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Mitochondria and aging in the vascular system

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Abstract

This review focuses on mitochondrial abnormalities that occur in the vasculature during aging and explores the link between mitochondrial oxidative stress, chronic low-grade vascular inflammation, increased rate of endothelial apoptosis, and development of vascular diseases in the elderly. Therapeutic strategies targeting the mitochondria for prevention of age-associated vascular dysfunction and disease in old age are considered here based on emerging knowledge of the vasoprotective effects of caloric restriction, caloric restriction mimetics, the GH/IGF-1 axis, and mitochondria-targeted antioxidants.

Keywords

Aging; Mitochondria; Endothelium

Introduction

Mitochondria play important roles in biosynthetic pathways, cellular energetics, cellular redox homeostasis, signaling, calcium buffering, and regulation of programmed cell death. Thus, age-related changes of mitochondria are likely to affect a host of cellular physiological functions simultaneously and contribute to the development of a wide range of age-related diseases. Earlier studies focused on the role of mitochondria in pathological alterations of the brain, heart, and skeletal muscle. However, there is increasing evidence that mitochondria play an important role in vascular pathophysiology as well. Age-specific mortality rates from heart disease and stroke increase exponentially with age throughout the later years of life, accounting for more than 40% of all deaths among people aged over 65. Understanding the role of mitochondria (including alterations in mitochondrial ATP production, regulation of calcium signaling and apoptosis, modulating the activity of sirtuins) in age-related vascular pathophysiological alterations holds promise for developing novel therapeutic approaches to reduce cardiovascular mortality in an aging population. In this review, the effects of aging on mitochondrial ROS production and mitochondrial biogenesis in the vasculature and the signaling role of mitochondria in the aged endothelial and smooth muscle cells are considered in terms of potential mechanisms involved in vascular pathological alterations in aging. The possible benefits of emerging therapeutic strategies that have the potential to promote mitochondrial health in the aged vasculature are also discussed (Fig. 1).

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Mitochondria as sources of reactive oxygen species in aging

Harman first proposed the free radical theory of aging in the 1950s and, in the 1970s, extended the theory to implicate mitochondrial production of reactive oxygen species (ROS, such as O_2^- and H_2O_2) as the main cause for age-related macromolecular damage [1]. Since then, a large body of evidence has been published both in support of and against the free radical theory of aging. Although antioxidant supplementation has not been conclusively shown to produce an extension of lifespan in mammals and genetic knockout mice for major cellular antioxidant enzymes show a relatively mild phenotype despite the significant increases in ROS levels, a key role of increased mitochondrial production of ROS in the development of various age-related diseases is supported by the available data. Considerable evidence has been published that with advanced age production of ROS significantly increases in the vasculature, both in endothelial and smooth muscle cells (recently reviewed elsewhere [2]). Recent studies suggest that mitochondrial oxidative stress has an important role in aging-induced vascular dysfunction, which is further exacerbated by an increased activity of cytoplasmic and cell membrane-associated oxidases (including NAD(P)H oxidases) [3,4]. Our recent understanding of the pathological mechanisms of atherosclerosis suggests that age-related oxidative stress leads to chronic low-grade vascular inflammation [4], which promotes the development of coronary artery disease and stroke in the elderly (see below). It is important to point out that age-related mitochondrial oxidative stress and inflammatory phenotypic changes are present both in endothelial and smooth muscle cells [4,5]. The role of mitochondrial oxidative stress in cardiac aging leading to mitochondrial protein oxidation and increased mitochondrial DNA mutations has been well documented (for a recent overview, see reference [6]). Further studies are evidently needed to gain more insight in the role of similar aging-induced mitochondrial DNA and protein damage present in vascular endothelial and smooth muscle cells. Another potentially important link between mitochondrial oxidative stress and cardiovascular aging is the induction of programmed cell death [7]. The available evidence suggests that age-related increases in oxidative stress are associated with an increased rate of endothelial apoptosis [17,21] and leads to microvascular rarefaction [8]. Mitochondrial-derived ROS may also impact vascular relaxation [9]. In that regard, it will be interesting to determine whether pharmacological agents that attenuate mitochondrial oxidative stress are able to prevent apoptotic cell death and/or improve vasomotor function in the aged vasculature. An interesting question that also should be addressed is the possible differences in the responses of endothelial and smooth muscle cells to increased age-related oxidative stress: age-related oxidative stress in the large vessels tends to promote vascular smooth muscle cell proliferation and invasion [10] rather than inducing apoptosis (see below).

The molecular mechanisms responsible for age-related mitochondrial oxidative stress in the vasculature are multifaceted and likely involve cell-autonomous effects, including dysregulation of antioxidant defenses such as peroxynitrite-mediated nitration and inhibition of MnSOD [11], decline in GSH content [12], Nrf2/ARE dysfunction [13], and a dysfunctional electron transport chain [3]. Recent studies showed that the mitochondrial enzyme p66^{Shc} is involved in the regulation of mitochondrial redox status, linking vascular oxidative stress to apoptosis [14]. It is significant that genetic depletion of p66^{Shc} in mice significantly reduces mitochondrial ROS production, which is associated with a 30% increase in lifespan and improved endothelial function [15–17]. In addition to cell-autonomous effects, age-related changes in endocrine regulation are likely to contribute to vascular mitochondrial oxidative stress in aging. Previous studies suggest the existence of a relationship between declining levels of growth hormone (GH) and insulin-like growth factor-1 (IGF-1, the synthesis of which is regulated by GH) and age-related cardiovascular impairment. Importantly, epidemiological studies provide strong evidence that GH and IGF-I deficiency in humans is associated with premature atherosclerosis and elevated

cardiovascular disease mortality [18]. There is increasing evidence that IGF-1 confers mitochondrial protection, which likely contributes to its vasoprotective effects in aging. This view is supported by the findings in mice with hypopituitary dwarfism (Ames dwarf), where low plasma IGF-1 levels are associated with increased endothelial ROS generation, mitochondrial oxidative stress, and down-regulation of major anti-oxidant enzymes [19], mimicking the vascular aging phenotype. The available data support the conclusion that supplementation of IGF-1 may exert vasculoprotective effects in aging [20,21], improving cardiac diastolic function [22], and preventing hippocampal microvascular rarefaction [8,23,24]. Recent reports demonstrate that treatment of aged rats with IGF-1 confers mitochondrial protection, including an attenuation of mitochondrial oxidative stress in parenchymal tissues [25]. We hypothesize that GH replacement and/or IGF-1 treatment in aging may also exert mitochondrial protective effects in the aged cardiovascular system as well. The findings that treatment of cultured endothelial cells with IGF-1 in vitro attenuates mitochondrial ROS production strongly support this hypothesis. Further studies are needed to determine the effects of IGF-1 on autophagy of dysfunctional mitochondria and apoptosis in the vasculature. Several traditional cardiovascular risk factors, including dietary factors, can also increase mitochondrial ROS production leading to mitochondrial damage. There is evidence that oxidized low-density lipoprotein, smoking [26], high methionine diet and hyperhomocysteinemia, angiotensin II [27,28], and hyperglycemia [29] may increase ROS production in mitochondria of endothelial cells. Thus, mitochondrial ROS production likely represents a common pathway through which environmental factors can influence the rate of vascular aging. In that regard, it is significant that, in blood vessels of successfully aging species, metabolic stressors elicit a lower level of mitochondrial oxidative stress than in arteries of shorter-living ones [29], suggesting a possible link between mechanisms regulating increased metabolic stress resistance and slower rate of aging in longer-living species.

Dysregulation of mitochondrial biogenesis in aging

Mitochondria are highly dynamic organelles, and their biogenesis is likely involved in the regulation of cell metabolism and signal transduction. Because mitochondria are particularly susceptible to damage over time, effective control of mitochondrial biogenesis and turnover is critical for the maintenance of energy production, the prevention of accumulation of oxidatively damaged macromolecules, and the promotion of healthy aging [30]. Dysfunction of mitochondrial biogenesis affects the whole organism during aging [30]. Recent advances show that, with age, mitochondrial biogenesis is also impaired in endothelial cells both in conduit arteries [3] and capillaries [31,32]. The available evidence suggests that mitochondrial biogenesis is also impaired in aged smooth muscle cells [3]. We posit that a decrease in mitochondrial biogenesis can reduce turnover of specific mitochondrial components resulting in the accumulation of oxidized lipids, proteins, and DNA in this cellular compartment, which would lead to a gradual deterioration of various mitochondrial functions, affecting biosynthetic pathways, cellular energetics, cellular redox homeostasis, signaling, calcium buffering, and regulation of apoptosis. It is likely that maintaining a normal turnover of mitochondria during aging would be critical to preventing the deleterious side effects of mitochondrial oxidative stress. Accordingly, maintenance of mitochondrial activity and biogenesis capacity during aging appears to be a key factor in caloric restriction-mediated prevention of age-related diseases in many organs [30,33]. There is good reason to believe that this is the case in the cardiovascular system as well.

The mechanisms regulating mitochondrial biogenesis and their impairment in the aged vasculature are only partially understood. Mitochondrial biogenesis is governed by a complex interaction of cellular pathways involved in sensing availability of nutrients, stress response, and redox state with endocrine regulatory mechanisms, and it requires the

transcriptional regulation of 20% of cellular proteins, coordinated by the interaction of the mitochondrial and nuclear genomes. The view that has emerged during the past decade is that many of the aforementioned endogenous and exogenous factors act through common effector pathways, which involve the peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1 α) and activation of PGC-1 α by nitric oxide, CREB, and AMPK. The available evidence suggests that, in the aged vasculature, due to an increased oxidative stress and down-regulation and uncoupling of eNOS [2], bioavailability of nitric oxide is significantly decreased. Lack of NO results in a down-regulation of PGC-1 α and other mitochondrial biogenesis factors and consequential dysregulation of constituents of the electron transport chain and other mitochondrial proteins in aged endothelial and smooth muscle cells [3]. Studies on animal models with genetic depletion of eNOS [34] and treated with pharmacological inhibitors of NO synthesis [35] support this model. An aging-associated reduction in AMPK activity may also be an important contributing factor in dysregulation of PGC-1 α transcription and impaired mitochondrial biogenesis associated with aging [36]. In addition to the aforementioned intra-cellular regulatory factors, age-related changes in endocrine regulation of cellular energetics (e.g., hypothyroidism, low IGF-1 levels [37]) may also be implicated in impaired mitochondrial biogenesis.

In addition to PGC-1 α -regulated mitochondrial biogenesis, another mechanism involved in regulating mitochondrial function during aging is mitochondrial fission/fusion, which permit a constant remodeling of mitochondrial architecture. Recent data suggest that disruption of mitofusin pathways in cells results in complete loss of mitochondrial fusion and severe defects of cellular functions, including poor cellular growth, widespread heterogeneity of mitochondrial membrane potentials, and decreased cellular respiration [38]. Although the role of mitochondrial fission and fusion in vascular aging is poorly understood [39], the available evidence suggest that mitochondrial oxidative stress induced in vitro in endothelial cells results in dysregulation of these processes, whereas lowering the mitochondrial ROS levels can restore the morphological changes in mitochondria and improve mitochondrial function [40].

Mitochondria as signaling organelles in aging

In the past decade, a new era in aging research has emerged that concerns the role of mitochondria in intracellular signaling. Retrograde regulation is the general term for the crosstalk between mitochondria and the nucleus, which encompasses cellular responses to alterations in mitochondrial function. This pathway of communication from mitochondria to the nucleus influences many cellular functions and is thought to play an important role in the aging process. The signaling cascades involved include altered ROS production, Ca²⁺ dynamics, which activate downstream effectors pathways and transcription factors, the energy-sensing TOR pathway, stress-induced protein kinases, and a mitochondrial stress response pathway similar to the unfolded protein response in the endoplasmic reticulum. The cross-talk between the aforementioned pathways and cellular redox signaling mechanisms in aging is an important area of ongoing research. Mitochondria-initiated apoptosis can be considered as an extreme example of mitochondrial signaling, when signals emanating from the mitochondria drive the programmed breakdown of the entire cell. Although there is increasing evidence suggesting an important role for the aforementioned mechanisms in aging (e.g., the link between mTOR signaling, cardioprotection by caloric restriction [41], and lifespan regulation [42]), their physiological/pathophysiological roles in the cardiovascular system are poorly understood.

The mitochondrial theory of aging [1] postulates that mitochondria-derived ROS contribute to a variety of macromolecular oxidative modifications. Accumulation of such oxidative damage has been proposed to be a primary causal factor in the aging process [1], as well as

in development of age-related diseases. However, there is an emerging view that mitochondria-derived ROS, in addition to causing oxidative damage, play important signaling roles as well. Numerous transcription factors, including NF- κ B, AP-1, Nrf-2, and P53, are known to be redox-sensitive. Here, we present recent advances on the role of mitochondria-derived ROS in activating NF- κ B signaling and inducing a pro-inflammatory vascular phenotype in aging. Our current understanding is that O₂⁻, overproduced in the aged mitochondria in vascular endothelial and smooth muscle cells, is dismutated to H₂O₂ by Mn-SOD and it is the increased release of H₂O₂, which is responsible for activating NF- κ B in the cytoplasm of aged cells [4]. This concept is supported by the fact that O₂⁻ is membrane-impermeable, whereas H₂O₂ easily penetrates the mitochondrial membranes. Indeed, scavenging of mitochondria-derived H₂O₂ attenuates NF- κ B activation in aged vessels [4], suggesting that the age-related decline in mitochondrial function is, at least in part, responsible for chronic low-grade vascular inflammation in aging [4]. Previous studies have shown that endothelial activation and pro-inflammatory gene expression in aging (e.g., up-regulation of adhesion molecules and iNOS) is promoted, at least in part, by an increased NF- κ B activation [4,43–45]. Chronic activation of NF- κ B and endothelial activation are known to predispose arteries to atherosclerosis [46]. Similar to the endothelial cells, age-related oxidative stress in vascular smooth muscle cells also lead to the development of a secretory phenotype promoting proliferation, invasion, matrix fragmentation, and collagenization (recently reviewed elsewhere [10]). Thus, we posit that attenuation of mitochondrial H₂O₂ release by pharmacological or molecular interventions in vivo should exert vasoprotective effects in aging. Recent studies have shown that, in aging mice, overexpression of human catalase in the mitochondria delays cardiac pathology and attenuates age-related oxidative stress and systemic inflammation [6]. Subsequent studies should determine whether attenuation of mitochondrial ROS production in the endothelial and smooth muscle also prevents age-related low-grade vascular inflammation.

Vascular mitochondria as therapeutic targets in aging

Pathways that attenuate mitochondrial oxidative stress, regulate mitochondrial biogenesis, and/or improve mitochondrial function have recently emerged as potential therapeutic targets for the amelioration of vascular dysfunction and prevention of the development of age-related vascular diseases. Caloric restriction is a dietary regimen, which improves health and slows the aging process in various organisms. There is increasing evidence that caloric restriction confers vasoprotection in aging and in pathological conditions associated with accelerated vascular aging (recently reviewed elsewhere [13]). Although the mechanisms underlying the beneficial vascular effects of caloric restriction are undoubtedly multifaceted, they likely include mitochondrial biogenesis [33] likely due to an increased NO bioavailability [47], an attenuation of mitochondrial ROS production [13,34], and consequential inhibition of NF- κ B activity [48]. The molecular mechanisms responsible for the beneficial cardiovascular effects of caloric restriction are not completely understood and may involve effects mediated by increased levels of adiponectin [49].

Recent studies demonstrated that the polyphenol resveratrol mimics vasoprotective effects of caloric restriction [30,50,51], including induction of mitochondrial biogenesis [52] and attenuation of mitochondrial oxidative stress [53,54] in endothelial cells. The effects of resveratrol are, in part, explained by its ability to activate and/or up-regulate SIRT-1 in these cells, which subsequently deacetylates and activates PGC-1 α . In fact, resveratrol is unable to modulate PGC1- α function in SIRT1^{-/-} cells [55]. Resveratrol can also activate the NF-E2-related factor 2 (Nrf2), a transcription factor that regulates expression of numerous ROS detoxifying and antioxidant genes involved in regulation of mitochondrial redox homeostasis [54]. It is significant that resveratrol treatment in aged animals exerts vasoprotective effects and substantially decreases oxidative stress and endothelial apoptosis

[50], an effect that likely involves both SIRT1 and Nrf2 mediated pathways. There is increasing evidence suggesting that AMPK regulates both mitochondrial content and mitochondrial antioxidant defenses in endothelial cells [56]. Thus, pharmacological activation of AMPK may also confer vasoprotective effects in aging.

Many of the adverse consequences of mitochondrial oxidative stress are mediated via production of the highly reactive oxidant peroxynitrite, the reaction product of NO and superoxide. Previous studies demonstrated that ONOO⁻ formation is substantially increased in the vasculature of aged animals [11,57]. The downstream targets of peroxynitrite-induced cytotoxicity are likely multiple (for a comprehensive review see reference [58]) and involve alterations in mitochondrial function. Because there are studies extant suggesting that pharmacological decomposition of peroxynitrite improves vascular dysfunction in aged rodents [59], further studies should determine the effects of peroxynitrite decomposition catalysts on vascular mitochondria in aging.

Several specific mitochondria-targeted antioxidants (e.g., antioxidants conjugated with triphenylphosphonium cation such as mitoquinone, mitovitamin E and mitophenyltertbutyl) are under development and experimental testing; however, their efficacy in attenuating mitochondrial oxidative stress in aging and inhibiting vascular inflammation have not been evaluated yet. These mitochondria-targeted antioxidants achieve concentrations in the mitochondrial matrix several-fold higher than those achieved in the cytosol because of the high negative membrane potential of the inner mitochondrial membrane. Although ongoing human clinical studies with mitochondria-targeted antioxidants involve only non-cardiovascular applications, preclinical studies show promise for prevention of cardiovascular diseases. Recent studies in animal models of hypertension [28] provide proof of concept that mitochondria-targeted antioxidants may confer vasoprotection in aging as well. A recent study reported that oral supplementation with the mitochondrial antioxidants alpha-lipoic acid and coenzyme Q10 reduces apoptosis in the cochlea-aged mice [60], and a similar treatment paradigm was shown to improve endothelial function in the aged aorta [61]. Future studies will undoubtedly exploit the unique biophysical and biochemical properties of mitochondria for the targeted delivery of drugs that affect mitochondrial function and reduce mitochondrial oxidative stress in the aged vasculature.

Perspectives

Although significant progress has been achieved in describing age-related alterations in mitochondrial function and phenotype in the cardiovascular system, there are several questions that have yet to be answered. Importantly, the specific roles for cell-autonomous and non-cell-autonomous mechanisms (e.g., hormonal effects) in age-related mitochondrial alterations need to be elucidated further. A consensus should be reached whether mitochondrial contributions to vascular aging occur primarily via increased mitochondrial ROS or other mechanisms by which mitochondria affect the aging process play an equally important role. For example, future studies should elucidate age-related alterations in mitochondrial autophagy and their contributions to vascular aging. New data on mitochondrial fusion/fission and cellular energetics in vascular aging are also needed. It seems to be likely that age-related changes in nuclear gene expression are different in the major vascular cell types (endothelial and smooth muscle cells, cells of the adventitia). Thus, future studies should consider cell-type specific differences in mitochondrial retrograde signaling in aging as well. There is a reasonable consensus that mitochondrial oxidative stress plays a central role in both induction of apoptotic death and activation of redox-sensitive molecular pathways (e.g., NF- κ B-mediated inflammatory processes). These pathways are under intense investigation as therapeutic targets for the prevention of

cardiovascular diseases. The concept that evolutionarily conserved pathways (such as SIRT, Nrf2/ARE) control the aging process in mammals, regulating cellular bioenergetics, mitochondrial function, and mitochondrial redox homeostasis lead to the proposal that pharmacological or nutritional modulation of these pathways may be effective in delaying the onset of age-dependent vascular disease.

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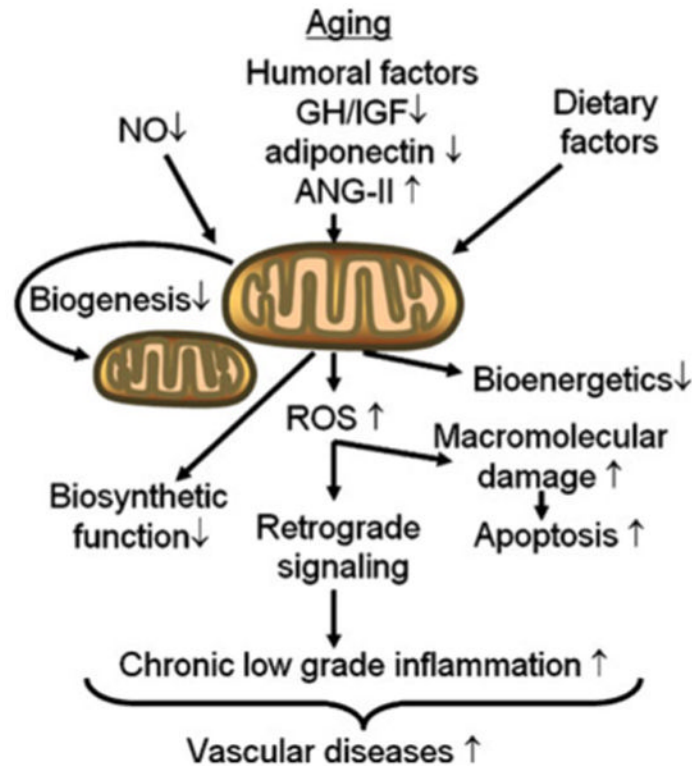


Fig. 1.

Age-related alterations in mitochondrial function affect endothelial function and phenotype. Aging-induced cell-autonomous mechanisms (e.g., an age-related decline in endothelial NO bioavailability) and age-related changes in the endocrine milieu (e.g., decreases in serum IGF-1 levels) disrupt pathways regulating biogenesis and self-renewal of mitochondria and impair mitochondrial functions. The central importance of aging-induced increases in mitochondrial release of ROS (H_2O_2 , peroxynitrite) in this flowchart is evident, which leads to macromolecular damage in the cells promoting endothelial apoptosis. ROS can react with various proteins and lipids such as LDL, forming highly damaging oxLDL. ROS-mediated activation of retrograde signaling pathways, including NF- κ B, lead to chronic low-grade vascular inflammation promoting the development of vascular diseases in the elderly