Mitochondria in Endothelial Dysfunction and the Relation to Cardiovascular Disease

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Abstract

Atherosclerosis is spreading more and more every year. Accordingly, more and more people suffer from this disease, especially its negative consequences. The pathogenesis of atherosclerosis involves many different mechanisms and processes. One of the players in this field is endothelial dysfunction, which captures the cells of the endothelial layer of blood vessels. Vascular dysfunction is a well-known risk factor for cardiovascular disease. Abnormalities include increased arterial stiffness as well as endothelial dysfunction associated with an atherogenic decrease in the expression and bioavailability of nitric oxide (NO). This article focuses on the evidence supporting the role of mitochondria in the maintenance of vascular function and the pathophysiology of mitochondrial-related vascular disorders. In addition, to identify possible gaps in current knowledge and find potential promising interventions, we will discuss lifestyle and nutraceutical strategies, as well as pharmaceutical treatments that improve vascular function through effects on mitochondria.

Significance | This review discusses the mitochondria's role in endothelial function informs strategies for preventing cardiovascular diseases through targeted interventions on mitochondrial health

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Introduction

Cardiovascular diseases (CVD) pose a significant health burden globally, with a high prevalence among the aging population. Endothelial dysfunction is a well-established risk factor for CVD, characterized by impaired nitric oxide bioavailability and arterial stiffness. Mitochondria play a crucial role in maintaining vascular function and are implicated in the pathophysiology of mitochondrial-related vascular disorders. Understanding the interplay between mitochondria and endothelial cells is essential for developing targeted interventions to improve vascular health and prevent CVD (Sanchis-Gomar et al., 2016; Hall et al., 2019; Mensah et al., 2017).

The research hypothesis of this review paper is that dysregulation of mitochondria in endothelial cells contributes to vascular dysfunction, promoting the development of cardiovascular diseases. By exploring the role of mitochondria in maintaining vascular homeostasis and the pathophysiology of mitochondrial-related vascular impairments, this review aims to uncover potential therapeutic targets for mitigating endothelial dysfunction and reducing the risk of CVD (Gutiérrez et al., 2013; Schwalm et al., 2016).

The primary objective of this review paper is to provide a comprehensive overview of the evidence supporting the involvement of mitochondria in vascular function and dysfunction. Additionally, the review aims to identify gaps in current understanding and explore potential interventions, including

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lifestyle modifications, nutraceutical strategies, and pharmaceutical treatments targeting mitochondria, to improve vascular function and prevent cardiovascular diseases. Through a thorough analysis of mitochondrial dynamics, autophagy pathways, mitochondrial-derived reactive oxygen species, mitochondrial calcium homeostasis, and potential interventions, the review seeks to offer insights into novel therapeutic approaches for managing vascular health and reducing CVD risk.

By addressing these objectives, the review paper aims to contribute to the current knowledge of the intricate relationship between mitochondria and endothelial dysfunction, shedding light on promising avenues for future research and clinical interventions in the field of cardiovascular health.

This article is focusing on the evidence confirming the role of mitochondria in maintaining vascular function and the pathophysiology behind mitochondria-related vascular impairments. Also, in order to identify possible gaps in the current knowledge and find potential prospective interventions, we will discuss lifestyle and nutraceutical strategies and pharmaceutical treatment methods that improve vascular function by targeting mitochondria.

Mitochondria in vascular endothelial cells

Location and content

In comparison with other cell types that demand a lot of energy, endothelial cells contain relatively few mitochondria. For example, mitochondrial content in rats is 2-6% in endothelium while in cardiac myocytes it is 32%. Depending on the vascular bed and its function, the cytoplasmic volume occupied with mitochondria may vary (Kluge et al., 2013). Endothelial cells in the blood-brain barrier, for instance, are highly active and thus have an increased content of mitochondria (8-11%). Research showed that mitochondrial signaling in the endothelium depends on the way mitochondria are distributed in the cell. According to a recent study, clustering of mitochondria close to the cellular nucleus and diffusion of ROS derived by the mitochondria into the nucleus mediates hypoxia-sensitive gene regulation in lung endothelium in rats (Doll et al., 2015).

Several translational studies have been carried out to investigate the role of mitochondrial distribution within the cell in human subjects and how it affects mitochondrial signaling. For example, in arterioles outside the myocardium mitochondria are attached to the cytoskeleton and express ROS as a result of cell damage caused by shear stress. ROS expression in this setting sets off release of nitric oxide and triggers flow-mediated dilation (Cvetkovska et al., 2013). Mitochondrial dysfunction is intricately linked to specific cardiovascular disease (CVD) outcomes through a variety of mechanisms. Impaired mitochondrial function in endothelial cells contributes to endothelial dysfunction, characterized by reduced nitric oxide bioavailability and increased oxidative stress, leading to impaired vasodilation and increased vascular tone. This endothelial dysfunction is a key driver of atherosclerosis, a common precursor to CVD that manifests as the buildup of plaque in arteries. Mitochondrial-derived reactive oxygen species play a crucial role in the inflammatory processes that contribute to arterial stiffening and endothelial dysfunction, promoting the progression of atherosclerosis. Additionally, mitochondrial dysfunction can disrupt calcium homeostasis in vascular smooth muscle cells, leading to increased vascular tone and contributing to hypertension and vascular remodeling. Furthermore, mitochondrial dysfunction in cardiomyocytes can impact cardiac function, leading to conditions such as heart failure and cardiomyopathy. Understanding the explicit connections between mitochondrial dysfunction and specific CVD outcomes is essential for developing targeted interventions to improve mitochondrial health and mitigate the risk of cardiovascular diseases.

Mitochondrial fusion and fission

Traditionally mitochondria are considered discrete organelles, however, it has been acknowledged that in many cell types, such as cardiac myocytes and endothelial cells, they gather into networks and have fusion and division cycles. A lot of research has been done on these dynamics. The transmembrane GTPases mitofusin-1 and mitofusin-2 (MFN1, MFN2) control the outer mitochondrial membrane fusion, while optic atrophy protein 1 (OPA1) mediates inner membrane fusion (Boengler et al., 2017). Dynamin-related protein-1 (DRP1) and Fission-1 (FIS1) are in charge of the division process. FIS1 interacts with DRP1 and attracts it to mitochondria in order to promote fission. Phosphatase calcineurin triggers translocation of DRP1 while phosphorylation at serine-656 by AMPK inhibits it (Jin et al., 2021).

Fusion and fission of mitochondria normally counterbalance each other under physiological conditions and there can be observed mitochondrial networks. Mitochondrial fusion contributes to biochemical and electrical connectivity and helps distribute proteins, mtDNA and metabolites. Mitochondrial division is also critical for normal functioning of the cells (Youle and van der Bliek, 2012) . Firstly, it allows mitochondrial segregation during cell division and rearrangement of mitochondria within a cell. Secondly, mitochondrial fission is also promoted as a result of cellular stress (Zorov et al., 2019). This adaptive response helps to mitigate stress by mitophagy of partially damaged mitochondria. Thirdly, mitochondrial division is also associated with apoptosis-related permeabilization of outer membrane and cytochrome c release. However, it is still not clear whether mitochondria-regulated apoptosis necessarily involves mitochondrial fission (Baechler et al., 2019).

According to newest studies, fusion and fission of mitochondria affect the endothelial cell function. Inhibited fusion as a result of silenced MFN1, for instance, results in weaker angiogenic responses to Akt-dependent endothelial nitric oxide synthase activation (eNOS) and vascular endothelial growth factor (VEGF). Under physiological conditions mitochondrial networks can be observed in endothelial cells (Kluge et al., 2013). However, they decompose if the cells are exposed to ischemia-reperfusion conditions, increased glucose levels or hydrogen peroxide. In experimental diabetes there was observed an increase in endothelial DRP1 and FIS1 expression, while OPA1 expression decreased. FIS1 or DRP1 silencing in the setting of increased glucose is presumed to maintain eNOS activity by means of reducing mitochondrial ROS (mtROS) (Kim et al., 2020).

Changes in mitochondrial fusion and fission have been observed in endothelial cells of individuals with cardiovascular risk factors. A recent study, for instance, revealed higher FIS1 levels and fragmentation of mitochondria in freshly isolated endothelial cells from subjects with diabetes. Additionally, there was observed a positive correlation between hypertension and polymorphisms in the OPA1 and MFN2 genes (Shenouda et al., 2011). These data suggest that there is an association between mitochondrial dynamics and CVD. This means that restoring normal mitochondrial dynamics may be a potential therapeutic target in vascular disease treatment.

Autophagy and mitophagy

Autophagy is a cell survival mechanism. It controls degradation and recycling of damaged organelles, proteins and macromolecules in order to provide energy when there is lack of nutrients under cellular stress (Das et al., 2012). During this process a double-membrane autophagosome is formed in the cell that absorbs intracellular components that are to be degraded. The autophagosome fuses with a lysosome where the inner membrane and the nutrients are recycled through acid hydrolases (Yim and Mizushima, 2020).

Selective degradation of mitochondria is called mitophagy. As a result of damage accumulation in mitochondrial networks, they undergo fission and rearrangement in order to separate populations of daughter mitochondria. Dysfunctional elements are targeted for degradation while healthy mitochondria with normal membrane potential are reincorporated into networks. This mechanism helps keep the cell clean of impaired mitochondria that otherwise could cause outer membrane permeabilization and result in apoptosis (Kim et al., 2007). Interestingly, mitophagy pathways also trigger PGC-1α and biogenesis in order to generate fresh replacements for the damaged organelles. So, mitochondrial dynamics, mitophagy and biogenesis are all parts of a holistic mechanism that controls the quality of mitochondria (Liu et al., 2021).

Mitophagy is primarily activated by membrane depolarization. Phosphatase and tensin homolog-induced putative kinase protein-1 (PINK1) are recruited into the mitochondria and degraded under physiological conditions. This process is set off by changes in mitochondrial membrane potential (Rakovic et al., 2013). When the membrane is depolarized, PINK is accumulated at the surface of mitochondria, while E3 ubiquitin ligase Parkin is imported into the organelle. This may lead to Beclin-1 de-repression and trigger mitophagy. As a result of protein ubiquitination at the mitochondria surface, p62 is bound and degraded. Then the mitochondria are engulfed in an autophagosome and recycled (Pickrell and Youle, 2015). A number of proteins regulate the formation and development of an autophagosome, such as microtubule-associated protein 1 light chain 3 (LC3)-I. This is a ubiquitin-resembling protein that ligates to phosphatidylethanolamine and forms LC3-II. NIX can also set off autophagy of mitochondria by attaching to the mitochondrial membrane and interacting with LC3 (Hanna et al., 2012).

There is a lot of evidence that dysregulations in autophagy and mitophagy aggravate the course of diseases. Studies show that impairments in PINK and Parkin-mediated autophagy in dopaminergic neurons, for instance, play an important role in the development of hereditary Parkinson's disease forms. Dysfunctional autophagy has also been associated with atherosclerosis, diabetes and shorter life expectancy (Doblado et al., 2021). When the autophagy in cardiac myocytes is impaired, it may accelerate the development of hypertensive heart disease. Depending on the stage of the process, abnormalities in autophagy may also contribute to the pathophysiology of atherosclerotic CVD. These data suggest that autophagy is an adaptive response mechanism in the endothelium. Thus, interventions aimed at promoting autophagy can enhance vascular function (Gatica et al., 2015).

Recently, there have been a number of studies aimed at describing the autophagy mechanisms in endothelium in the setting of energy crisis or oxidative stress. Following exposure to hydrogen peroxide or the glycolysis blocker 2-deoxyglucose, AMPK was activated in cultured endothelial cells, leading to formation of autophagosomes as evidenced by conversion of LC3-I to LC3-II (Carresi et al., 2021). Mitochondria-targeted irradiation of endothelial cells leads to oxidative stress which induces translocation of Parkin to depolarized mitochondria and promotes formation of LC3-II. When endothelial cells are exposed to hemin, it induces peroxidation of lipids, promotes depolarization of mitochondria and triggers mitophagy. It is worth mentioning that Beclin-1/LC3-II-induced autophagy also helps remove ox-LDL from the endothelial cells (Zuo et al., 2020). Consequently, autophagy and

mitophagy can be considered a crucial mechanism in the endothelial cells responding to oxidative stress.

In aging, disordered quality control in mitochondria has been associated with impairments of endothelium. Cell culture aging models demonstrated alterations in mitochondrial dynamics, and depletion of membrane potential. The lifespan of cultured endothelial cells can be extended by overexpression of proteins involved in autophagy, such as LC3B, ATG5, and ATG12, as they enhance mitochondrial fitness resulting in lowered damage to mtDNA, and increased ATP production and membrane potential (Kumar and Jurkunas, 2021). In a recent study, aged mice exhibited altered endothelium-dependent dilation in aortic tissue as well as overexpression of superoxide, and dysfunctional autophagic flux. There was also observed a reduction in Beclin-1 expression and increase in p62 which indicates that p62 degradation was blunted (normally this happens when an autophagosome is formed). Treatment with trehalose — a dietary supplement that promotes autophagy — reversed these processes in mice (Fernández et al., 2018).

There is still a lack of research around the relation between CVD and autophagy in humans. Patients with dilated cardiomyopathy demonstrated an alteration in autophagy marker expression in myocardial tissue. Changes were observed in the release of Beclin-1, ATG5, and LC3-II. Mechanic cardiac unloading restores these markers. During a recent study, autophagy in skeletal muscle biopsied from elderly women with excessive weight was examined (Gatica et al., 2022). The study confirmed earlier findings in mice, showing that exercise promotes autophagy. It is known for a fact that exercise helps to maintain endothelial function. However, there are still some doubts on whether improved autophagy adds to this effect. A recent translational study revealed lower Beclin-1 and higher p62 protein expression in endothelial cells obtained by biopsy from old healthy patients, consistent with dysfunctional autophagy (Okutsu et al., 2021). In addition, there was observed an association between decreased Beclin-1 level and dysfunctional endothelium-dependent dilation in the forearm. All in all, the existing data indicates that improving endothelial autophagy can be an effective method of preventing and treating vascular disease (Donato et al., 2018).

Role of mitochondrial-derived reactive oxygen species (ROS) in vascular dysfunction

Mitochondria have a significant impact on oxidative stress level. Several studies confirmed the critical influence of ROS on vascular dysfunction. Human and preclinical trials demonstrated that antioxidants that target mitochondria enhanced vascular function. Other studies also demonstrated that alterations in mitochondrial function affect cardiac function and autonomic regulation, and thus can promote the development of CVDs (Peoples et al., 2019).

One of the main cells signaling roles mitochondria play in the endothelium is the expression of ROS. A number of different ROS is produced in the endothelium, for example, hydroxyl radicals (OH•), peroxynitrite (ONOO−), superoxide (O−2O2−), hydrogen peroxide (H2O2) and other nitrosative and oxidative radicals (Nita and Grzybowski, 2016). Excess production of oxidants and/or lack of antioxidant capacity leads to oxidative stress, which in its turn reduces nitric oxide (NO) bioavailability and results in endothelial dysfunction. NADPH oxidase (NOX) and xanthine oxidase also contribute to ROS production and promote oxidative stress. Additionally, a cross-talk between mtROS and NOX-produced ROS may take place (Münzel et al., 2017). Although MtROS are essential for cellular homeostasis, their overexpression affects bioavailability of NO in different ways, including NO scavenging that takes place during a reaction between O−2O2− and NO to form ONOO−. This process uncouples nitric oxide synthase (eNOS) in the endothelium and promotes nitrosative and oxidative stress in the cell. Increased ratio eNOS monomer-to-eNOS dimers was previously associated with vascular aging. Furthermore, the essential eNOS cofactor BH4 can be oxidized by O−2O2− to BH2, which results in uncoupling of eNOS and leads to eNOS generating even more O−2O2− and less NO (Herb and Schramm, 2021).

MtROS also contribute to inflammatory processes, leading to arterial stiffening and endothelial dysfunction. Lack of NO results in increased vascular smooth muscle cell (VSMC) tone. Together with collagen deposition and degradation of elastin these factors lead to stiffening of the arterial wall and result in higher arterial stiffness. These factors contribute to the pathogenesis of CVD. There is not much evidence proving the association between arterial stiffness and VSMC dysfunction (Kirkman et al., 2021). However, some preclinical trials show that intrinsic increase in VSMC stiffness can aggravate arterial stiffening in aging. There is not enough research around the possible mediating role of mtROS in this relationship. Arterial stiffening is supported by inflammation and oxidative stress through various mechanisms, such as structural transformation, alterations in gene production and release of inflammatory mediators into the arterial wall (Ungvari et al., 2018). MtROS also promote the generation of mitochondrial DNAs (mtDNAs) that are more susceptible to the development of resistant and age-induced hypertension. MtDNA abnormalities lead to reduction of mtDNA copy number and suppress the release of respiratory subunits, subsequently damaging vascular compliance and resulting in the development of hypertension. Mitochondrial expression of important peptides that can protect endothelial function (such as humanin) may also be affected by changes in mtDNA. Below we will look through sources of mtROS, their cross-talk with other ROS sources and discuss the main cellular antioxidant mechanisms that can prevent overexpression of ROS (Yu and Bennett, 2016).

Mitochondria and Ca2+ Homeostasis in the Endothelium

Different aspects of endothelial function depend on calcium levels in cytosol. For example, receptor agonists like serotonin and acetylcholine increase cytosolic calcium, promote the binding of calcium/calmodulin and thus activate eNOS (Filippini et al., 2019). Ca2+/calmodulin-dependent protein kinase II (CamKII) is also activated by calcium and regulates phosphorylation state, expression of eNOS genes and actin cytoskeletal elements that have an impact on cell barrier function, motility and shape. Furthermore, calcium has an impact on angiogenic functions of the endothelium and VEGF signaling (Yaniv et al., 2013).

As evidenced by several previous studies, mitochondria and the endoplasmic reticulum (ER) are both involved in Ca2+ traffic regulation and thus have an impact on the main aspects of endothelial function. Mitochondria act as buffers of intracellular Ca2+ levels and control uptake and release of calcium by ER (Rizzuto et al., 2009). Despite the fact that most of the calcium is stored in the ER, ca. 0,25 of cellular Ca2+ is localized to mitochondria. When the cell is stimulated, constant calcium flux through mitochondria is required for store-operated entry and refilling of ER calcium store. Calcium microdomains produced as a result of localized calcium absorption and formed depending on the location of mitochondria in relation to the plasma membrane, play an important role in these processes (Pinton et al., 2008).

Mitochondria-related Ca2+ dynamics is highly controlled. Overall, the presence of multiple Ca2+ uptake and release channels allow to regulate calcium levels in mitochondria within the physiological range of calcium in cytosol. When calcium level exceeds the buffering threshold, Ca+ in mitochondria quickly increases and may set off mitochondria-induced apoptosis (Boyman et al., 2020). Suppression of the sodium/calcium exchanger in endothelial cells may be one of the factors affecting calcium extrusion and leading to hydrogen peroxide-related increases in Ca2+ levels in mitochondria. Since mitochondrial calcium flux are important for maintaining ER calcium stores, decreased Ca2+ extrusion may lead to ER calcium store depletion, activate the unfolded protein response and result in apoptosis (Alevriadou et al., 2017).

The mitochondria-associated membrane (MAM) is a specific region in the ER that mediates the interaction with mitochondria. According to recent studies, the MAM attaches the ER to mitochondria and contains proteins involved in metabolism of lipids and calcium. The membranes regulate Ca2+ traffic that plays a crucial role in mitochondrial energy metabolism and apoptosis (Morciano et al., 2018).

A recent study demonstrated that MAMs may affect pulmonary hypertension. If the mitochondria-ER dynamics in the VSMC of the pulmonary artery are impaired as a result of reticulon protein Nogo-B, it affects the mechanism of hypoxia-induced apoptosis, leading to formation of proliferative lesions, commonly observed in pulmonary hypertension. It is worth mentioning that under this condition there was also observed an increase in Nogo-B expression in the endothelium (Zhuan et al., 2020). It leads to an assumption that pulmonary hypertension can induce a proliferative phenotype in endothelial cells, making them resistant to apoptosis. However, the pool of data around the role of Nogo-B and other aspects of MAM functioning is still insufficient (Boyman et al., 2020).

Physiological oscillations in cytosolic and mitochondrial Ca2+ levels play an important role in the regulation of various mitochondrial functions, such as biogenesis, motility, dynamics, energy metabolism and ROS expression. Calcium triggers oxidative phosphorylation and Krebs cycle enzymes, subsequently increasing ATP release (Ray et al., 2020). Mitochondrial motility is controlled by cytosolic calcium through the Miro-Milton protein complex which connects mitochondria to microtubules by interacting with the outer mitochondrial membrane. Being a Ca2+ sensor, Miro controls mitochondrial retention in regions with higher Ca2+ concentrations. Calcium influences mitochondrial dynamics through calcineurin which triggers DRP1 and promotes fission of mitochondria. Finally, Ca2+ in mitochondria increases PGC-1α expression and thus plays an important role in mitochondrial biogenesis (Tilokani et al., 2018).

It has been observed that in pathological states changes in mitochondrial Ca2+ levels add to endothelial responses. For example, when exposed to hyperglycemic conditions, cultured human endothelial cells show alterations in histamine signaling as a result of increased cytosolic and mitochondrial calcium (Negri et al., 2021). Calcium uptake and expression of ROS in mitochondria are involved in shedding of cell membrane receptors for tumor necrosis factor-alpha TNF-α as a result of TNF-α stimulation. This mechanism moderates the degree of endothelial activation in pro-inflammatory states (Dada and Sznajder, 2011).

A recent study evaluated the transient receptor potential vanilloid type 4 (TRPV4) channel, a mechanosensitive calcium ion channel in cell membrane, in human myocardial arterioles. The samples were isolated from subjects with coronary artery disease. The study demonstrated that TRPV4 channel activation promoted expression of mtROS, Ca2+ entry, and endothelium-induced vasodilation (Peng et al., 2020). The study suggests that TRPV4 channels are localized closely to mitochondria that are associated with cytoskeletal components and illustrate the impact of calcium microdomains on ROS signaling mechanisms in human mitochondria. Still, there is a need for more research on mitochondrial Ca2+ flux in endothelial cells (Germande et al., 2022).

Imbalance of Mitochondrial Calcium and Vascular Damage

Intracellular calcium balance plays an important role in supporting many cell functions in the endothelium, as well as cell and VSMC integrity. When Ca2+ levels are increased because of agonists, this leads to activation of eNOS and thus to release of NO. Ca2+-mediated signaling is essential for modulating vasomotive activity of VSMCs (Dalal et al., 2020). The interaction between mitochondria and cellular ER is a central mechanism regulating Ca2+ levels in the cell. Calcium levels in mitochondria are important not only for intracellular Ca2+ homeostasis, but also for mitochondrial metabolism, cell biogenesis, morphology and signaling. All these factors can influence vascular functioning (Tanwar et al., 2021).

A number of preclinical studies have associated Ca2+ balance in mitochondria with viability and function of vascular cells. Exposure to high glucose increases mitochondrial calcium content in cultured HUVECs significantly after histamine stimulation. Simulated ischaemia-reperfusion injury triggers changes in mitochondrial calcium levels in aortic endothelial cells in humans (Chen et al., 2017). The same process has been observed in cells following an exposure to high ROS concentrations. Mitochondrial Ca2+ levels have also increased as a result of inhibited Ca2+ release following exposure to abnormally high H2O2 concentrations which suppress the mitochondrial Na+/Ca2+ exchanger. All these states induce Ca2+ overload which affects mitochondrial morphology and Ca2+ signaling, resulting in replicative senescence of the endothelial cells and eventually leading to apoptosis (Beckhauser et al., 2016). High calcium levels can also induce apoptosis as a result of cytochrome C release and stimulate ROS production by activating mPTP and respiratory complexes. It has also been observed that hexokinase is involved in mitochondrial calcium homeostasis in endothelial cells. Hexokinase is an enzyme that phosphorylates glucose in glycolysis. In the endothelium the enzyme inhibits the voltage-dependent anion channel Ca2+ transporter (Delierneux et al., 2020). According to several preclinical studies, higher hexokinase release reduces mitochondrial calcium and thus decreases ROS in coronary endothelial cells under hyperglycemia. Thus, hexokinases may be a potential target for therapy. There is a need for more studies examining the effect of mitochondrial calcium regulation on vascular endothelial function (Pan et al., 2018).

Potential interventions

Mitochondria-directed antioxidants

Mitochondria-directed antioxidants hold significant potential as a therapeutic target. MtROS play an important role in signaling, thus, it is important to develop a strategy for ROS regulation within physiological levels rather than eliminate them completely. One of the strategies targeting mtROS is linking antioxidant compounds to lipophilic cations like triphenylphosphonium (TPP) (Vaka et al., 2022). The mitochondria-targeted antioxidant MitoQ (the TPP-modified ubiquinone) reduces to ubiquinol in the matrix of the mitochondria. The SOD mimetic tempol or alpha tocopherol have been directed to mitochondria through a similar approach (Jiang et al., 2020).

MitoQ has demonstrated efficacy in pre-clinical trials of Parkinson's disease, adriamycin-induced toxicity, sepsis and ischemia-reperfusion conditions. In cultured endothelial cells, MitoQ has alleviated oxidative damage as well as apoptosis. The antioxidant has also inhibited amyloid light chain-related damage in isolated arterioles, enhanced endothelial function and decreased hypertension in rats (Zhou et al., 2018). MitoTempol was shown to lower blood pressure and support improvement of endothelium-dependent vasodilation in hypertensive mice. Clinical studies assessing MitoQs efficacy for the treatment of chronic hepatitis C and Parkinson's disease have demonstrated mixed results. It is still unclear if MitoQ or other TPP-linked compounds will help to maintain endothelial function or alleviate CVD in human subjects (McLachlan et al., 2014).

Lipoic Acid and Acetyl-L-Carnitine

Lipoic acid is a natural antioxidant and a coenzyme that plays an important role in the normal functioning of Krebs cycle. L-carnitine is involved in energy metabolism. It transports fatty acids into mitochondria. In humans, carnitine supplementation is usually dietary in the form of acetyl-L-carnitine. It is delivered into mitochondria and enhances its function to a greater degree compared with L-carnitine (Pagano et al., 2020). The combination of these drugs was shown to reverse impairments in ATP production, mitochondrial membrane potential and ROS expression in various tissues according to a study that involved animal models of hypertension, diabetes, and aging. Additionally, lipoic acid mitigates inflammatory activation, contributes to NO production and increases the anti-apoptotic protein BCL-2 levels. Oral supplementation with acetyl-L-carnitine or lipoic acid increases bioavailability of NO and decreases formation of atherosclerotic lesions in animal models (Mongirdienė et al., 2022). One can find lipoic acid, acetyl-L-carnitine, and L-carnitine as food supplements. Their efficacy in improving vascular function has been confirmed by various studies. In subjects with diabetes or metabolic syndrome, the endothelial function can be improved by lipoic acid, while L-carnitine reduces endothelial damage caused by free fatty acids. Still, it remains unknown whether the endothelial function improvements caused by these compounds reflect their beneficial effect on mitochondrial function (McMackin et al., 2007).

Interventions involving AMPK and PGC-1α activation

Interventions aimed at increasing AMPK and PGC-1α activity have constantly shown beneficial effects on endothelial function in animal models. Short-term calorie limitation in old mice, for

instance, reversed oxidative stress and endothelial dysfunction. Furthermore, calorie limitation contributes to biogenesis in mitochondria and promotes MnSOD production (Rius-Pérez et al., 2020). 2-deoxyglucose is a glycolysis inhibitor that activates AMPK, promotes autophagy and has a protective effect against cell death in cultured endothelial cells. Thiazolidinediones, such as pioglitazone, also activate PGC-1α and promote mitochondrial biogenesis. Metformin has also been reported to activate AMPK, reduce MPT pore opening, prevent apoptosis in endothelial cells and inhibit endothelial dysfunction development (Timm and Tyler, 2020).

It is well known that physical activity and weight loss have a favourable effect on endothelial vasodilation in subjects with such risk factors as obesity or diabetes. A study among elderly women showed that weight loss and exercise program promoted PGC-1α expression in skeletal muscle. It is still unknown if metformin really contributes to AMPK activation in physiological conditions. However, it has been observed to reverse impairments of endothelial function in diabetic patients. Although all of these interventions improve metabolism and reduce risk factors, it is unclear whether improvements in the endothelial function are due to enhanced mitochondrial function (Byun and Lee, 2020).

Sirtuins

Recent studies show that sirtuins are important regulators of mitochondrial activity, metabolism, and lifespan, thus they may be targets for treatment. Being NAD+-dependent deacetylases, sirtuins are responsible for the regulation of gene expression and enzyme functions. Function of Sirtuin 1 (SIRT1) is to deacetylate and activate PGC-1α, which is beneficial for lipid and glucose metabolism as well as mitochondrial gene expression (Kupis et al., 2016). Sirtuin 3, localizing specifically to mitochondria, deacetylates mitochondrial enzymes and regulates energetics of mitochondria. Besides, SIRT3 activates and deacetylates MnSOD, which may lead to the reduction of superoxide levels in mitochondria (Zhang et al., 2020).

In respect of the cardiovascular system, numerous studies indicate that SIRT1 protects against ischemic damage and heart hypertrophy besides deacetylating and activating endothelial eNOS as well as regulating angiogenesis (Md Fakruddin et al. 2020, Chowdhury et al. 2018). Clinical research demonstrated reduction of SIRT1 expression in endothelium isolated from the brachial artery and its correlation with endothelium-dependent vasodilation in elderly humans (Man et al., 2019).

There is information concerning possible beneficial effects of mitochondrial SIRT3 in endothelium, but it remains unknown if they are due to changes in mitochondria function. For instance, endothelial-specific NF-κB inhibition in a mice model enhances mitochondrial biogenesis, reduces inflammation, improves blood flow and insulin sensitivity, and increases longevity. Vascular tissue from these mice demonstrates high levels of SIRT3 (Martino et al., 2022). Aging in humans is characterized by reduction of SIRT3 expression in skeletal muscles, but it is associated only with sedentary lifestyle and not observed in active individuals. As of today, it is unknown how specific activation of SIRT3 may impact endothelial function since research is lacking both in animal models or humans. However, it is possible that SIRT1 and SIRT3 activators may prove beneficial in combating cardiovascular and metabolic diseases (He et al., 2019).

Resveratrol

Resveratrol or 3,4',5-trihydroxystilbene can be found in various foods, like grapes or red wine. According to experimental research, this polyphenolic compound can be an activator of SIRT1. Further research suggested that it also activates AMPK, which explains its favorable effect. Animal studies showed that the positive effects of resveratrol are similar to those of low-calorie diet and target mitochondrial biogenesis, insulin sensitivity, and in the long run longevity (Perrone et al., 2017).

Mitochondrial biogenesis in the endothelium is induced by resveratrol that increases NRF-1 and PGC-1α in a process depending on the activation of eNOS and SIRT1; the same effects have been observed in the aorta in diabetes (db/db) mouse model. Resveratrol acts as antioxidant in the endothelium and, according to a study, it increases MnSOD expression and reduces levels of hydrogen peroxide in the cell (Csiszar et al., 2009). Moreover, resveratrol has demonstrated cytoprotective features and can prevent apoptosis during oxidative stress as it upregulates BCL-2. Thanks to these multiple atheroprotective properties, resveratrol therapy has become widely applicable. In obesity, oral therapy with resveratrol proved to significantly improve endothelium-dependent vasodilation. Clinical trials are in process now that examine resveratrol effects on metabolism and vascular therapy; however, long-term results of resveratrol therapy on cardiovascular condition, mitochondrial or vascular function are still unknown (Gal et al., 2021).

Inhibition of Mitochondrial Fission

Clinical research suggests that increased levels of mitochondrial fission and autophagy impairment in the endothelium are linked with pathological conditions like diabetes mellitus. It has been demonstrated that mitochondrial division inhibitor-1 (Mdivi-1), which inhibits DRP-1 fission protein, also decreases permeabilization of the mitochondrial outer membrane, release of cytochrome c, and apoptosis. It has also been shown that Mdivi-1 limits ischemia reperfusion damage in isolated myocytes and reduces a myocardial infarction size in a mice model. However, it remains unknown how it may influence endothelial function (Hu et al., 2022).

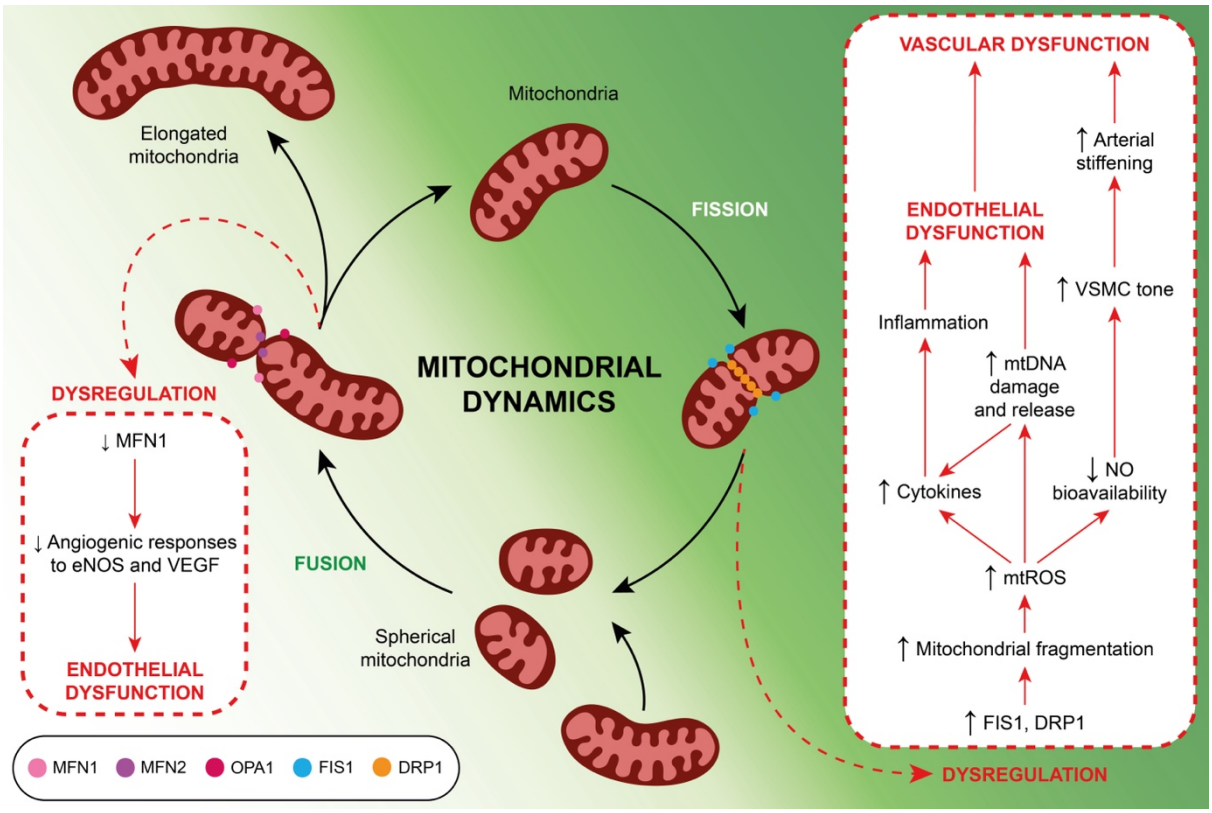

Figure 1. The scheme of the causative relationships between the dysregulation of mitochondrial dynamics and endothelial dysfunction.

Mitochondrial Membrane Depolarizing Agents

Uncoupling proteins, UCP2 among them, cause mild cell membrane depolarization and lower production of mitochondrial reactive oxygen species. In a rat model, UCP2 overexpression enhanced endothelial function, which suggests that uncoupling agents may be used therapeutically (Hass and Barnstable, 2021). Research showed that another uncoupling agent, carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP), lowered membrane potential and enhanced endothelial function in arterioles in diabetes mellitus patients. The uncoupling agent 2,4-dinitrophenol had been used in antiobesity therapy in humans. It proved highly toxic, which put an end to its clinical trials; however, there remains a possibility that less active, self-limiting agents may be used for mitochondrial therapy (Tanpradit et al., 2016).

Micro RNAs

Micro-RNAs have recently been proved to play a significant role in coordinating quite a few biological processes. Micro-RNA-210 has been shown to repress iron-sulfur cluster assembly proteins (ISCU1/2) that are important for expressing components in Krebs cycle and the electron transport chain in pulmonary vascular endothelial cells. Increased levels of miR-210 during hypoxia and HIF-1α activation led to repression of iron-sulfur enzymes in mitochondria and endothelial mitochondria respiration. Such response might be of importance for adaptation of mammals to hypoxia and the Pasteur effect (Arora et al., 2015). Considering the fact that micro-RNAs have recently become therapeutic targets, the research results show that focusing on miR-210 may lead to a broader regulation of endothelial function and mitochondrial respiration (Chan and Loscalzo, 2010).

Discussion

Mitochondrial dysfunction plays a critical role in the pathogenesis of vascular diseases, contributing to endothelial dysfunction, arterial stiffness, and the development of cardiovascular diseases. The intricate interplay between mitochondria and endothelial cells underscores the importance of targeting mitochondrial health as a potential therapeutic strategy for improving vascular function and reducing CVD risk. Understanding the clinical implications of mitochondrial dysfunction provides valuable insights into novel treatment avenues for managing vascular health.

Mitochondrial dysfunction in endothelial cells leads to impaired nitric oxide bioavailability, increased oxidative stress, and altered calcium homeostasis, all of which are key factors in endothelial dysfunction and vascular damage. Targeting mitochondrial pathways involved in oxidative stress, autophagy, and mitochondrial dynamics holds promise for restoring endothelial function and mitigating the progression of vascular diseases. Therapeutic interventions aimed at improving mitochondrial health, such as mitochondria-targeted antioxidants, AMPK activation, sirtuin modulation, and inhibition of mitochondrial fission, have shown potential benefits in preclinical studies.

Translating these findings into clinical practice poses challenges but offers exciting opportunities for developing personalized and targeted treatments for patients at risk of cardiovascular diseases. Customizing interventions to address specific mitochondrial deficits in individual patients may lead to more effective and precise therapeutic outcomes. Moreover, monitoring mitochondrial function through biomarkers and advanced imaging techniques could aid in assessing treatment responses and optimizing patient care. By harnessing the therapeutic potential of mitochondrial-targeted interventions, clinicians can pave the way for a new era of precision medicine in the management of vascular diseases, ultimately improving patient outcomes and quality of life.

In Figure 1, we provided the summary of the concept of mitochondria dynamics and processes of endothelial dysfunctions.

Conclusions and Challenges

The role of mitochondria in the pathogenesis of cardiovascular diseases does not raise questions. As one of the most important organelles, mitochondria are involved in several key processes of cell life. Endothelial cells are no exception. Knowing how various disorders in the work and functioning of mitochondria affect the development of various pathologies, it was logical to assume that they do not stand aside in the case of cardiovascular diseases. According to the newest studies, fusion and fission of mitochondria affect endothelial cell function. Also received a lot of data indicating the positive effects of the use of drugs aimed at reducing mitochondrial oxidative stress.

While the studies discussed in this review provide valuable insights into the role of mitochondria in endothelial dysfunction and cardiovascular diseases, there are several limitations that need to be acknowledged. One significant challenge lies in translating promising preclinical findings into effective clinical interventions. Preclinical studies often use animal models or cell cultures that may not fully represent human physiology, leading to potential discrepancies in outcomes when applied to human populations. Additionally, the complexity of mitochondrial dynamics and their interactions with other cellular processes make it challenging to identify specific targets for therapeutic interventions that can be reliably translated to clinical settings. Furthermore, the variability in individual responses to treatments, differences in disease progression, and comorbidities among patients pose challenges in achieving consistent and reproducible outcomes in clinical trials. Therefore, while preclinical studies offer valuable insights, further research and carefully designed clinical trials are necessary to validate the efficacy and safety of mitochondrial-targeted interventions in improving vascular health and reducing

cardiovascular disease risk in human populations. Adequately addressing these limitations will be critical in bridging the gap between preclinical research and clinical practice to develop effective treatments for cardiovascular diseases.

Therapeutic interventions targeting mitochondrial dysfunction hold great promise in managing vascular health and reducing cardiovascular disease risk. However, several limitations and challenges need to be considered when developing and implementing such interventions. One significant limitation is the potential off-target effects of mitochondrial-targeted therapies, which may disrupt essential cellular processes and lead to unforeseen adverse effects. Mitochondrial dynamics are highly complex, involving a delicate balance between fusion, fission, autophagy, and mitophagy, and perturbing this balance could have unintended consequences on cellular function.

Another challenge is the variability in individual responses to mitochondrial-targeted therapies, as genetic factors, lifestyle habits, and underlying health conditions can influence treatment outcomes. Additionally, the lack of standardized biomarkers to assess mitochondrial function and dysfunction poses a challenge in monitoring the efficacy of interventions and evaluating their long-term effects on vascular health.

Moreover, the bioavailability and pharmacokinetics of mitochondrial-targeted compounds may impact their effectiveness in reaching and exerting therapeutic effects within the mitochondria of endothelial cells. Ensuring adequate delivery and retention of therapeutic agents specifically to the mitochondria while minimizing systemic side effects presents a technical challenge in drug development.

Furthermore, the complexity of mitochondrial biology and the interconnected nature of mitochondrial pathways with various cellular processes require a comprehensive understanding of the mechanisms involved to develop targeted and effective interventions. Limited knowledge about specific molecular targets and signaling pathways within mitochondria may hinder the development of precise and efficient therapeutic strategies.

Addressing these limitations will be crucial in the successful translation of mitochondrial-targeted interventions from preclinical studies to clinical practice. Collaborative efforts between researchers, clinicians, and pharmaceutical developers will be essential in overcoming these challenges and advancing towards personalized and effective treatments for mitigating mitochondrial dysfunction and improving vascular health in individuals at risk for cardiovascular diseases.

Author contributions

A.V.P. prepared the original draft; V.N.S., M.A.P., I.A.S., A.Y.P., and A.N.O. reviewed and edited; V.A.K. illustrated.

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Competing financial interests

The authors have no conflict of interest.

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