Mitochondria are cellular power stations and essential organelles for maintaining cellular homeostasis. Dysfunctional mitochondria have emerged as a key factor in the occurrence and development of cardiovascular disease. This review focuses on advances in the relationship between mitochondrial dysfunction and cardiovascular diseases such as atherosclerosis, heart failure, myocardial ischemia reperfusion injury, and pulmonary arterial hypertension. The clinical value and challenges of mitochondria-targeted strategies, including mitochondria-targeted antioxidants, mitochondrial quality control modulators, mitochondrial function protectors, mitochondrial biogenesis promoters, and recently developed mitochondrial transplants, are also discussed. (Am J Pathol 2023, 193: 1485–1500; https://doi.org/10.1016/j.ajpath.2023.06.013)
Interestingly, mitochondria not only serve as cellular power stations to control cells’ life activities but also play a central role in regulating various biologic processes. For example, mitochondrial components and metabolites such as nucleic acids, ATP, and proteins act as damage-associated molecular patterns to activate pattern recognition receptors on the surface of inflammatory cells, thereby promoting inflammation (Figure 1). Mechanically, mtDNA acts as damage-associated molecular patterns to promote inflammation by activating cyclic GMP–AMP synthase/stimulator of interferon response cGAMP interactor 1 signaling, inflammasome signaling, and Toll-like receptor 9. In addition, other mitochondrial components and products such as cytochrome c, cardiopilin, N-formyl peptides, ATP, and heme have been shown to promote inflammation.

In cells, strict quality control guarantees the normal physiological function of mitochondria (Figure 1). The proteome quality control system of mitochondria ensures that protein import, folding, and degradation are precise. Mitochondrial fission maintains the number and distribution of mitochondria, and mitochondrial fusion maintains normal mitochondrial function through exchanging contents from fused mitochondria. Mitophagy selectively removes dysfunctional or redundant mitochondria through the ubiquitin- or receptor-mediated pathway. Studies have shown that mitochondrial spheroid responds to mitochondrial oxidation independently of mitophagy, acting as a novel mitochondrial quality control (MQC) mechanism.

**Mitochondrial Dysfunction and CVD**

**ETC Dysfunction and CVD**

The ETC conducts electron transfer to generate ATP during oxidative phosphorylation. In myocardial ischemia, damaged ETC reduces cell death by decreasing cytochrome c release and activating calpain 1 through calcium overload and oxidative stress. The activation of calpain 1 mediates vascular remodeling and fibrosis in pulmonary arterial hypertension (PAH) via hypoxia-inducible factor-1α and participates in Coxsackievirus B3—induced myocardial injury by mitochondrial ROS-induced NOD-, LRR-, and pyrin domain—containing 3 inflammasome activation. Therefore, blocking ETC can enhance the tricarboxylic acid cycle and fatty acid oxidation (FAO) to reduce myocardial ischemia/reperfusion injury (MI). In PAH rats, the activities of complexes I and III are decreased, leading to metabolic shifts, a hallmark of PAH pathology. In patients with atrial fibrillation (AF), the activity of complexes I and II is decreased due to the reduced expression of the complex I subunit NDUF8 and posttranslational modifications of complex II subunits. In the ischemic heart, the activity of complexes I to IV and the expression of ETC proteins is decreased due to the increased expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), which is improved after blood flow is recovered. These data show that the decreased activity of ETC complexes is strongly linked to the development of CVD.

**Oxidative Stress and CVD**

Under physiological conditions, about 0.2% to 2% of electrons leak from ETC. When the ETC is impaired, a large number of electrons leak out to generate large amounts of ROS. ROS contribute to the occurrence and development of CVD through the following pathways. The first pathway is uncoupling of nitric oxide synthase (NOS): during the conversion of arginine to L-citrulline to produce NO, various cofactors, notably tetrahydrobiopterin, are required to stabilize NOS. ROS can deplete tetrahydrobiopterin and directly bind to NO to form ONOO−, leading to the uncoupling of NOS. This process has been considered as a hallmark of CVD. A second pathway is inflammation: ROS up-regulates pro-inflammatory factors such as IL-1β, IL-6, and tumor necrosis factor-α; activates the NOD-, LRR-, and pyrin domain—containing 3 inflammasome and inflammasome; and then promotes the development of CVD. The final pathway is a vicious circle: excessive ROS inhibit mitochondrial Na+/Ca2+ exchange and damage mtDNA, resulting in mitochondrial dysfunction, which in turn leads to more ROS production.

**mtDNA Damage and CVD**

mtDNA is susceptible to damage by ROS compared with nuclear DNA due to its lack of histone protection and its proximity to the ETC. A prospective, population-based cohort analysis of 21,870 participants found that the reduction of mtDNA copy numbers is an independent risk factor for cardiovascular events. In addition, mtDNA copy numbers are inversely associated with the risk of heart failure (HF) events, the occurrence of adverse stroke events, and the risk of AF. Mechanically, mtDNA damage induces excessive ROS to promote mitochondrial dysfunction, resulting in more serious mtDNA damage, thus forming a vicious cycle. Consistent with this notion, mtDNA mutant mice at a later stage exhibit higher levels of ROS than at an early stage. Moreover, mtDNA damage reduces ATP synthesis and the MMP, which increases the opening of mitochondrial permeability transition pore (mPTP) and apoptosis. Furthermore, mtDNA, as damage-associated molecular patterns, activate cyclic GMP–AMP synthase/stimulator of interferon response cGAMP interactor 1 and Toll-like receptor 9 signaling, promoting cardiovascular inflammation and accelerating progression of CVD. Notably, mtDNA mutant mice exhibit hyperlipidemia, as well as decreased subcutaneous fat and body weight, suggesting that mtDNA damage leads to the systemic metabolic switch, which might be a potential way to promote the progression of CVD.
Mitochondrial Quality Control Dysfunction and CVD

Deregulation of Mitophagy and CVD

Mitophagy is divided into ubiquitin-mediated mitophagy and receptor-mediated mitophagy. Mitophagy and mitochondrial function are impaired in patients with HF and in HF mouse models. When mitophagy is restored, the damaged mitochondria are removed, and mitochondrial function is improved, contributing to a positive effect on HF. In addition, Parkin−/− mice develop pathologic cardiovascular hypertrophy and left ventricular dysfunction as early as 2 months of age, accompanied by mitochondrial dysfunction and enhanced oxidative stress. Similarly, the administration of cytosolic p53 (a Parkin inhibitor) in mice inhibits mitophagy and leads to myocardial mitochondrial dysfunction. Moreover, Parkin−/− mice are more prone to myocardial infarction and have lower survival rates than wild-type mice, and Parkin-regulated mitophagy is impaired in aging hearts. These results suggest that impaired mitophagy leads to the accumulation of damaged mitochondria and the development of CVD.

Deregulation of Mitochondrial Fission and CVD

Mitochondrial fission is a process of self-maintenance and repair in which mitochondrial components are separated by asymmetric division and subsequently removed by mitophagy or by symmetric division into two functional mitochondria. In the myocardial infarction region, excessive mitochondrial fission is caused by hypoxia, and the inhibition of mitochondrial fission delays myocardial senescence and HF. This phenomenon is also observed in MIRI. Furthermore, right ventricular (RV) fibroblasts and myocytes in PAH exhibit excessive mitochondrial fission, whereas mitochondrial fission inhibitors (mdivi-1 and P110) protected RV function in RV-IR in a PAH rat model.

Generally, GTPase dynamin-related protein 1 (Drp1) and dynamin proteins (DNM1, DNM2, and DNM3) are considered to be required in mitochondrial fission. However, recent studies showed that mitochondrial fission is impaired by the down-regulation of Drp1 instead of dynamins. When Drp1 is deficient, mitochondria become larger, and the expression of oxidative phosphorylation protein is reduced, resulting in impaired mitochondrial respiration and energy production. In addition, Drp1 induces excessive opening of mPTP through Bcl-2−associated X protein/phosphate carrier protein and leucine-rich repeat serine/threonine-protein kinase 2-hexokinase 2, resulting in mitochondrial swelling and mitochondrial membrane rupture, and ultimately causing cell death. Drp1 also enhances the activation of endothelial proinflammatory signaling NF-κB, the adhesion of monocytes and leukocytes, and the production of mitochondrial ROS. Drp1 is therefore the key molecule for mitochondrial fission and function homeostasis. Targeting with Drp1 might be a potential novel strategy for the prevention and treatment of CVD.

Deregulation of Mitochondrial Fusion and CVD

Mitochondrial fusion begins with the juxtaposition and tethering of adjacent mitochondria, in which mitofusin (Mfn) 1 and 2 and optic atrophy 1 (OPA1) are key mediators. OPA1 regulates apoptosis and mitochondrial...
respiration. The decreased expression of OPA1 leads to decreased nuclear antioxidant gene expression and mtDNA copy numbers in failing hearts.28 In addition, mitochondrial fusion proteins regulate calcineurin and Notch signaling, which are essential for cardiac differentiation.29

Endothelial cell (EC) migration and differentiation as well as vascular smooth muscle cell (VSMC) phenotypic transformation are hallmarks of CVD. The deficiency of Mfn1 and Mfn2 attenuates EC viability, migration, and differentiation.30,31 Mfn1 is down-regulated in patients with idiopathic dilated cardiomyopathy32 as well as Mfn2 in postinfarction remodeling33 and cardiac hypertrophy.34 The decreased expression of Mfn2 leads to proliferation–apoptosis imbalance, mitochondrial fragmentation, and overproliferation of pulmonary artery smooth muscle cells (PASMCs), promoting the development of PAH.35–37 Therefore, the up-regulation of Mfn2 is valuable in attenuating CVD.

**Mitochondrial Dysfunction and Specific CVD**

Normal physiological function of mitochondria, as cellular power stations, is a prerequisite for cell life activities. Under stress, mitochondrial function is impaired. Specifically, the mitochondrial ETC is damaged, leading to impaired ATP production and massive ROS production, which in turn damage mtDNA and then accelerate the progression of CVD. In addition, MQC (mitophagy, mitochondrial fission, and mitochondrial fusion) is also impaired, disrupting mitochondrial homeostasis, leading to an enormous accumulation of dysfunctional mitochondria and promoting the progression of CVD.

The mechanism of specific CVD progression in dysfunctional mitochondria is different (Table 1).9,22,23,29,55,38–61 Oxidative stress plays a key role in the progression of atherosclerosis, HF, MIRI, and PAH. However, rather than inducing cell death and inflammation to promote the progression of atherosclerosis, dysfunctional mitochondria can also influence the progression of HF by affecting the metabolism of substances. In addition, mitochondrial quantity (mitophagy, mitochondrial fission, and mitochondrial fusion) influences both MIRI and PAH progression, but mitochondrial mass (oxidative stress, calcium overload, and inflammation) plays a more important role in MIRI than in PAH.

**Atherosclerosis**

Atherosclerosis, a major contributor to CVD, is characterized by the dysfunction or injury of ECs, formation of foam cells, and massive release of pro-inflammatory cytokines. Emerging evidence showed that dysfunctional mitochondria are linked to atherosclerosis primarily through the following mechanisms. The first is oxidative stress: ROS directly damages lipids, proteins, and DNA, causing cell dysfunction and even death. Under oxidative stress, low-density lipoprotein is oxidized, which leads to EC dysfunction, the formation of macrophage-derived foam cells, the transformation of VSMC phenotype, and impaired mitochondrial respiratory activity and ATP production. In addition, NO is oxidized to ONOO⁻, and NOS is uncoupling, which benefits the pathophysiological progression of atherosclerotic lesions. In addition, oxidative stress increases the expression of matrix metalloproteinases, resulting in atherosclerotic plaque development and rupture. The second mechanism is mtDNA damage: Patients with atherosclerosis exhibit a positive correlation between mtDNA mutations and the progression of atherosclerosis.38 The deletion of mtDNA also leads to an increased risk of all-cause mortality in patients with coronary artery disease.39 Mechanically, mtDNA damage promotes inflammation and oxidative stress, cell death, and the alternation of metabolism. The third mechanism is cell death: The abnormal death of ECs disrupts normal endothelial structure; it promotes lipid deposition, expression of adhesion molecules, adhesion of monocytes, and the subsequent transformation of macrophage-derived foam cells, leading to plaque rupture and thrombus formation. The death of VSMCs leads to plaque thinning, resulting in plaque instability and the eventual rupture of plaques. Interestingly, the roles of macrophage death depend on the stages of atherosclerotic lesions. In the early stage, it exerts an antiatherosclerotic effect, while it promotes the formation of a necrosis core in advanced lesions. The fourth mechanism is inflammation: In the 1980s, inflammatory cells were discovered within the plaques and since then, the inflammatory theory of

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**Table 1 Mitochondrial Dysfunction in Specific Cardiovascular Disease**

<table>
<thead>
<tr>
<th>Cardiovascular disease</th>
<th>Mitochondrial dysfunction</th>
</tr>
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<tbody>
<tr>
<td>Atherosclerosis</td>
<td>ROS ↑, mtDNA damage,38,39 cell death, inflammation</td>
</tr>
<tr>
<td>Heart failure</td>
<td>ROS ↑, inflammation, calcium overload, FAO ↓, glycolysis ↑, ketone body oxidation ↑45–51</td>
</tr>
<tr>
<td>Myocardial ischemia reperfusion injury</td>
<td>ROS ↑, calcium overload,52 inflammation ↑, impaired mitochondrial fission22,53 and fusion, impaired mitophagy54,55</td>
</tr>
<tr>
<td>Pulmonary arterial hypertension</td>
<td>ROS ↑,9,56 impaired mitophagy,57–60 impaired mitochondrial fission73,29,61 and fusion15</td>
</tr>
</tbody>
</table>

FAO, fatty acid oxidation; mtDNA, mitochondrial DNA; ROS, reactive oxygen species.
atherosclerosis has emerged. Subsequently, an increasing number of animal experiments and clinical trials have consolidated the inflammatory theory. To date, multiple inflammatory cells and their subtypes have been identified. Among the inflammatory cells involved in atherosclerosis, macrophages bear the brunt, phagocytosing oxidized low-density lipoprotein to transform into foam cells. Lymphocytes and neutrophils then enter the vasculature and release a variety of mediators that affect plaque stability. The rupture of plaque can lead to the development of ischemic heart disease, stroke, and other serious life-threatening diseases.

Heart Failure

HF, a bioenergetic disease, is the end stage of CVD. The role of mitochondrial dysfunction in promoting HF by increasing intracellular ROS, inflammation, and calcium homeostasis imbalance has been described in detail in the literature. This section provides a thorough description of the role of dysfunctional mitochondria on substrate utilization impairment and its potential roles in the regulation of HF.

The heart is an energy-consuming organ. Fatty acid (FA), glucose, and ketone body provide most of the ATP to meet energy needs. Among them, FA is the main energy donor, providing 50% to 70% of the ATP of the heart. When FA transporters (CD36 and FA transport protein-1) are activated, FA enters cells and is esterified into fatty acyl-CoA, and then forms long-chain acylcarnitine with the action of carnitine palmitoyltransferase I. After transport to the mitochondria, the long-chain acylcarnitine is modified and oxidized with the help of carnitine palmitoyltransferase II to produce CoASAc. In the fetal heart, glucose is absorbed through glucose transporter 1, whereas the absorption of glucose in the adult heart is mainly mediated by glucose transporter 4. Pyruvate is produced during glycolysis, which then enters the mitochondria via the mitochondrial pyruvate carrier and produces CoASAc under the action of pyruvate dehydrogenase; this is followed by addition to the tricarboxylic acid cycle. Ketone bodies are an important energy substance in the heart, among which β-hydroxybutyrate is particularly important. It enters the cell through monocarboxylate transporter 1 (SLC16A1) and oxidizes under the action of β-hydroxybutyrate dehydrogenase 1. It is then transformed into CoASAc by succinyl-CoA:3-oxoacid-CoA transferase and enters the tricarboxylic acid cycle. However, in the failing heart, metabolism is altered and metabolic reprogramming occurs, characterized by decreased FAO, increased glucose uptake, and ketone body oxidation (Figure 2).

The heart of CPT1b−/− mice is more prone to hypertrophy and failure.40 Stimulating mitochondrial FAO plays a positive role in the prevention of HF. Interestingly, activation of peroxisome proliferator-activated receptor α, a regulator of lipid metabolism, results in increased mitochondrial FA uptake and FAO.41 Moderate restoration of FAO exhibits a positive effect on HF, whereas excessive FA uptake and oxidation can impair cardiac function as well as produce lipotoxicity in the heart.42

Although glucose intake is increased in the failing heart, most of its metabolism is through the glycolytic rather than the oxidative pathway. This may be related to the downregulation of glucose transporter 4, suggesting that regulation of glucose metabolism may provide new possibilities for the treatment of HF.43 Sodium-glucose cotransporter 2 inhibitors have been used in the treatment of diabetes by inhibiting glucose reabsorption, lowering the renal glucose threshold, and promoting glucose excretion from the urine. These agents reduce the risk of hospitalization and improve

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**Figure 2** Metabolism of fatty acid, glucose, and ketone bodies in the failing heart. In the failing heart, the oxidation of fatty acid and glucose decreases, while the oxidation of ketone body increases. Fatty acid, glucose, and ketone body produce CoASAc under the action of various channel carriers and enzymes, and then enter the tricarboxylic acid (TCA) cycle to produce ATP. BDH1, β-OHB dehydrogenase 1; CPT1, carnitine palmitoyltransferase-1; FAT, fatty acid transporter; GLUT, glucose transporter; MPC, mitochondrial pyruvate carrier; PDH, pyruvate dehydrogenase; SCOT, succinyl-CoA:3-oxoacid-CoA transferase; SLC16A1, solute carrier family 16 member 1.
the prognosis of patients with HF. In addition, sodium-glucose cotransporter 2 inhibitors delay the progression of atherosclerosis and reduce the incidence of AF and the risk of cardiovascular events.

Ketone bodies, intermediates of FAO, include acetocetate, acetone, and β-hydroxybutyrate. The oxidation of the ketone body is increased in the failing heart, which is related to increased expression of ketone body transport proteins as well as the key enzyme for ketone body oxidation. However, increased ketone body oxidation did not affect FA and glucose metabolism and also did not improve cardiac metabolism efficiency.

Ketone body therapy has positive effects on CVD, such as improving endothelial and mitochondrial function, inhibiting oxidative stress and inflammation, and regulating cardiovascular risk factors, including lipids, body weight, blood pressure, and glucose. Long-term supplementation of ketone ester slows the development of mouse HF, reduces pathologic remodeling of the heart, and significantly increases the expression of two ketolytic enzymes and ketone transporter associated with ketone body metabolism in the heart. Clinical trial results also show that supplementation of 3-hydroxybutyrate improves hemodynamics and cardiac function in patients with HF.

Myocardial Ischemia Reperfusion Injury

MIRI refers to the restoration of blood flow to the myocardium after a period of ischemia, which further aggravates the damage. Due to hypoxia, cellular metabolism is switched to anaerobic glycolysis. During this process, lactic acid is produced, and the cell pH is decreased, which activates the Na+/H+ exchanger. In addition, due to the reduction of ATP synthesis, the activity of the sodium pump is decreased, and the intracellular Na+ concentration is increased. When blood flow is restored and oxygen is regained, ATP synthesis is restored, and the Na+ concentration difference between inside and outside the cell is increased. The Na+/H+ ion exchanger is then activated, and the cellular Na+/Ca2+ exchange is increased, resulting in calcium overload. The increase of ROS, pH, and calcium overload promotes the opening of the mPTP and the release of cytochrome c, causing abnormal mitochondrial mass and cardiomyocyte apoptosis (Figure 3). Thus, MIRI is closely associated with abnormal mitochondrial function (oxidative stress, calcium overload, and inflammation) and abnormal mitochondrial quantity (mitochondrial fission, mitochondrial fusion, and mitophagy).

The first factor in MIRI, abnormal mitochondrial function, includes oxidative stress, calcium overload, and inflammation. A high ratio of NAD+/NADH is the prerequisite for electron transfer to maintain MMP and mitochondrial ATP synthesis. Following myocardial hypoxia for an extended time, the NAD+/NADH ratio and the electron carrier coenzyme Q content are decreased. Therefore, electrons cannot be efficiently transferred into the mitochondrial respiratory chain when blood is restored. This leads to the massive synthesis of ROS and oxidative stress, causing cardiomyocyte damage. In addition, high levels of ROS prolong the opening of mPTP, which leads to the massive release of ROS and forms a vicious cycle. Abnormal mitochondrial function also includes calcium overload. Under physiological conditions, Na+/Ca2+ exchangers transport Ca2+ intracellularly to extracellularly, which is ATP required. During myocardial ischemia, ATP synthesis is reduced, resulting in the intracellular accumulation of Na+. When the oxygen supply is restored, Na+/Ca2+ exchangers mediate the reverse transport; that is, Na+ is transported out and Ca2+ is transported into cardiomyocytes. In addition, catecholamine is increased during ischemia-reperfusion, which activates the G protein-phospholipase C–mediated cellular signaling pathway to promote Ca2+ release from the endoplasmic reticulum, leading to the overload of intracellular Ca2+. These excessive Ca2+ impair cell membrane and mitochondrial ATP synthesis, exacerbating MIRI. A third factor in abnormal mitochondrial function is inflammation. The prolonged opening of mPTP during reperfusion and cell membrane damage leads to mtDNA released into the cytoplasm, which acts as damage-associated molecular patterns to promote pro-inflammatory cytokine activation as well as expression, inducing inflammation.

The second factor in MIRI is abnormal mitochondrial quantity, including mitochondrial fission, mitochondrial fusion, and mitophagy. Under physiological conditions, Drp1 is inactive and present in the cytoplasm. Under stress, it undergoes posttranslational modifications (eg, phosphorylation), which leads to changes in conformation and subsequently binds to receptors on the mitochondrial membrane, inducing mitochondrial fission. Ser616 and Ser637 are the two main phosphorylation sites of Drp1. Ser616 phosphorylation enhances the binding of Drp1 with membrane receptors to enhance mitochondrial fission, whereas Ser637 phosphorylation impairs the oligomerization of Drp1 and inhibits mitochondrial fission. During myocardial reperfusion, Ser616 phosphorylation is increased while Ser637 phosphorylation is decreased, leading to excessive mitochondrial fission. As a result, MMP dissipation and mPTP opening are increased, which promote cardiomyocyte apoptosis via decreased ATP synthesis and increased cytochrome c release. Abnormal mitochondrial quantity also includes mitochondrial fusion, which occurs under the regulation of OPA1, Mfn1, and Mfn2. Moderate mitochondrial fusion inhibits apoptosis triggered by excessive mitochondrial fission and maintains mitochondrial homeostasis. However, in myocardial reperfusion, calcium overload leads to decreased expression of OPA1 and Mfn2, inhibiting mitochondrial fusion. Mitophagy is the final factor in abnormal mitochondrial quantity. During myocardial ischemia-reperfusion, the reduction of MMP promotes the aggregation and phosphorylation of PINK1 in the outer mitochondrial membrane, which
promotes the aggregation of Parkin and excessive mitophagy. In addition, the mitophagy receptor FUN14 domain-containing 1 (FUNDC1)-mediated mitophagy is also altered. During myocardial ischemia-reperfusion, casein kinase 2α, the upstream inhibitory factor of FUNDC1, is upregulated to inhibit FUNDC1-mediated mitophagy.\textsuperscript{54} Casein kinase 2α also blocks MMP depolarization-induced, FUNDC1-mediated mitophagy.\textsuperscript{55} The imbalance of mitophagy leads to the untimely clearance of dysfunctional mitochondria and increases apoptosis of cardiomyocytes.

Pulmonary Arterial Hypertension

PAH is a syndrome involving pulmonary artery endothelium, myocardium, and epicardium with mean pulmonary artery pressure >20 mm Hg, in which pulmonary blood flow is restricted and pulmonary vascular resistance is increased. When mitochondrial dysfunction occurs, large amounts of ROS are produced. In the PAH rat model, the activity of complexes I to III is reduced, and the production of ROS is increased.\textsuperscript{59} ROS contributes to pulmonary vasoconstriction by increasing Ca\textsuperscript{2+} concentration during acute hypoxia. However, in spontaneous PAH rats, the expression of superoxide dismutase-2 is reduced, resulting in the reduction of ROS production.\textsuperscript{56}

Impaired MQC also plays a key role in PAH. Mitochondrial fragmentation is increased in PASMCs of patients with PAH, indicating the insufficient removal of dysfunctional mitochondria by mitophagy.\textsuperscript{57} UCP2\textsuperscript{−/-} (a protein that regulates mitophagy) mice exhibit increased mitophagy, higher RV systolic pressure, spontaneous pulmonary vascular remodeling, more severe right heart hypertrophy, and pulmonary artery pressure.\textsuperscript{58,59} The mitophagy protein light chain-3B is also increased in patients with PAH. However, the knockout of light chain-3B leads to increased ROS, higher sensitivity to hypoxia-induced PAH, and higher pulmonary artery pressure.\textsuperscript{60} Further studies are therefore needed to identify the roles of mitophagy on PAH.

Increased Drp1 expression is also observed in PAH rat models and in patients with PAH. The activation of Drp1 increases mitochondrial fission, promoting RV fibroblast proliferation and collagen production. High-mobility group box-1, a biomarker of PAH, promotes Drp1 phosphorylation and then induces PASMC proliferation/migration.\textsuperscript{61} When Drp1 is inhibited, mitochondrial fission, PASMC proliferation, and RV hypertrophy are suppressed, thereby delaying...
Mitochondrial Therapeutic Strategies and CVD
Mitochondria-Targeted Antioxidants

Dysfunctional mitochondria produce large amounts of ROS, which promotes the development of CVD. Antioxidants are therefore a particularly important potential target for the treatment of CVD. Antioxidants include nontargeted antioxidants and mitochondria-targeted antioxidants (MTAs). Previous studies show that nontargeted antioxidants such as β-carotene, vitamin A, vitamin C, and vitamin E have positive effects on the treatment of CVD. However, with further research, some studies have shown that supplementation with these nontargeted antioxidants does not reduce the morbidity and mortality of CVD and that over-supplementation can even lead to the further development of CVD. Recently, increasing attention has been focused on MTAs such as lipophilic cation-linked MTAs, liposome-encapsulated antioxidants, and peptide-based mitochondrial antioxidants.

Mitoquinone (mitoQ), a lipophilic cation-linked MTA, can enter mitochondria and shows positive efficacy in CVD. Mechanistically, mitoQ improves mitochondrial function, regulates redox-related noncoding RNAs, reduces peroxide production, improves endothelial function, and delays cardiac dysfunction. In patients with hypertension, long-term supplementation of mitoQ significantly reduces oxidative stress and inflammation levels and improves endothelial function and aortic stiffness to benefit cardiovascular health. More important, mitoQ supplementation also improves endothelial function and reduces aortic stiffness to lower the risk of CVD in the elderly healthy population. These results show that mitoQ can be beneficial in the treatment of CVD as well as in its prevention. Interestingly, recent studies found that the combination of mitoQ with moderate endurance training is more effective for patients with hypertension. These patients treated with mitoQ and moderate endurance training exhibit increased serum antioxidant (glutathione peroxidase and superoxide dismutase) and NO levels and reduced left ventricular hypertrophy and diastolic blood pressure. However, reduced left ventricular hypertrophy and diastolic blood pressure are not achieved in patients treated with mitoQ or moderate endurance training alone. In addition, studies showed that mitoQ can inhibit platelet activation and slow cardiovascular progression by suppressing platelet adhesion induced by CD63 and P-selectin, as well as platelet aggregation induced by collagen and convulxin. These data indicate that mitoQ might be a very valuable molecule for inhibiting the progression of CVD and improving its outcomes.

Liposome-encapsulated antioxidants can deliver oxidants into mitochondria without changing the structure and function of the oxidants, thereby improving their effects. For example, liposome-encapsulated curcumin significantly improves its bioavailability and increases antioxidant capacity compared with direct oral curcumin. In the rat ischemia model, antioxidants encapsulated with liposomes enhance their ability to inhibit oxidative stress and significantly reduce reperfusion injury. Although liposome-encapsulated MTAs display good antioxidant capacity, they inhibit endosome degradation. The MITO-Porter, composed of lipid and plasmid DNA, was developed to deliver MTAs. The effectiveness of the MITO-Porter in delivering MTAs has been shown in fluorescent probe experiments and has been successful in various animal studies. Despite the lack of clinical data, researchers believe that MITO-Porter will bring positive effects to the clinical treatment of CVD.

SS-peptide is an antioxidant activity peptide that can enter mitochondria. Its cardiovascular efficacy has been extensively reported in animal models and clinical trials. For example, Bendavia (SS-31) inhibits mitochondrial respiratory chain uncoupling and ROS production in ECs, protects ATP synthesis and mitochondrial function, and also reduces EC apoptosis under oxidative stress. In addition, SS-31 binds to cardiolipin on the mitochondrial membrane and inhibits cytochrome c peroxidase activity, significantly improving mitochondrial function and reducing IRI. In ApoE−/− mice, the administration of SS-31 not only increased the activity of the endogenous antioxidant superoxide dismutase but also reduced cholesterol deposition through the down-regulation of LOX-1 and CD36, inhibiting the development of atherosclerosis. In the clinical treatment of CVD, the positive roles of SS-peptide have also been shown. For example, in myocardial infarction patients after percutaneous coronary intervention, myocardial infarct size was significantly reduced after intravenous administration of SS-31. In addition, elamipretide (MTP-131, a type of SS-peptide) can increase the activity of complexes I and V to improve the mitochondrial respiratory chain and mitochondrial oxygen flux in human failing hearts, reducing left ventricular end-diastolic volume and improving outcomes for patients with HF. Furthermore, MTP-131 is found to reduce myocardial infarction patients’ serum high-temperature requirement serine peptidase 2 levels to inhibit caspase-mediated apoptosis and improve patient prognosis. Moreover, no serious adverse events have been observed in MTP-131—treated HF patients.

MQC Modulators

Recent results from animal models suggest that modulating MQC might offer a new potential treatment for CVD. For example, melatonin, an endogenous hormone secreted by the pineal gland, promotes mitophagy and mitochondrial fusion by activating the ATP-activating AMP-activated
protein kinase (AMPK)-OPA1 signaling pathway to maintain myocardial function and attenuate MIRI. In addition, melatonin reduces mitochondrial fission and reactivates sirtuin 6 and AMPK-PGC-1α/AKT signaling to enhance mitophagy and ultimately reduce MIRI. It also significantly reduces the size of atherosclerotic lesions and improves plaque stability by activating mitophagy. Taken together, melatonin slows cardiovascular progression and improves the quality of life of cardiovascular patients. More important, it is clinically safe.

Irisin, an exercise-induced myokine, is inversely associated with cardiovascular development and serves as a novel biomarker of cardiovascular risk. Mechanically, irisin protects cell viability and mitochondrial function after myocardial infarction by activating OPA1-mediated mitophagy. It also reduces the mitochondrial fission of VSMCs and inhibits osteoblast transformation by regulating the AMPK/Drp1 signaling pathway. In addition, pre-treatment with gastrodin (a Chinese medicinal herb component) reduced infarct size in ischemia-reperfusion male C57BL/6 mice via increased mitophagy levels during myocardial IR, whereas this effect was significantly reduced when mitophagy was inhibited with chloroquine. The pretreatment of electroacupuncture modulates mitophagy via the mechanistic target of rapamycin complex 1 (mTORC1)—Unc-51-like kinase 1 (ULK1)—FUNDC1 pathway, which attenuates MIRI, and this therapeutic effect is blocked by rapamycin (inhibitor of mammalian target of rapamycin).

Recently, studies have found that regulation of mitochondrial dynamics also has a positive impact on the development of CVD. For example, hydralazine (a vasodilator) reduces acute MIRI by inhibiting Drp1-mediated mitochondrial fission in a dose-dependent manner. Klotho (an antiaging protein) reduces doxorubicin-induced cardiotoxicity by significantly delaying doxorubicin-induced phosphorylation of Drp1 Ser616 and then reducing apoptosis and mitochondrial fission. Mitochondrial fission inhibitor mdivi-1 attenuates MIRI and improves prognosis by selectively inhibiting mitochondrial fission. In addition, it restores mitochondrial activity to reverse angiotensin II–induced VSMD phenotype switching and alleviate hypertension. Notably, Drp1-deficient mice exhibit the accumulation of mitochondrial elongation and dysfunction, and myocardial injury after IR is more pronounced, which is associated with mitochondrial fission and mitophagy imbalance. Therefore, further studies to balance mitochondrial dynamics with mitophagy are required. Donepezil reduces myocardial infarct size and arrhythmias by balancing mitochondrial dynamics with mitophagy during IR in mice, regardless of the timing of administration. These results indicate that donepezil has the potential to balance these two relationships.

Interestingly, studies have shown that food and exercise can affect MQC and CVD. Intake of a high-protein diet sharply increases amino acid levels in the body, which inhibit mitophagy through the mTORC1 signaling pathway and increases cardiovascular risk. The addition of fish oil to the high-fat diet of ApoE−/− mice significantly increased the expression of Mfn2 and OPA1 and decreased the expression of Fis1, improving EC function. In addition, when male Wistar rats are subjected to aerobic exercise, Mfn1 and Mfn2 expression are increased and Drp1 expression is decreased, resulting in a protective effect on myocardial IR.

Protection of Mitochondrial Function

With the deepening understanding of the role of mitochondria in CVD, the protection of mitochondria has gradually become regarded as an important measure for the treatment of CVD. Studies have shown that substances such as NAD, glutamine, and coenzyme Q10 (CoQ10) play active roles in CVD by improving mitochondrial function. NAD is not only involved in electron transport in the mitochondrial respiratory chain as an electron carrier but also protects mitochondria by regulating sirtuin 3 to reduce production of mitochondrial ROS. Indeed, during the progression of CVD, NAD metabolism is deranged and mitochondrial function is protected by supplementation of NAD precursors (nicotinamide and nicotinamide riboside), showing positive cardiovascular effects. For example, nicotinamide supplementation increases the expression of tricarboxylic acid— and oxidative phosphorylation—related genes in the heart to restore cardiac energy supply and improve prognosis. Long-term nicotinamide supplementation reduces HF morbidity and cardiac mortality in the population. Nicotinamide riboside supplementation in failing hearts reverses the failing heart phenotype by improving mitochondrial function, and improves mitochondrial function in peripheral blood mononuclear cells of patients with advanced HF. Furthermore, clinical studies have shown that NAD supplementation also has positive effects on cardiovascular patients by improving metabolism, reducing inflammation, and delaying aging, which have been comprehensively described in the literature.

Glutamine, a nonessential amino acid, can stabilize mitochondrial membrane potential and protect mitochondrial physiological function, as well as reduce ROS production and mitochondrial damage. Numerous clinical trials have shown the positive cardiovascular effects of glutamine supplementation. For example, glutamine supplementation after strenuous exercise significantly reduced the levels of oxidized low-density lipoprotein, cholesterol, and IL-6 to reduce the risk of atherosclerosis. Glutamine supplementation reduces myocardial injuries, maintains myocardial cell viability, and reduces MIRI in patients undergoing elective cardiac surgery requiring extracorporeal circulation. In addition, glutamine supplementation reduces the levels of heat shock protein-27 and heat shock protein-70 to maintain normal metabolism in patients with AF, reducing the progression of AF.
CoQ10, an electron transmitter in the mitochondrial ETC, is essential for the synthesis of mitochondrial ATP. It acts as an antioxidant to enhance endothelial function and also reduces lipid and lipoprotein levels to improve the prognosis of CVD.  

In a 2-year clinical trial, 420 patients with moderate to severe HF were randomized to the CoQ10 and placebo groups; the results showed that CoQ10 is not only safe for patients with HF but also improves the prognosis of patients with HF and reduces the incidence of HF hospitalization and cardiovascular mortality. Further studies found that the incidence of AF in patients with HF is also reduced by CoQ10. In addition, CoQ10 levels are lower in patients with hypertension, while supplementation with CoQ10 reduces diastolic and systolic blood pressure in patients with hypertension.

Stimulation of Mitochondrial Biogenesis

In addition to protecting the normal function of mitochondria, another important therapeutic strategy is to boost the number of intracellular mitochondria by enhancing mitochondrial biogenesis, which reduces the biologic damage caused by mitochondrial damage or deficiency. PGC-1α, the main regulator of mitochondrial biogenesis, is activated by AMPK phosphorylation and sirtuin 1 deacetylation, up-regulates the expression of nuclear respiratory factor 1 and 2 and estrogen-related receptors, and then up-regulates the expression of mitochondrial transcription factor A and oxidative phosphorylation genes, which increases the synthesis of mitochondrial proteins and promotes mitochondrial biogenesis. In addition, PGC-1α regulates oxidative stress, inflammation, and apoptosis, providing another new direction for the treatment of CVD. However, Karamanlidis et al found that overexpression of PGC-1α in HF mice increased acute mortality and produced no improvement in cardiac function. Therefore, more studies are needed before PGC-1α is used as the clinical target to treat CVD. The endothelial NOS/NO/cGMP system has also been found to regulate mitochondrial biogenesis; this action may be related to PGC-1α, but the exact mechanism is not yet clear.

Previous studies have shown that MIRI is related to mitochondrial biogenesis damage. Melatonin can restore mitochondrial biogenesis through the AMPK/PGC-1α pathway and reduce reperfusion injury, and this therapeutic effect is weakened when PGC-1α is silent. Similar results have been reported in diabetic mice. In addition, metformin regulates the AMPK signaling pathway and enhances PGC-1α expression, which promotes mitochondrial biogenesis, contributing to protecting cardiomyocytes from oxidative damage and delaying endothelial senescence. Pioglitazone has been shown to improve diabetes mitochondrial biogenesis through the peroxisome proliferator-activated receptor-γ/PGC-1α pathway, inhibiting the development of adverse atrial structures. Trevellin and Vettor et al found that expression of endothelial NOS and mitochondrial biogenesis are increased in swim-training wild-type mice, whereas increased mitochondrial biogenesis is not observed in swim-training eNOS−/− mice. These findings indicate that the endothelial NOS/NO system might play key roles in the regulation of mitochondrial biogenesis. Consistent with this hypothesis, inhalation of NO in persistent PAH of newborn lambs promoted mitochondrial biogenesis and improved mitochondrial dysfunction. Taken together, these data suggest that targeted stimulation of mitochondrial biogenesis might provide novel strategies for the prevention and treatment of CVD.

Mitochondrial Transplantation

In 2006, Spees et al reported the existence of mitochondrial transfer between cells and found that stem cell–derived mitochondria transfer into damaged cells, restoring aerobic respiration. Subsequently, an increasing number of studies have shown that mitochondrial transplantation exists between cells under normal physiological conditions (Figure 4). Scientists subsequently proposed that mitochondrial transplantation could be used to treat mitochondrial-related diseases. In 2017, Emani et al first reported the use of autologous mitochondrial transplantation for myocardial recovery at Boston Children’s Hospital for patients requiring extracorporeal membrane oxygenation support after cardiac surgery. In recent years, with the deepening of the study of mitochondrial transplantation, both in vitro (Figure 5) and in vivo mitochondrial transplantation have been on the rise.

Increasing evidence shows that mitochondrial transplantation plays an important role in the treatment of CVD. Mitochondrial transplantation has been found in animal and clinical trials to attenuate IRI through the following mechanisms: (1) mitochondrial transplantation reduces oxidative stress and apoptosis in posts ischemic cells of the heart and brain, increases complex IV expression and ATP production, causes metabolic reprogramming, and reduces the size of infarcts, (2) reducing the levels of tumor necrosis factor-α, IL-6, and high-sensitivity C-reactive protein, and improving inflammation in an MIRI animal model, and (3) increasing the expression of mitochondrial function–related proteins, cellular respiration, and energy production. In addition, mitochondrial transplantation inhibits cardiomyocyte apoptosis, a key event in RV hypertrophy and exhaustion, suggesting its clinical value for the treatment of failing hearts. In a rat PAH model, mitochondrial transplantation increases the ATP concentrations and improves RV function and pulmonary artery remodeling characterized by VSMCs exhibiting a nonproliferative contractile phenotype and the diminished pulmonary artery response to α-adrenergic stimulation.

Although mitochondrial transplantation has positive effects on CVD, there are still many challenges, including the storage of mitochondria. Even with mitochondria kept on ice, the activity of isolated mitochondria is significantly reduced after 1 hour. Currently, new storage agents have...
been invented, such as the Euro-Collins solution and the University of Wisconsin Solution. Immunologic response after transplantation is another challenge. Inflammatory cytokines and chemokines are up-regulated and allograft rejection is significantly increased during allogeneic mitochondrial transplantation. Autologous mitochondrial transplantation does not cause inflammatory and immune responses in the recipient. However, autologous mitochondrial transplantation is inappropriate in patients with pre-existing congenital or acquired mitochondrial diseases. Although the use of mitochondrial donation was approved by the United Kingdom as early as 2015, many challenges, including technology, policy, and clinic issues, still need to be explored.

Prospects

Mitochondria are currently considered important targets for the design and development of drugs for many diseases, including CVD. Although laboratory research and preclinical trials have shown positive results, clinical mitochondrial...
therapy for the treatment of patients with CVD has not been approved. Specific, sensitive, efficient, clinically meaningful methods to evaluate mitochondrial function need to be explored. The most direct and effective way to evaluate mitochondria is tissue biopsy. After isolating mitochondria, mitochondrial respiration, ATP synthesis, and membrane potential are measured in vitro to evaluate mitochondrial function. However, these measurements cannot be repeated because of tissue damage. Measuring the bioenergetics of blood and the markers of mitochondrial dysfunction in circulation avoids the tissue damage caused by direct isolation of mitochondria and the shortcomings of unrepeatable measurement.127 However, these methods are difficult to restrict to specific tissues or organs. In recent years, with the rapid development of imaging technology, it has become possible to measure the mitochondrial function of specific tissues in vivo. For example, magnetic resonance spectroscopy is flexible to assess mitochondrial metabolism, and near-infrared spectroscopy and multi-wavelength spectroscopy might be appropriate to assess mitochondrial function in the body.128,129 Hyperpolarized 13C magnetic resonance spectroscopy can be used as a direct, noninvasive assessment of myocardial mitochondrial Krebs cycle activity, tricarboxylic acid cycle flux or activity, and medium-chain FAO in vivo.130,131 Alternatively, the MMP in vivo can be evaluated by using [18F]-triphenylphosphonium under positron emission tomography scans.132 How to target drugs to mitochondria and restrict them to act within the mitochondria has been a long-standing problem. In recent years, MTAs based on the spontaneous accumulation of cations in MMP have been developed, which could address the bursting of non-mitochondria—derived ROS by nontargeted antioxidants. The main difficulty of this approach, however, is that dysfunctional mitochondria have decreased MMP, which prevents the accumulation of these cation-based targeted antioxidants. In addition, delivering the drug into specific cells or tissue still needs to be addressed. In the absence of tissue specificity, external drug delivery cannot be controlled to the damaged tissue, which decreases the bioavailability of the drug at the lesion site and leads to off-target effects. Nanoparticle-mediated drugs may avoid this obstacle; animal experiments showed that the use of nanoparticle-mediated drugs alleviated myocardial IR injury.133 In addition, elderly subjects are prone to CVD, and the multifactorial nature of CVD often requires the concurrent use of multiple drugs. Therefore, additional studies are needed to study drug-drug interactions.

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