cardiac function in IRI.			[59]
Deferoxamine (DFO)	Decreases ferroptosis, improving IRI.			[60]
Overexpression of ubiquitin-specific protease 22 (USP22)	Decreases ferroptosis in myocardial IRI.			[61]
Fer-1 or dexrazoxane	Prevents ferroptosis, alleviating IRI.			[63]
Inhibition of ELAV-like RNA Binding Protein 1 (*ELAVL1*)	Suppresses iron and ROS increase, increasing GPX4 and GSH, avoiding ferroptosis in myocardial cells via inhibiting autophagy.			[64]
Vitexin	Reduce ROS production and even increase Mfn2 expression, reducing the recruitment of DRP1 and infarct size.			[65]

Mitochondrial fission is mediated by a cytosolic dynamin family member dynamin-related protein 1 (DRP1) that translocates from the cytosol and binds to fission protein 1 (FIS1), mitochondrial dynamics of 49 kDa protein (MiD49), MiD of 51 kDa protein (MiD51) and mitochondrial fission factor (MFF) on the OMM [40]. Then, DRP1 produces coiled coils around mitochondria, which promotes a conformational change of DRP1 in a GTP-dependent

manner, resulting in membrane constriction to cut both the IMM and OMM [67]. Thus, this interaction oligomerizes, surrounds, and contracts the mitochondria to divide them into 2 individual mitochondria [67]. It has been shown that this process often occurs at sites where mitochondria come into contact with the endoplasmic reticulum [43]. Since DRP1 is the critical protein to induce fission, its regulation is essential, so it is regulated post-translationally at different levels, including protein phosphorylation, sumoylation, ubiquitination, and S-nitrosylation [68]. Under normal conditions, there is a balance between fission and fusion processes, and any alteration in them or the overactivation of one over the other induces abnormalities that impact mitochondrial structure and function [43].

Regarding IRI, it has been shown that during ischemia, DRP1 induces the fragmentation of mitochondria, and even the restitution of O_2 does not prevent this fragmentation but, on the contrary, induces a rapid translocation (in less than 5 min) of DRP1 from the cytosol to the mitochondria [53]. Furthermore, DRP1, MFF, and FIS have also been shown to increase their expression during coronary ligation-induced I/R in murine models and cellular models treated with hypoxia/reoxygenation (H/R) [69,70]. In contrast, the expression of OPA1, Mfn1, and Mfn2 is decreased [54], indicating the promotion of fission over fusion. It was also shown that in IRI, the expression of fusion factors such as OPA1, Mfn1, and Mfn2 is reduced, but fission factor proteins such as DRP1 and MFF are increased in a Yap-Hippo pathway-dependent manner [71]. One strategy to reduce IRI was to reduce mitochondrial fission, inducing mitochondrial fusion through OPA1, Mfn1, and Mfn2 induction, which was achieved with melatonin treatment, where this compound augments OPA1, reducing IRI induced by coronary artery ligation and H/R of cardiomyocytes isolated from mice. Thus, melatonin reduces infarct area and decreases cardiac dysfunction, myocardial inflammation, and OS [54,55]. Contrary to this, it has been shown that the ablation of proteins such as Mfn1 and Mfn2 have been shown to inhibit the mPTP opening, reducing MI size during acute I/R injury (30 min ischemia) [72]. The mechanism shows that Mfn2 tethers mitochondria to the SR, inducing microdomains through Ca^{2+} transit in a pleiotropic function unrelated to fusion [73,74]. Further exploration of the different timings of decrease or increase in mitochondrial fusion proteins in IRI is needed, as the protective effect of these proteins appears to be time-dependent. On the other hand, inhibition of fission during reperfusion is beneficial [53], as demonstrated by the treatment with Dynasore, a non-specific inhibitor of dynamins and DRP1, or mitochondrial division inhibitor 1 (Mdivi-1) and selective DRP1 inhibitor peptide (P110), selective inhibitors of DRP1, which function as myocardium cardioprotective during IRI [56]. These compounds preserve mitochondrial morphology, deplete cytosolic Ca^{2+}, and inhibit mPTP opening, preventing apoptosis in cardiomyocytes [53]. Moreover, excessive mitochondrial fission is detrimental to the myocardium. For example, excessive mitochondrial fission leads to mitochondrial dysfunction and decreased cardiac contractility [56], and a persistent inhibition of mitochondrial fission via DRP1 depletion by genetic or repetitive application of Mdivi-1 reduces IRI [57]. Therefore, the inhibition of mitochondrial fission by DRP depletion1 could be a target for inducing a cardioprotective effect in IRI.

Interestingly, epigallocatechin gallate, an inhibitor of OMA1 Zinc metallopeptidase (OMA1, OMA1 is a protein that degrades OPA1, reducing mitochondrial fusion), inhibits mitochondrial fission, thereby preventing apoptosis and reducing cell death after I/R, as another pleiotropic function unrelated to mitochondrial fusion to reduce MI size [58]. Previous research suggests that reducing fission relative to fusion could be a useful approach to reducing IRI damage.

Ferroptosis and Mitochondrial Dynamics in Myocardial IRI

Cell Death by Ferroptosis

Ferroptosis was described as a cell death associated with OS due to iron accumulation and GSH decrease generally induced by GPX4 depletion [18]. In these conditions, ROS significantly increases, producing oxidative damage in different biomolecules, such as lipids. Since cellular membranes are made of lipids, a rupture of the membranes is obtained, reducing membrane integrity and altering membrane fluidity. This decreases mitochondrial cristae and induces mitochondria size reduction. If lipid peroxides are not reduced, they undergo cyclization, producing aldehydes such as MDA and 4-HNE, which form adducts with proteins and DNA, increasing oxidative cell damage and promoting cell death via ferroptosis [18,28]. Thus, these processes differ from the typical characteristics of apoptosis, necrosis, or other cell deaths, such as autophagy [18], involving the dysregulation of cellular iron metabolism and depletion of antioxidant systems, generating ROS overproduction, lipid peroxidation, redounding in cellular oxidative damage, and cell death. Mitochondria play a crucial role in ferroptosis induced by OS in cardiomyocytes. For instance, mitochondrial DNA depletion or ROS mitochondrial decrease has been shown to inhibit ferroptosis induced by RAS-selective lethal 3 (RSL3), a GPX4 inhibitor and ferroptosis-inducer [75,76], and even mitochondrial TCA cycle or ETS diminution prevents ferroptosis induced by cysteine deprivation or erastin [77]. Ferroptosis was also shown to be dependent on mitochondrial free iron levels associated with high amounts of lipid peroxidation, and the inhibition of mitochondrial iron overload by overexpression of mitochondrial ferritin reduced OS-induced mitochondrial lipid peroxidation and ferroptosis-mediated cell death [78]. Also, targeted inhibition of mitochondrial ROS, using mitoquinone (mitoQ), 10-(6′-Plastoquinonyl) decyl-

triphenylphosphonium (SKQ1), or mitochondrial catalase also largely inhibited ferroptosis [78]. Thus, these results demonstrated that mitochondrial iron overload and lipid ROS accumulation are inductors of ferroptosis induced by OS in cardiomyocytes, but their depletion reduces ferroptosis.

Ferroptosis in IRI

In myocardial damage by IRI, ferroptosis contributes to significant cell death; for example, Gao *et al*. [59] demonstrated that transferrin transport and glutaminolysis induce ferroptosis triggered by complete amino acid or cysteine deprivation. Since this group identified glutaminolysis as a ferroptosis inducer, they tested whether Compound 968, a glutaminolysis inhibitor, can reduce myocardial IRI in an *ex vivo* cardiac model. They found that at the end of reperfusion, hearts treated with Compound 968 or deferoxamine (DFO, an iron chelator and ferroptosis inhibitor) improved cardiac function, pointing to ferroptosis as a potential therapeutic target for myocardial I/R. Interestingly, ferroptosis cell death is significantly present in myocardial reperfusion than in ischemia, where ischemic injury presents infarction and creatine kinase (CK) release, but no ferroptosis markers such as acyl-CoA synthetase long-chain family member 4 (ACSL4), GPX4, iron, and MDA, on the contrary, myocardial reperfusion showed high levels of ACSL4, iron, and MDA associated with a decrease of GPX4 [59]. Moreover, the reduction of ferroptosis with DFO was reduced in rat hearts with I/R compared to ischemia-treated rat hearts [60]. Overexpression of ubiquitin-specific protease 22 (USP22) also decreases ferroptosis in myocardial IRI, where this overexpression deubiquitinated SIRT1, which induces p53 depletion, increasing the cysteine transporter solute carrier family 7 member 11 (SLC7A11) and therefore increased GSH levels, decreasing ROS production, lipid peroxidation, and iron accumulation, preventing ferroptosis [61]. On the contrary, it has been shown that during myocardial I/R, ubiquitin-specific protease 7 (USP7) induces p53 deubiquitination, activating transferrin receptor 1 (TfR1), which is a specific marker of ferroptosis, charging cellular iron uptake. Inhibiting USP7 leads to ubiquitination and decreased p53 and TfR1, decreasing iron content and increasing GPX4, preventing ferroptosis [62]. Fang *et al*. [63] demonstrated that ferroptosis contributes to a substantial portion of cell death, above apoptosis, necrosis, and autophagy in cardiomyopathy and IRI. This group of researchers found that inhibiting ferroptosis improves the survival of mice with doxorubicin-induced cardiomyopathy. In brief, their research demonstrated that the ferroptosis inhibitor Fer-1 reduced doxorubicin-induced mortality; however, mortality was not significantly reduced in mice treated with emricasan (an apoptosis inhibitor), necrostatin-1 (Nec-1, a specific inhibitor of necroptosis), or 3-methyladenine (3-MA, an autophagy inhibitor). When this group examined the role of ferroptosis in IRI, they found that ferroptosis is present and that if it is prevented using ferroptosis inhibitors such as Fer-1 or dexrazoxane (an iron chelator), the damage caused by IRI in the myocardium is decreased [63]. This group of researchers also found that hemoxygenase (HO-1) is overexpressed during IRI, which induces iron overload through heme degradation and lipoperoxidation. Iron overload promotes ROS accumulation (via the Fenton and Haber-Weiss reaction), which promotes lipid oxidation. It has also been shown that elevated ROS production can induce iron overload in cardiomyocytes in positive feedback on the increase of the OS [79]. It is demonstrated that OS promotes the expression of HO-1 via Nrf2, and reducing HO-1 abolishes ferroptosis. Although these data remark that OS induces iron overload via HO-1, it should be noted that iron overload can occur through additional mechanisms, such as nuclear receptor coactivator 4 (NCOA4), which mediates ferritinophagy and lysosomal iron release [80]. It is essential to mention that HO-1 is activated via Nrf2, playing a cytoprotective role as an antioxidant, since along with the release of iron, HO-1 also releases biliverdin, an organic antioxidant and ROS scavenger. Still, these levels of activation should be moderate, since excessive and/or prolonged activation of HO-1 increases iron labile, leading to ferroptotic cell death [81]. In this regard, Miyamoto *et al*. [82] demonstrated that HO-1 silencing prevents ferroptosis induced during I/R in cardiomyocytes.

Ferroptosis and Mitochondrial Dynamics in Myocardial IRI

Interestingly, a relationship exists between increased mitochondrial iron and mitochondria dynamics, where the augment of cellular iron causes disturbances in mitochondrial dynamics and hampers the balance between mitochondrial fission and fusion through ROS increase [83,84]. It has been shown that the deficiency of mitochondrial proteins such as asparagine- glutamic acid (Glu)-Glu-threonine (NEET), which are a group of [2Fe–2S] proteins that regulate iron and ROS levels, increases mitochondrial labile iron, disrupts mitochondrial dynamics, promoting fission, triggering mitophagy and ferroptosis [83–85]. However, iron chelators can alleviate this effect due to the NEET deficiency, avoiding high levels of mitochondrial labile iron [85] and leading to mitochondrial elongation due to a DRP1 decrease [84]. An increase in ROS production can promote endoplasmic reticulum stress (ERS), playing an essential role in ferroptosis, where the transcription factor 4 (ATF4)-C/EBP homologous protein (CHOP) pathway is activated, and ferroptosis inducers cause the unfolded protein response (UPR). Specifically, diabetic rats subjected to myocardial IRI show high levels of ROS induced by the NADPH oxidase (NOX) 2 prooxidant enzyme [86]. This model also showed that ferroptosis markers, such as GPX4 are depleted along with ERS markers, such as ATF4 and CHOP, which are increased, showing a direct relationship

between ferroptosis and ERS. Interestingly, inhibition of ferroptosis by Fer-1 decreases ferroptosis and ERS during myocardial IRI in diabetic rats, suggesting that inhibition of ferroptosis by Fer-1 might also decrease ERS, improving cardiac function during myocardial I/R in diabetic rats [87]. Since the endoplasmic reticulum is key to mitochondrial fission, ERS-associated ferroptosis would also be expected to play a key role in mitochondrial dynamics, something that has not been studied; however, it warrants further investigation. It has been shown that mitochondrial dynamics is related to autophagy via mitochondrial fission, and recently, an association was demonstrated between autophagy and ferroptosis. For instance, Chen *et al.* [64] found that in myocardial IRI, forkhead box C1 (FOXC1) transcriptionally activated ELAV-like RNA Binding Protein 1 (*ELAVL1*), a gene involved in the regulation of genetic expression, which induces iron increase together GPX4 and GSH decrease, and increases Becline-1 and Light Chain 3 (LC3) II/LC3 I ratio but diminished p62 levels; however, inhibition of *ELAVL1* suppresses iron and ROS increase but increasing GPX4 and GSH, and reverse Becline and LC3 II/LC3 I increase, augmenting p62 avoiding ferroptosis in myocardial cells via inhibiting autophagy. The latter was demonstrated using 3-MA, which induces GPX4 and GSH augment, but iron, lipoperoxidation, and ROS decrease in H/R. Thus, the study and understanding of ferroptosis regulation mechanisms could be a potential tool to develop new therapeutic strategies in treating diseases such as IRI. Thus, the relationship to mitochondrial dynamics boosts this strategy. For instance, it has been shown that OPA-1 is related to cristae remodeling, facilitating the assembly of electron transport supercomplexes, improving mitochondrial respiratory efficiency, and regulating mitochondrial cytochrome c release. Thus, OPA1 is a protein related to mitochondrial structure and OXPHOS, where this protein promotes mitochondrial fusion when the IMM potential is intact [88,89]. However, $\Delta\Psi$ loss activates the metalloprotease OMA1, cleaving the long isoform of optic atrophy 1 (L-OPA1) to the short isoform of optic atrophy 1 (S-OPA1), inducing mitochondrial fragmentation [89]. It has been shown that mitochondrial ETS inhibitors like valinomycin, oligomycin, and carbonyl cyanide m-chlorophenyl hydrazine promote the cleavage of L-OPA1 to S-OPA1 [88]. This cleavage process of OPA1 is activated during mitochondrial stress response and is an early indicator of OS [90]. Thus, OS and after the depletion of $\Delta\Psi$, mitochondrial fragmentation is triggered, inducing loss of OXPHOS and activating OMA1. OMA1 activation promotes L-OPA1 proteolysis and mitochondrial fragmentation [88]. During OMA1 activation, this protein also undergoes autocatalytic degradation at the N- and C-terminal regions, restoring mitochondrial potential following the removal of stress stimuli or after mitochondrial damage is repaired. If ROS production persists, mitochondrial fragmentation results in cell death [91]. Thus, the increased processing of L-OPA1 to S-OPA1 by activated OMA1 is a general response to cellular OS [92]. Due to S-OPA1 being activated during OS, a cellular condition that induces ferroptosis, it has been proposed that S-OPA1 increase might be a prosurvival adaptation in ferroptosis induced by oxidant-stressed cells following IRI. Regarding the latter, it has been shown that cells that cannot cleave L-OPA1 to S-OPA1 are more susceptible to OS and, consequently, to ferroptosis cell death [93]. The latter is because high quantities of L-OPA1 generate a significant $O_2^{\bullet -}$ increase, producing sensitivity to mitochondrial permeability transition. Furthermore, OMA1 silencing increases oxidant-induced cell death, such as ferroptosis [93]. Thus, an increase in S-OPA1 and OMA1 may be a target to avoid oxidant-induced ferroptosis due to OS followed by IRI.

Mitochondrial fission depletion maintains mitochondrial function, providing mitochondrial quality. Nrf2 links ferroptosis and mitochondrial fission since Nrf2 upregulates proteasomal genes that induce the degradation of DRP1, inhibiting mitochondrial fission [94]. Moreover, ferroptosis induced by cisplatin in intestinal injury involves the overexpression of DRP1 and Fis1. However, vitamin D3 treatment alleviates ferroptosis by increasing antioxidant capacity, decreasing ROS production and MDA, attenuating excessive mitochondrial fission, and boosting mitochondrial ATPase activity [95]. Ferroptosis is also induced in non-small cell lung cancer cells by erastin by activating mitochondrial fission [96]. Mitochondrial fission and ferroptosis are also activated by inhibiting carbonic anhydrase 9 in malignant mesothelioma [97]. Similarly, in cholangiocarcinoma, the inhibition of SHANK-Associated RH Domain-Interacting Protein (*SHARPIN*) gene expression induced ROS accumulation associated with ferroptosis and mitochondrial fission by suppressing the p53/SLC7A11/GPX4 signaling pathway [98]. These findings exhibit the relationship between mitochondrial fission and ferroptosis. Under myocardial IRI following acute myocardial infarction, damaged mitochondria generate less ATP and produce ROS, which is detrimental to cell survival. For instance, it has been described that fission is highly regulated over mitochondrial fusion because there is an increase in the levels of DRP1 and a decrease in Mfn2, causing the overproduction of ROS and the mitochondrial membrane potential reduction, causing aberrant and dysfunctional mitochondria and apoptosis [65]. Interestingly, compounds such as vitexin, a flavonoid derived from the leaf of hawthorn, reduce ROS production and even increase Mfn2 expression, reducing the recruitment of DRP1 and OS in myocardial IRI [65]. Although this work did not study additional ferroptosis markers, such as GPX4, ferroptosis may play a critical role. Thus, it is necessary to continue exploring ferroptosis associated with mitochondrial dynamics in myocardial IRI, having as an essential target the machinery of fusion and fission in cell death, such as ferroptosis during I/R to avoid MI. Although relating mitochondrial dy-

namics with ferroptosis in myocardial IRI has been poorly explored, it is very certain that there exists a close relationship between both processes and that they may open new avenues of research to counteract the damage to the myocardium during the IRI process.

Conclusions

Myocardial IRI is a public health problem with millions of deaths per day. Although there are different strategies to reduce its effects, such as PPCI or thrombolytic drugs, the damage to the myocardium remains substantial, and this is because both in ischemia and reperfusion, there are metabolic changes, such as OS or Ca^{2+} overload, that induce cell death, such as ferroptosis. This review proposes new horizons for treating myocardial IRI, such as mitochondrial dynamics associated with decreased ferroptosis in myocardial IRI as therapeutic targets. Although they have been little explored, they suggest great benefits. For example, it has been described that during IRI there is an increase in fission and a deficiency in fusion. Restoring this imbalance could decrease OS and, therefore, death by ferroptosis. Treatments such as melatonin that reduce OS and promote increased fusion decrease cell death. However, it has not been found whether increased fusion decreases OS, decreases OS increases fusion, or both processes are independent. Even so, as shown in the review, essential molecules of mitochondrial fusion, such as OPA1, are crucial sensors of the redox state, so the evidence would lean toward a dependence between mitochondrial dynamics and ferroptosis through OS in IRI. So, this relationship remains to be discovered in future research, which would provide better tools for treating IRI.

Availability of Data and Materials

Not applicable.

Author Contributions

ACG developed the concept, drafted and revised the manuscript, approved the final version for publication, participated sufficiently in the work, and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The author declares no conflict of interest.

References

[1] Salari N, Morddarvanjoghi F, Abdolmaleki A, Rasoulpoor S, Khaleghi AA, Hezarkhani LA, *et al*. The global prevalence of myocardial infarction: a systematic review and meta-analysis. BMC Cardiovascular Disorders. 2023; 23: 206. https://doi.org/10.1186/s12872-023-03231-w.

[2] Saleh M, Ambrose JA. Understanding myocardial infarction. F1000Research. 2018; 7: F1000 Faculty Rev–1378. https://doi.org/10.12688/f1000research.15096.1.

[3] Salaudeen MA, Bello N, Danraka RN, Ammani ML. Understanding the Pathophysiology of Ischemic Stroke: The Basis of Current Therapies and Opportunity for New Ones. Biomolecules. 2024; 14: 305. https://doi.org/10.3390/biom14030305.

[4] Lasorsa F, Rutigliano M, Milella M, d'Amati A, Crocetto F, Pandolfo SD, *et al*. Ischemia-Reperfusion Injury in Kidney Transplantation: Mechanisms and Potential Therapeutic Targets. International Journal of Molecular Sciences. 2024; 25: 4332. https://doi.org/10.3390/ijms25084332.

[5] Khan MA, Hashim MJ, Mustafa H, Baniyas MY, Al Suwaidi SKBM, AlKatheeri R, *et al*. Global Epidemiology of Ischemic Heart Disease: Results from the Global Burden of Disease Study. Cureus. 2020; 12: e9349. https://doi.org/10.7759/cureus.9349.

[6] Ibáñez B, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. Journal of the American College of Cardiology. 2015; 65: 1454–1471. https://doi.org/10.1016/j.jacc.2015.02.032.

[7] Hausenloy DJ, Yellon DM. Ischaemic conditioning and reperfusion injury. Nature Reviews. Cardiology. 2016; 13: 193–209. https://doi.org/10.1038/nrcardio.2016.5.

[8] Munk K, Andersen NH, Schmidt MR, Nielsen SS, Terkelsen CJ, Sloth E, *et al*. Remote Ischemic Conditioning in Patients With Myocardial Infarction Treated With Primary Angioplasty: Impact on Left Ventricular Function Assessed by Comprehensive Echocardiography and Gated Single-Photon Emission CT. Circulation. Cardiovascular Imaging. 2010; 3: 656–662. https://doi.org/10.1161/CIRCIMAGING.110.957340.

[9] Chong B, Jayabaskaran J, Jauhari SM, Chan SP, Goh R, Kueh MTW, *et al*. Global burden of cardiovascular diseases: projections from 2025 to 2050. European Journal of Preventive Cardiology. 2024; zwae281. https://doi.org/10.1093/eurjpc/zwae281.

[10] Wu MY, Yiang GT, Liao WT, Tsai APY, Cheng YL, Cheng PW, *et al*. Current Mechanistic Concepts in Ischemia and Reperfusion Injury. Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology. 2018; 46: 1650–1667. https://doi.org/10.1159/000489241.

[11] Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiological Reviews. 2008; 88: 581–609. https://doi.org/10.1152/physrev.00024.2007.

[12] Bugger H, Pfeil K. Mitochondrial ROS in myocardial ischemia reperfusion and remodeling. Biochimica et Biophysica Acta. Molecular Basis of Disease. 2020; 1866: 165768. https://doi.org/10.1016/j.bbadis.2020.165768.

[13] Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. The Journal of Clinical Investigation. 2013; 123: 92–100. https://doi.org/10.1172/JCI62874.

[14] Hashmi S, Al-Salam S. Acute myocardial infarction and myocardial ischemia-reperfusion injury: a comparison. International Journal of Clinical and Experimental Pathology. 2015; 8: 8786–8796.

[15] Agyapong ED, Pedriali G, Ramaccini D, Bouhamida E, Tremoli E, Giorgi C, *et al*. Calcium signaling from sarcoplasmic reticulum and mitochondria contact sites in acute myocardial infarction. Journal of Translational Medicine. 2024; 22: 552. https://doi.org/10.1186/s12967-024-05240-5.

[16] Bertero E, Popoiu TA, Maack C. Mitochondrial calcium in cardiac ischemia/reperfusion injury and cardioprotection. Basic Research in Cardiology. 2024; 119: 569–585. https://doi.org/10.1007/s00395-024-01060-2.

[17] Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiological Reviews. 2014; 94: 909–950. https://doi.org/10.1152/physrev.00026.2013.

[18] Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, *et al*. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell. 2012; 149: 1060–1072. https://doi.org/10.1016/j.cell.2012.03.042.

[19] Cruz-Gregorio A, Aranda-Rivera AK. Quercetin and Ferroptosis. Life (Basel, Switzerland). 2023; 13: 1730. https://doi.org/10.3390/life13081730.

[20] Martínez-Reyes I, Chandel NS. Mitochondrial TCA cycle metabolites control physiology and disease. Nature Communications. 2020; 11: 102. https://doi.org/10.1038/s41467-019-13668-3.

[21] Casanova A, Wevers A, Navarro-Ledesma S, Pruimboom L. Mitochondria: It is all about energy. Frontiers in Physiology. 2023; 14: 1114231. https://doi.org/10.3389/fphys.2023.1114231.

[22] Spinelli JB, Haigis MC. The multifaceted contributions of mitochondria to cellular metabolism. Nature Cell Biology. 2018; 20: 745–754. https://doi.org/10.1038/s41556-018-0124-1.

[23] Larrañaga-SanMiguel A, Bengoa-Vergniory N, Flores-Romero H. Crosstalk between mitochondria-ER contact sites and the apoptotic machinery as a novel health meter. Trends in Cell Biology. 2025; 35: 33–45. https://doi.org/10.1016/j.tcb.2024.08.007.

[24] Wu C, Zhang Z, Zhang W, Liu X. Mitochondrial dysfunction and mitochondrial therapies in heart failure. Pharmacological Research. 2022; 175: 106038. https://doi.org/10.1016/j.phrs.2021.106038.

[25] Wang Y, Mohsen AW, Mihalik SJ, Goetzman ES, Vockley J. Evidence for physical association of mitochondrial fatty acid oxidation and oxidative phosphorylation complexes. The Journal of Biological Chemistry. 2010; 285: 29834–29841. https://doi.org/10.1074/jbc.M110.139493.

[26] Wang Y, Palmfeldt J, Gregersen N, Makhov AM, Conway JF, Wang M, *et al*. Mitochondrial fatty acid oxidation and the electron transport chain comprise a multifunctional mitochondrial protein complex. The Journal of Biological Chemistry. 2019; 294: 12380–12391. https://doi.org/10.1074/jbc.RA119.008680.

[27] Vargas-Mendoza N, Angeles-Valencia M, Morales-González Á, Madrigal-Santillán EO, Morales-Martínez M, Madrigal-Bujaidar E, *et al*. Oxidative Stress, Mitochondrial Function and Adaptation to Exercise: New Perspectives in Nutrition. Life (Basel, Switzerland). 2021; 11: 1269. https://doi.org/10.3390/life11111269.

[28] Aranda-Rivera AK, Cruz-Gregorio A, Arancibia-Hernández YL, Hernández-Cruz EY, Pedraza-Chaverri J. RONS and Oxidative Stress: An Overview of Basic Concepts. Oxygen. 2022; 2: 437–478.

[29] Holmström KM, Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signalling. Nature Reviews. Molecular Cell Biology. 2014; 15: 411–421. https://doi.org/10.1038/nrm3801.

[30] Jastroch M, Divakaruni AS, Mookerjee S, Treberg JR, Brand MD. Mitochondrial proton and electron leaks. Essays in Biochemistry. 2010; 47: 53–67. https://doi.org/10.1042/bse0470053.

[31] Liu Y, Lu S, Wu LL, Yang L, Yang L, Wang J. The diversified role of mitochondria in ferroptosis in cancer. Cell Death & Disease. 2023; 14: 519. https://doi.org/10.1038/s41419-023-06045-y.

[32] Paradies G, Petrosillo G, Pistolese M, Ruggiero FM. Reactive oxygen species generated by the mitochondrial respiratory chain affect the complex III activity via cardiolipin peroxidation in beef-heart submitochondrial particles. Mitochondrion. 2001; 1: 151–159. https://doi.org/10.1016/s1567-7249(01)00011-3.

[33] Mailloux RJ, Jin X, Willmore WG. Redox regulation of mitochondrial function with emphasis on cysteine oxidation reactions. Redox Biology. 2013; 2: 123–139. https://doi.org/10.1016/j.redox.2013.12.011.

[34] Zhang D, Wang F, Li P, Gao Y. Mitochondrial Ca^{2+} Homeostasis: Emerging Roles and Clinical Significance in Cardiac Remodeling. International Journal of Molecular Sciences. 2022; 23: 3025. https://doi.org/10.3390/ijms23063025.

[35] Kehrer JP. The Haber-Weiss reaction and mechanisms of toxicity. Toxicology. 2000; 149: 43–50. https://doi.org/10.1016/s0300-483x(00)00231-6.

[36] Jena AB, Samal RR, Bhol NK, Duttaroy AK. Cellular Red-Ox system in health and disease: The latest update. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie. 2023; 162: 114606. https://doi.org/10.1016/j.biopha.2023.114606.

[37] Andrés CMC, Pérez de la Lastra JM, Juan CA, Plou FJ, Pérez-Lebeña E. Chemistry of Hydrogen Peroxide Formation and Elimination in Mammalian Cells, and Its Role in Various Pathologies. Stresses. 2022; 2: 256–274.

[38] Priya Dharshini LC, Vishnupriya S, Sakthivel KM, Rasmi RR. Oxidative stress responsive transcription factors in cellular signalling transduction mechanisms. Cellular Signalling. 2020; 72: 109670. https://doi.org/10.1016/j.cellsig.2020.109670.

[39] Chen W, Zhao H, Li Y. Mitochondrial dynamics in health and disease: mechanisms and potential targets. Signal Transduction and Targeted Therapy. 2023; 8: 333. https://doi.org/10.1038/s41392-023-01547-9.

[40] Al Ojaimi M, Salah A, El-Hattab AW. Mitochondrial Fission and Fusion: Molecular Mechanisms, Biological Functions, and Related Disorders. Membranes. 2022; 12: 893. https://doi.org/10.3390/membranes12090893.

[41] Zong Y, Li H, Liao P, Chen L, Pan Y, Zheng Y, *et al*. Mitochondrial dysfunction: mechanisms and advances in therapy. Signal Transduction and Targeted Therapy. 2024; 9: 124. https://doi.org/10.1038/s41392-024-01839-8.

[42] Wang Y, Dai X, Li H, Jiang H, Zhou J, Zhang S, *et al*. The role of mitochondrial dynamics in disease. MedComm. 2023; 4: e462. https://doi.org/10.1002/mco2.462.

[43] Adebayo M, Singh S, Singh AP, Dasgupta S. Mitochondrial fusion and fission: The fine-tune balance for cellular homeostasis. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2021; 35: e21620. https://doi.org/10.1096/fj.202100067R.

[44] Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. Science (New York, N.Y.). 2012; 337: 1062–1065. https://doi.org/10.1126/science.1219855.

[45] Gao S, Hu J. Mitochondrial Fusion: The Machineries In and Out. Trends in Cell Biology. 2021; 31: 62–74. https://doi.org/10.1016/j.tcb.2020.09.008.

[46] Cogliati S, Frezza C, Soriano ME, Varanita T, Quintana-Cabrera R, Corrado M, *et al*. Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory effi-

ciency. Cell. 2013; 155: 160–171. https://doi.org/10.1016/j.cell.2013.08.032.

[47] Lhuissier C, Desquiret-Dumas V, Girona A, Alban J, Faure J, Cassereau J, *et al*. Mitochondrial F0F1-ATP synthase governs the induction of mitochondrial fission. iScience. 2024; 27: 109808. https://doi.org/10.1016/j.isci.2024.109808.

[48] Ding Q, Qi Y, Tsang SY. Mitochondrial Biogenesis, Mitochondrial Dynamics, and Mitophagy in the Maturation of Cardiomyocytes. Cells. 2021; 10: 2463. https://doi.org/10.3390/cells10092463.

[49] Tang BL. Sirt1 and the Mitochondria. Molecules and Cells. 2016; 39: 87–95. https://doi.org/10.14348/molcells.2016.2318.

[50] Wai T, Langer T. Mitochondrial Dynamics and Metabolic Regulation. Trends in Endocrinology and Metabolism: TEM. 2016; 27: 105–117. https://doi.org/10.1016/j.tem.2015.12.001.

[51] Baba T, Kashiwagi Y, Arimitsu N, Kogure T, Edo A, Maruyama T, *et al*. Phosphatidic acid (PA)-preferring phospholipase A1 regulates mitochondrial dynamics. The Journal of Biological Chemistry. 2014; 289: 11497–11511. https://doi.org/10.1074/jbc.M113.531921.

[52] Stamenkovic A, O'Hara KA, Nelson DC, Maddaford TG, Edel AL, Maddaford G, *et al*. Oxidized phosphatidylcholines trigger ferroptosis in cardiomyocytes during ischemia-reperfusion injury. American Journal of Physiology. Heart and Circulatory Physiology. 2021; 320: H1170–H1184. https://doi.org/10.1152/ajpheart.00237.2020.

[53] Ong SB, Subrayan S, Lim SY, Yellon DM, Davidson SM, Hausenloy DJ. Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. Circulation. 2010; 121: 2012–2022. https://doi.org/10.1161/CIRCULATIONAHA.109.906610.

[54] Zhang Y, Wang Y, Xu J, Tian F, Hu S, Chen Y, *et al*. Melatonin attenuates myocardial ischemia-reperfusion injury via improving mitochondrial fusion/mitophagy and activating the AMPK-OPA1 signaling pathways. Journal of Pineal Research. 2019; 66: e12542. https://doi.org/10.1111/jpi.12542.

[55] Ding M, Feng N, Tang D, Feng J, Li Z, Jia M, *et al*. Melatonin prevents Drp1-mediated mitochondrial fission in diabetic hearts through SIRT1-PGC1α pathway. Journal of Pineal Research. 2018; 65: e12491. https://doi.org/10.1111/jpi.12491.

[56] Disatnik MH, Ferreira JCB, Campos JC, Gomes KS, Dourado PMM, Qi X, *et al*. Acute inhibition of excessive mitochondrial fission after myocardial infarction prevents long-term cardiac dysfunction. Journal of the American Heart Association. 2013; 2: e000461. https://doi.org/10.1161/JAHA.113.000461.

[57] Coronado M, Fajardo G, Nguyen K, Zhao M, Kooiker K, Jung G, *et al*. Physiological Mitochondrial Fragmentation Is a Normal Cardiac Adaptation to Increased Energy Demand. Circulation Research. 2018; 122: 282–295. https://doi.org/10.1161/CIRCRESAHA.117.310725.

[58] Nan J, Nan C, Ye J, Qian L, Geng Y, Xing D, *et al*. EGCG protects cardiomyocytes against hypoxia-reperfusion injury through inhibition of OMA1 activation. Journal of Cell Science. 2019; 132: jcs220871. https://doi.org/10.1242/jcs.220871.

[59] Gao M, Monian P, Quadri N, Ramasamy R, Jiang X. Glutaminolysis and Transferrin Regulate Ferroptosis. Molecular Cell. 2015; 59: 298–308. https://doi.org/10.1016/j.molcel.2015.06.011.

[60] Tang LJ, Luo XJ, Tu H, Chen H, Xiong XM, Li NS, *et al*. Ferroptosis occurs in phase of reperfusion but not ischemia in rat heart following ischemia or ischemia/reperfusion. Naunyn-Schmiedeberg's Archives of Pharmacology. 2021; 394: 401–410. https://doi.org/10.1007/s00210-020-01932-z.

[61] Ma S, Sun L, Wu W, Wu J, Sun Z, Ren J. USP22 Protects Against Myocardial Ischemia-Reperfusion Injury via the SIRT1-p53/SLC7A11-Dependent Inhibition of Ferroptosis-Induced Cardiomyocyte Death. Frontiers in Physiology. 2020; 11: 551318. https://doi.org/10.3389/fphys.2020.551318.

[62] Tang LJ, Zhou YJ, Xiong XM, Li NS, Zhang JJ, Luo XJ, *et al*. Ubiquitin-specific protease 7 promotes ferroptosis via activation of the p53/TfR1 pathway in the rat hearts after ischemia/reperfusion. Free Radical Biology & Medicine. 2021; 162: 339–352. https://doi.org/10.1016/j.freeradbiomed.2020.10.307.

[63] Fang X, Wang H, Han D, Xie E, Yang X, Wei J, *et al*. Ferroptosis as a target for protection against cardiomyopathy. Proceedings of the National Academy of Sciences of the United States of America. 2019; 116: 2672–2680. https://doi.org/10.1073/pnas.1821022116.

[64] Chen HY, Xiao ZZ, Ling X, Xu RN, Zhu P, Zheng SY. ELAVL1 is transcriptionally activated by FOXC1 and promotes ferroptosis in myocardial ischemia/reperfusion injury by regulating autophagy. Molecular Medicine (Cambridge, Mass.). 2021; 27: 14. https://doi.org/10.1186/s10020-021-00271-w.

[65] Xue W, Wang X, Tang H, Sun F, Zhu H, Huang D, *et al*. Vitexin attenuates myocardial ischemia/reperfusion injury in rats by regulating mitochondrial dysfunction induced by mitochondrial dynamics imbalance. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie. 2020; 124: 109849. https://doi.org/10.1016/j.biopha.2020.109849.

[66] Vásquez-Trincado C, García-Carvajal I, Pennanen C, Parra V, Hill JA, Rothermel BA, *et al*. Mitochondrial dynamics, mitophagy and cardiovascular disease. The Journal of Physiology. 2016; 594: 509–525. https://doi.org/10.1113/JP271301.

[67] Zerihun M, Sukumaran S, Qvit N. The Drp1-Mediated Mitochondrial Fission Protein Interactome as an Emerging Core Player in Mitochondrial Dynamics and Cardiovascular Disease Therapy. International Journal of Molecular Sciences. 2023; 24: 5785. https://doi.org/10.3390/ijms24065785.

[68] Kaur S, Khullar N, Navik U, Bali A, Bhatti GK, Bhatti JS. Multifaceted role of dynamin-related protein 1 in cardiovascular disease: From mitochondrial fission to therapeutic interventions. Mitochondrion. 2024; 78: 101904. https://doi.org/10.1016/j.mito.2024.101904.

[69] Chen Z, Chua CC, Gao J, Chua KW, Ho YS, Hamdy RC, *et al*. Prevention of ischemia/reperfusion-induced cardiac apoptosis and injury by melatonin is independent of glutathione peroxidase 1. Journal of Pineal Research. 2009; 46: 235–241. https://doi.org/10.1111/j.1600-079X.2008.00654.x.

[70] He B, Zhao Y, Xu L, Gao L, Su Y, Lin N, *et al*. The nuclear melatonin receptor RORα is a novel endogenous defender against myocardial ischemia/reperfusion injury. Journal of Pineal Research. 2016; 60: 313–326. https://doi.org/10.1111/jpi.12312.

[71] Ma S, Dong Z. Melatonin Attenuates Cardiac Reperfusion Stress by Improving OPA1-Related Mitochondrial Fusion in a Yap-Hippo Pathway-Dependent Manner. Journal of Cardiovascular Pharmacology. 2019; 73: 27–39. https://doi.org/10.1097/FJC.0000000000000626.

[72] Hall AR, Burke N, Dongworth RK, Kalkhoran SB, Dyson A, Vicencio JM, *et al*. Hearts deficient in both Mfn1 and Mfn2 are protected against acute myocardial infarction. Cell Death & Disease. 2016; 7: e2238. https://doi.org/10.1038/cddis.2016.139.

[73] de Brito OM, Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature. 2008; 456: 605–610. https://doi.org/10.1038/nature07534.

[74] Chen Y, Csordás G, Jowdy C, Schneider TG, Csordás N, Wang W, *et al*. Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomyocyte bioenergetic responses via interorganelle Ca(2+) crosstalk. Circulation Research. 2012; 111: 863–875. https://doi.org/10.1161/CIRCRESAHA.112.266585.

[75] Jelinek A, Heyder L, Daude M, Plessner M, Krippner S, Grosse

R, *et al*. Mitochondrial rescue prevents glutathione peroxidase-dependent ferroptosis. Free Radical Biology & Medicine. 2018; 117: 45–57. https://doi.org/10.1016/j.freeradbiomed.2018.01.019.

[76] Oh SJ, Ikeda M, Ide T, Hur KY, Lee MS. Mitochondrial event as an ultimate step in ferroptosis. Cell Death Discovery. 2022; 8: 414. https://doi.org/10.1038/s41420-022-01199-8.

[77] Gao M, Yi J, Zhu J, Minikes AM, Monian P, Thompson CB, *et al*. Role of Mitochondria in Ferroptosis. Molecular Cell. 2019; 73: 354–363.e3. https://doi.org/10.1016/j.molcel.2018.10.042.

[78] Chen Y, Guo X, Zeng Y, Mo X, Hong S, He H, *et al*. Oxidative stress induces mitochondrial iron overload and ferroptotic cell death. Scientific Reports. 2023; 13: 15515. https://doi.org/10.1038/s41598-023-42760-4.

[79] Fan X, Li A, Yan Z, Geng X, Lian L, Lv H, *et al*. From Iron Metabolism to Ferroptosis: Pathologic Changes in Coronary Heart Disease. Oxidative Medicine and Cellular Longevity. 2022; 2022: 6291889. https://doi.org/10.1155/2022/6291889.

[80] Zhou H, Zhou YL, Mao JA, Tang LF, Xu J, Wang ZX, *et al*. NCOA4-mediated ferritinophagy is involved in ionizing radiation-induced ferroptosis of intestinal epithelial cells. Redox Biology. 2022; 55: 102413. https://doi.org/10.1016/j.redox.2022.102413.

[81] Chiang SK, Chen SE, Chang LC. A Dual Role of Heme Oxygenase-1 in Cancer Cells. International Journal of Molecular Sciences. 2018; 20: 39. https://doi.org/10.3390/ijms20010039.

[82] Miyamoto HD, Ikeda M, Ide T, Tadokoro T, Furusawa S, Abe K, *et al*. Iron Overload via Heme Degradation in the Endoplasmic Reticulum Triggers Ferroptosis in Myocardial Ischemia-Reperfusion Injury. JACC. Basic to Translational Science. 2022; 7: 800–819. https://doi.org/10.1016/j.jacbts.2022.03.012.

[83] Sabouny R, Shutt TE. Reciprocal Regulation of Mitochondrial Fission and Fusion. Trends in Biochemical Sciences. 2020; 45: 564–577. https://doi.org/10.1016/j.tibs.2020.03.009.

[84] Upadhyay M, Agarwal S. Ironing the mitochondria: Relevance to its dynamics. Mitochondrion. 2020; 50: 82–87. https://doi.org/10.1016/j.mito.2019.09.007.

[85] Mittler R, Darash-Yahana M, Sohn YS, Bai F, Song L, Cabantchik IZ, *et al*. NEET Proteins: A New Link Between Iron Metabolism, Reactive Oxygen Species, and Cancer. Antioxidants & Redox Signaling. 2019; 30: 1083–1095. https://doi.org/10.1089/ars.2018.7502.

[86] Wang C, Zhu L, Yuan W, Sun L, Xia Z, Zhang Z, *et al*. Diabetes aggravates myocardial ischaemia reperfusion injury via activating Nox2-related programmed cell death in an AMPK-dependent manner. Journal of Cellular and Molecular Medicine. 2020; 24: 6670–6679. https://doi.org/10.1111/jcmm.15318.

[87] Li W, Li W, Leng Y, Xiong Y, Xia Z. Ferroptosis Is Involved in Diabetes Myocardial Ischemia/Reperfusion Injury Through Endoplasmic Reticulum Stress. DNA and Cell Biology. 2020; 39: 210–225. https://doi.org/10.1089/dna.2019.5097.

[88] Griparic L, Kanazawa T, van der Bliek AM. Regulation of the mitochondrial dynamin-like protein Opa1 by proteolytic cleavage. The Journal of Cell Biology. 2007; 178: 757–764. https://doi.org/10.1083/jcb.200704112.

[89] Babbar M, Sheikh MS. Metabolic Stress and Disorders Related to Alterations in Mitochondrial Fission or Fusion. Molecular and Cellular Pharmacology. 2013; 5: 109–133.

[90] Wang R, Mishra P, Garbis SD, Moradian A, Sweredoski MJ, Chan DC. Identification of new OPA1 cleavage site reveals that short isoforms regulate mitochondrial fusion. Molecular Biology of the Cell. 2021; 32: 157–168. https://doi.org/10.1091/mbc.E20-09-0605.

[91] Gilkerson R, De La Torre P, St Vallier S. Mitochondrial OMA1 and OPA1 as Gatekeepers of Organellar Structure/Function and Cellular Stress Response. Frontiers in Cell and Developmental Biology. 2021; 9: 626117. https://doi.org/10.3389/fcell.2021.626117.

[92] MacVicar T, Langer T. OPA1 processing in cell death and disease - the long and short of it. Journal of Cell Science. 2016; 129: 2297–2306. https://doi.org/10.1242/jcs.159186.

[93] Lee H, Smith SB, Sheu SS, Yoon Y. The short variant of optic atrophy 1 (OPA1) improves cell survival under oxidative stress. The Journal of Biological Chemistry. 2020; 295: 6543–6560. https://doi.org/10.1074/jbc.RA119.010983.

[94] Sabouny R, Fraunberger E, Geoffrion M, Ng ACH, Baird SD, Screaton RA, *et al*. The Keap1-Nrf2 Stress Response Pathway Promotes Mitochondrial Hyperfusion Through Degradation of the Mitochondrial Fission Protein Drp1. Antioxidants & Redox Signaling. 2017; 27: 1447–1459. https://doi.org/10.1089/ars.2016.6855.

[95] Liu X, Wang S, Jin S, Huang S, Liu Y. Vitamin D_3 attenuates cisplatin-induced intestinal injury by inhibiting ferroptosis, oxidative stress, and ROS-mediated excessive mitochondrial fission. Food & Function. 2022; 13: 10210–10224. https://doi.org/10.1039/d2fo01028c.

[96] Liu M, Fan Y, Li D, Han B, Meng Y, Chen F, *et al*. Ferroptosis inducer erastin sensitizes NSCLC cells to celastrol through activation of the ROS-mitochondrial fission-mitophagy axis. Molecular Oncology. 2021; 15: 2084–2105. https://doi.org/10.1002/1878-0261.12936.

[97] Li Z, Jiang L, Chew SH, Hirayama T, Sekido Y, Toyokuni S. Carbonic anhydrase 9 confers resistance to ferroptosis/apoptosis in malignant mesothelioma under hypoxia. Redox Biology. 2019; 26: 101297. https://doi.org/10.1016/j.redox.2019.101297.

[98] Zeng C, Lin J, Zhang K, Ou H, Shen K, Liu Q, *et al*. SHARPIN promotes cell proliferation of cholangiocarcinoma and inhibits ferroptosis via p53/SLC7A11/GPX4 signaling. Cancer Science. 2022; 113: 3766–3775. https://doi.org/10.1111/cas.15531.