A large body of work has shown that endothelial dysfunction contributes to the pathogenesis of cardiovascular disease, and considerable effort has been made to define operative mechanisms. Recent work has emphasized the importance of mitochondria for endothelial function. Although they have long been recognized for their role in bioenergetics, mitochondria participate in a host of other cellular processes. In contrast to cardiac myocytes and other cell types, energy requirements in the endothelium are relatively low, and glycolysis is the major source of ATP production. It is now recognized that endothelial mitochondria play a prominent role in signaling cellular responses to environmental cues. An important mode of mitochondrial signaling is the regulated production of reactive oxygen species (ROS). Cardiovascular risk factors are associated with excess mitochondrial ROS production that promotes inflammation and decreases nitric oxide (NO) bioavailability.

This article reviews the key aspects of mitochondrial biology in endothelial cells, including subcellular location, biogenesis, dynamics, autophagy, reactive oxygen species production and signaling, calcium homeostasis, regulated cell death, and heme biosynthesis. In each section, we introduce key concepts and then review studies showing the importance of that mechanism to endothelial control of vasomotor tone, angiogenesis, and/or inflammatory activation. We particularly highlight the small number of clinical and translational studies that have investigated each mechanism in human subjects. Finally, we review interventions that target different aspects of mitochondrial function and their effects on endothelial function. The ultimate goal of such research is the identification of new approaches for therapy. The reviewed studies make it clear that mitochondria are important in endothelial physiology and pathophysiology. A great deal of work will be needed, however, before mitochondria-directed therapies are available for the prevention and treatment of cardiovascular disease. (Circ Res. 2013;112:1171-1188.)

Key Words: apoptosis ● biogenesis ● endothelium ● mitochondria ● mitophagy
this *Circulation Research* Review Series, we do not provide a detailed discussion of each area or extensively review findings in cardiac myocytes or other cell types. Instead, we provide an introduction to the key concepts and describe experimental work in endothelial cells. Each section concludes with a review of the available human studies relating mitochondrial and endothelial dysfunction in cardiovascular vascular disease. Our final section describes experimental and clinical studies of mitochondria-directed interventions and their effects on endothelial function.

**Mitochondrial Content and Subcellular Localization in Endothelial Cells**

Mitochondrial content in endothelial cells is modest compared with other cell types with higher energy requirements. In the rat, for example, mitochondria occupy 2% to 6% of cytoplasmic volume in endothelial cells compared with 32% in cardiac myocytes. Mitochondrial content varies by vascular bed and with other cell types with higher energy requirements. In the rat, for example, mitochondria occupy 2% to 6% of cytoplasmic volume in endothelial cells compared with 32% in cardiac myocytes. Mitochondrial content in endothelial cells is modest compared with cardiac myocytes or other cell types. Instead, we provide an introduction to the key concepts and describe experimental work in endothelial cells. Each section concludes with a review of the available human studies relating mitochondrial and endothelial dysfunction in cardiovascular vascular disease. Our final section describes experimental and clinical studies of mitochondria-directed interventions and their effects on endothelial function.

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**Mitochondrial Biogenesis and PGC-1α in the Endothelium**

Mitochondrial content depends on the balance between mitochondrial biogenesis and mitophagy (Figure 1). The formation of new mitochondria is a complex and incompletely understood process involving replication of mitochondrial DNA (mtDNA) and expression of nuclear and mitochondrial genes. The peroxisome proliferator–activated receptor-γ coactivator-1α (PGC-1α) plays a primary role in this process. PGC-1α activates nuclear respiratory factor-1 and nuclear respiratory factor-2 to coordinate expression of nuclear genes required for biogenesis. PGC-1α also regulates mitochondrial energy metabolism, and it is known that genetic deletion causes heart failure. Furthermore, cardiac PGC-1α expression is altered in diabetes and pressure overload and contributes to the pathogenesis of heart failure in these settings.

In addition to its role in mitochondrial biogenesis, PGC-1α also regulates expression of numerous genes related to glucose and lipid metabolism in cardiac myocytes and other cell types. In general, it is activated in states of increased energy demand and serves to augment cellular capacity for ATP production. PGC-1α also regulates expression of vascular endothelial growth factor-1 and stimulates angiogenesis. Given the energy demands in cardiac myocytes, it is perhaps not surprising that PGC-1α plays an important role in regulating metabolism, and it is known that genetic deletion causes heart failure. Furthermore, cardiac PGC-1α expression is altered in diabetes mellitus and pressure overload and contributes to the pathogenesis of heart failure in these settings.

Because energy demands are lower, it is interesting to consider that PGC-1α might play a different role in endothelial cells. It is known that PGC-1α is expressed in endothelial cells and regulates mitochondrial biogenesis. Additionally, PGC-1α orchestrates cellular defenses against oxidative stress that include many mitochondrial proteins. In this regard, overexpression of PGC-1α by adenoviral transfection increases expression of uncoupling protein (UCP)-2 and mitochondrial antioxidant enzymes, including manganese superoxide dismutase (MnSOD), catalase, and thioredoxin 2, whereas silencing PGC-1α has the opposite effect. PGC-1α overexpression protects against apoptosis, limits inflammatory activation, prevents activation of c-Jun N-terminal kinase, and improves NO release in response to cell deformation by shear stress. In this setting, ROS release signals NO production and flow-mediated dilation.
bioavailability.\textsuperscript{16,17} It further has been argued that PGC-1α−
induced mitochondrial biogenesis protects against oxidative
stress by supplying undamaged mitochondria that produce
less ROS.\textsuperscript{18}

Despite the favorable effects on endothelial phenotype, it
is uncertain whether augmenting PGC-1α will prove useful
as an antiatherosclerosis strategy. Unexpectedly, PGC-1α\textsuperscript{−/−}
mice bred onto an apolipoprotein E\textsuperscript{−/−} background did not have
increased lesion formation.\textsuperscript{19} Interpretation of this finding is
complicated, however, because PGC-1α\textsuperscript{−/−} mice are lean and
hyperactive, which would tend to limit atherogenesis. In
addition, increased expression of PGC-1β may compensate
for the loss of PGC-1α.\textsuperscript{20} Studies with endothelium-specific
or double knockout animals may be required to sort out
these possibilities.\textsuperscript{11} A very recent pilot study showed that
endothelial-specific overexpression of PGC-1α protects
against angiotensin II−induced hypertension.\textsuperscript{21}

With regard to clinical studies, it is well-established that
PGC-1α gene expression and mitochondrial mass are lower in
skeletal muscle of patients with diabetes mellitus and are asso-
ciated with insulin resistance and impaired bioenergetics.\textsuperscript{22–24}
With regard to the endothelial cells, mitochondrial content is
lower in tissue collected from patients with pulmonary hyper-
tension.\textsuperscript{25} A recent study showed lower mitochondrial mass
in arterioles isolated from patients with diabetes mellitus by
biopsy of subcutaneous fat.\textsuperscript{26} Epidemiological studies have
shown that PGC-1α polymorphisms are associated with hy-
pertension,\textsuperscript{27} carotid atherosclerosis,\textsuperscript{28} and coronary artery
disease,\textsuperscript{29} suggesting links with vascular disease. However,
such observational studies do not prove decreased mitochon-
drial mass or function in the endothelium is important, given
that PGC-1α regulates so many other aspects of metabolism.
Overall, however, the available studies suggest that interven-
tions that activate PGC-1α and stimulate mitochondrial bio-
genesis protect against cardiovascular disease, and efforts are
underway to identify small molecules that may be developed
as drugs.\textsuperscript{30}

**Mitochondrial Dynamics in Endothelial Cells**

Although traditionally viewed as discrete organelles, it is now
recognized that mitochondria form networks and undergo cy-
cycles of fusion and fission in many cell types, including cardiac
myocytes\textsuperscript{31} and endothelial cells (Figure 1).\textsuperscript{18} This process of
mitochondrial dynamics has been extensively reviewed.\textsuperscript{18,32}
Briefly, fusion of the outer mitochondrial membrane is medi-
ated by the transmembrane GTPases mitofusin-1 (MFN1) and
mitofusin-2 (MFN2), whereas fusion of the inner mem-
brane is controlled by optic atrophy protein 1 (OPA1). Fission is
mediated by dynamin-related protein-1 (DRP1) and fis-
sion-1 (FIS1). FIS1 recruits DRP1 to mitochondria to initiate
fission.\textsuperscript{33} DRP1 translocation is inhibited by phosphoryla-
tion at serine-656 by AMPK and is activated by the phosphatase
calcineurin.\textsuperscript{34}

Under physiological conditions, fusion and fission are
balanced and mitochondrial networks are present. Fusion
facilitates distribution of metabolites, proteins, and mtDNA
and helps maintain electrical and biochemical connectivity. Likewise, fission is important for normal cell function. For example, fission is required for cell division and movement of mitochondria within the cell, and is involved in the elimination of senescent mitochondria. Fission is also an adaptive response to cellular stress that facilitates the isolation and removal of damaged mitochondrial components by mitophagy. Finally, mitochondrial fission occurs concomitantly with outer membrane permeabilization and release of cytochrome c during apoptosis, although it remains controversial whether fission is a necessary step in mitochondria-dependent apoptosis.

Recent studies suggest that mitochondrial dynamics are relevant to endothelial cell function. For example, silencing mitofusin-1 and inhibiting fusion reduce angiogenic responses to vascular endothelial growth factor and Akt-dependent action of endothelial NO synthase (eNOS). Networks are present in endothelial cells under physiological conditions, but fragmentation is observed after exposure to hydrogen peroxide, high glucose, and ischemia-reperfusion injury. Endothelial expression of DRP1 and FIS1 is increased and expression of optic atrophy protein 1 is decreased in experimental diabetes mellitus. Silencing FIS1 or DRP1 under conditions of high glucose maintains eNOS activity and NO bioavailability, likely by decreasing mitochondrial ROS.

There is emerging evidence that mitochondrial dynamics are altered in the endothelium of patients with cardiovascular risk factors. For example, a recent study showed mitochondrial fragmentation and increased FIS1 in freshly isolated endothelial cells from patients with diabetes mellitus. Polymorphisms in the optic atrophy protein 1 and mitofusin-2 genes are associated with hypertension. These studies suggest links between mitochondrial dynamics and vascular disease, and raise the possibility that interventions directed toward restoring normal dynamics might have therapeutic benefit.

**Autophagy and Mitophagy in Endothelial Cells**

Autophagy is a cellular mechanism for controlled degradation of proteins, macromolecules, and organelles. Autophagy provides energy when the cell is deprived of nutrients and eliminates damaged organelles under conditions of cellular stress. This process involves formation of the double-membrane autophagosome that engulfs and isolates cellular components targeted for degradation. The autophagosome subsequently merges with a lysosome, where the contents and inner membrane are degraded by acid hydrolases and recycled for use by the cell. Mechanisms of autophagy have been extensively reviewed. In addition, a review by Kubli and Gustafsson appears in this Series on Mitochondria in Health and Disease.

Mitophagy refers to the selective autophagy of mitochondria (Figure 1). As mitochondrial damage accumulates, networks undergo rearrangement and fission to yield different populations of daughter mitochondria. Those with normal membrane potential and preserved components can be reinserted into networks, whereas dysfunctional progeny are targeted for elimination. It has been suggested that this process purges the cell of vulnerable mitochondria that otherwise would undergo outer membrane permeabilization and induce apoptosis. It is interesting that stimuli for mitophagy also activate PGC-1α and biogenesis, thus providing fresh replacements for eliminated mitochondria. Thus, dynamics, mitophagy, and biogenesis provide a unified mechanism for mitochondrial quality control.

Membrane depolarization is an important trigger for mitophagy. Under physiological conditions, phosphatase and tensin homolog–induced putative kinase protein-1 (PINK1) is imported into mitochondria and degraded via a process that depends on mitochondrial membrane potential. Membrane depolarization leads to accumulation of PINK1 at the mitochondrial surface and recruitment of the E3 ubiquitin ligase Parkin, and may derepress beclin-1 to activate mitophagy. Ubiquitylation of mitochondrial surface proteins leads to binding and degradation of p62 and targets the mitochondria for enclosure in an autophagosome and eventual degradation. Autophagosome formation and maturation involves a number of proteins, including microtubule-associated protein 1 light chain 3 (LC3)-I, which is a ubiquitin-like protein that conjugates phosphatidylethanolamine to form LC3-II. Mitochondria also can be targeted for autophagy through the action of NIX, which associates with the mitochondrial membrane and interacts with LC3.

A growing body of work suggests that impaired autophagy and mitophagy contribute to the pathogenesis of disease. PINK1 and Parkin-mediated mitophagy have received considerable attention because impairment of this mechanism in dopaminergic neurons plays a role in hereditary forms of Parkinson’s disease. Dysregulation of autophagy in a variety of tissues also has been linked to diabetes mellitus, atherosclerosis, and reduced lifespan. Impaired autophagy in cardiac myocytes contributes to the pathogenesis of hypertensive heart disease. Depending on the specific setting and degree of impairment, loss of autophagy may be protective or have pathological effects with regard to atherosclerotic cardiovascular disease. Overall, however, autophagy seems to be an adaptive response in the endothelium, and interventions that promote autophagy tend to improve vascular function.

Several recent studies have specifically examined mitophagy in endothelial cells under conditions of oxidative stress and energy deprivation. Exposure of cultured endothelial cells to hydrogen peroxide or the glycolysis blocker 2-deoxyglucose activates AMPK and stimulates autophagosome formation as reflected by conversion of LC3-I to LC3-II. Oxidative damage induced by mitochondria-targeted irradiation of endothelial cells promotes Parkin translocation to depolarized mitochondria and increases LC3-II formation. Exposure of endothelial cells to hemin promotes lipid peroxidation, leads to mitochondrial depolarization, and stimulates mitophagy. Interestingly, beclin-1/LC3-II–mediated autophagy is also a mechanism for clearance of oxidized low-density lipoprotein in endothelial cells. Thus, autophagy and mitophagy seem to be important responses to oxidative stress in the endothelium.

Altered mitochondrial quality control has been related to endothelial dysfunction in aging. For example, disordered mitochondrial dynamics and loss of membrane potential are observed in cell culture models of senescence. Overexpression of the autophagy proteins autophagy-related
Supporting previous work in mice, a recent study showed that exercise activates autophagy in skeletal muscle obtained by biopsy from older overweight women. Although it is uncertain whether exercise improves autophagy in skeletal muscle obtained by biopsy from older overweight women. Although it is well-known that exercise improves endothelial function, it remains uncertain whether enhanced autophagy contributes to this effect. A recent translational study showed that p62 protein expression was higher and beclin-1 expression was lower in endothelial cells taken by biopsy from older healthy human subjects, consistent with impaired autophagy. Furthermore, lower beclin-1 expression correlated with impaired endothelium-dependent dilation in the forearm. Overall, the available experimental and human data suggest that enhancing autophagy in the endothelium would be protective against vascular disease.

Mitochondrial ROS in Endothelial Cells

**Sources of Mitochondrial ROS**

Given their importance for cell signaling and vascular disease, there is considerable interest in the sources and regulation of mitochondrial ROS in endothelial cells (Figure 2). Although the primary site of oxygen consumption in mitochondria is cytochrome c oxidase (complex IV), it has been estimated that superoxide anion formation at complexes I and III accounts for 0.1% to 2% of the total. These estimates are based on studies of isolated mitochondria, however, and the percentage attributable to ROS production in endothelial cells in vivo remains uncertain.

In addition to complexes I and III, several other sources of mitochondrial ROS have been identified. For example, nicotinamide adenine dinucleotide phosphate oxidase (NOX) 4 is highly expressed in endothelial cells and has been localized to mitochondria in other tissues, although mitochondrial localization in endothelial cells remains uncertain. As has been recently reviewed for ROS signaling and contributes to endothelial cell senescence, migration, angiogenesis, and adaptive responses to hypoxia, inflammation, and oxidative stress.

The monoamine oxidase (MAO) family of enzymes is localized to the outer mitochondrial membrane and generates ROS during catabolism of catecholamines. ROS produced by MAO-A has been implicated in maladaptive hypertrophy and apoptosis of cardiac myocytes exposed to norepinephrine. MAO-derived ROS are also associated with adverse cardiac remodeling and heart failure in response to pressure overload in mice. Hydrogen peroxide produced by MAO-A in vascular smooth muscle cells contributes to serotonin-induced vasoconstriction. Although endothelial cells are known to express MAO, its importance for endothelial function is poorly understood.

The growth factor adaptor protein p66Shc is another source of ROS that functions in mitochondrial signaling. p66Shc generates hydrogen peroxide by oxidizing cytochrome c. Under physiological conditions, p66Shc may be contained within a high-molecular-weight inhibitory protein complex within mitochondria or the cytoplasm. Specific proapoptotic signals induce release and migration of p66Shc into the mitochondrial intermembrane space. p66Shc is also activated when the concentration of reduced cytochrome c is relatively high, such as when complex IV activity is reduced by hypoxia or because of physiological inhibition by NO, as discussed further below. Furthermore, p66Shc is activated via phosphorylation by protein kinase CβII in the setting of high glucose and contributes to the development of diabetic endothelial dysfunction. Thus, p66Shc transduces environmental signals into ROS that signal apoptosis and other cellular responses.

Another regulator of mitochondrial ROS is the mitochondrial ATP-sensitive potassium channel (mitoK<sub>ATP</sub>). A consensus structure for this inner membrane channel is lacking but remains the focus of intense study. Studies in cardiac myocytes have shown that mitoK<sub>ATP</sub> is activated by superoxide and hydrogen peroxide and is regulated in a redox-dependent manner by reactive nitrogen species. This channel is perhaps best known for its role in facilitating ROS-mediated ischemic preconditioning in cardiac myocytes, possibly by acting as an uncoupling agent that decreases membrane potential and mitochondrial calcium overload, although the precise mechanism remains unclear. In endothelial cells, mitoK<sub>ATP</sub> is less well-studied, but pharmacological mitoK<sub>ATP</sub> activation protects against ischemic cell death in cultured endothelial cells and preserves endothelial vasodilator function in Langendorff-perfused guinea pig hearts subjected to ischemia-reperfusion. Inhibition of mitoK<sub>ATP</sub> channels also blunts high-glucose-induced endothelial cell apoptosis.

In healthy human volunteers and patients with atherosclerosis, 20 minutes of ischemia followed by reperfusion produces a marked impairment in endothelium-dependent dilation in the forearm. This impairment can be prevented by short cycles of cuff occlusion and release before the ischemic insult. The benefit of such ischemic preconditioning is blocked by the nonselective K<sub>ATP</sub> channel blocker glibenclamide and is mimicked by the mitoK<sub>ATP</sub> opener diazoxide. These human studies are limited by the nonselective and off-target effects of these pharmacological interventions but strongly suggest that mitoK<sub>ATP</sub> activation may contribute to ischemic preconditioning in the human endothelium.

It is interesting that mitochondrial ROS production can be triggered by ROS from other enzymatic sites by a process termed ROS-induced ROS release. For example, ROS produced by NOX can stimulate mitochondrial ROS
production by a mechanism that involves opening of the mitoKATP channel, membrane depolarization, and the mitochondrial permeability transition (MPT) pore. In this situation, inhibiting mitoKATP may be protective and preserves NO production in endothelial cells exposed to angiotensin II. Thus, ROS produced by NOX, uncoupled NO synthase, and neighboring mitochondria, and other sources can contribute to the generation of mitochondrial ROS production in a multidirectional fashion. ROS-induced ROS release may amplify and target ROS signals under physiological conditions but, when excessive, may contribute to the pathogenesis of endothelial dysfunction.

Mitochondrial ROS Signaling
Mitochondria-derived hydrogen peroxide, which is generated via dismutation of superoxide by MnSOD or produced directly by NOX4 or p66Shc, can diffuse across membranes and function in physiological signaling, as has been extensively reviewed. For example, experimental studies have shown that shear stress–induced vasodilation, hypoxia signaling, autophagy, and proinflammatory activation depend on mitochondrial ROS. In humans, however, there are very limited data about mitochondrial ROS in physiological cell signaling in the vasculature. The lack of benefit and, at times, harmful effects of high-dose nonselective antioxidants as treatment for cardiovascular disease in clinical trials might be interpreted as indirect evidence that ROS play a physiological role in the vasculature.

A few clinical studies have more directly implicated ROS signaling in endothelial function. Flow-mediated dilation in arterioles isolated from human myocardium depends on the production of ROS by the electron transport chain. In healthy human volunteers, flow-mediated dilation of the brachial artery is blunted by oral antioxidant treatment, which might reflect scavenging of mitochondrial ROS. A study of arteries isolated from adipose tissue of patients with coronary artery disease demonstrated that mitochondrial ROS compensate for the loss of NO and promote flow-mediated dilation. Interpretation of those results is complicated, however, because hydrogen peroxide scavengers, such as N-acetylcysteine, improve endothelium-dependent dilation in coronary arteries of patients with atherosclerosis. The relative importance of mitochondrial ROS for endothelial cell signaling is likely to vary according to vascular bed and to depend on risk factor burden.

Excess Mitochondrial ROS Production
Altered mitochondrial membrane potential is an important factor that drives excess mitochondrial ROS production in the setting of risk factors. Membrane depolarization may activate ROS production by increasing the activity of complexes I and III. Membrane hyperpolarization also leads to higher ROS production and may be particularly relevant to metabolic disease states in which there is nutrient excess and low demand for ATP. These conditions are associated with an increase in the NADH/NAD⁺ ratio and a decrease in the rate of electron flow that prolongs the lifespan of reactive intermediates at complexes I and III. These intermediates promote the reduction of oxygen to superoxide anion. It has been suggested that high glucose
and fatty acid levels in obesity and diabetes mellitus drive mitochondrial ROS production by this mechanism.\textsuperscript{67}

It is known that nutrient excess alters mitochondrial metabolism in cardiomyocytes and contributes to heart failure.\textsuperscript{58,89} In endothelial cells, where energy requirements are lower, excess substrate stimulates mitochondrial ROS production to signal a change in endothelial phenotype. For example, exposure of endothelial cells to high glucose or fatty acid concentrations decreases NO bioavailability and activates nuclear factor-kB and protein kinase C.\textsuperscript{87,90} These responses are prevented by mitochondrial membrane depolarization, inhibitors of the electron transport chain, and overexpression of UCP1, which may lower membrane potential.\textsuperscript{90} Interestingly, p66Shc-derived ROS have been implicated in glucose-induced endothelial dysfunction in endothelial cells.\textsuperscript{66} Leukocytes and arterioles from diabetic individuals are protected against the development of endothelial dysfunction by streptozotocin injection are protected against the development of endothelial dysfunction and display increased antioxidant activity.\textsuperscript{26} Interestingly, a recent study showed that expression of electron transport chain components or expression of defective components that produce more ROS. Risk factors, including older age, cigarette smoking, hypercholesterolemia, and hyperglycemia, are associated with increased mtDNA damage in cardiovascular tissues.\textsuperscript{93} mtDNA damage correlates with the extent of atherosclerosis in mouse models and human tissues.\textsuperscript{24} It has recently been proposed that glycation of mitochondrial proteins might also alter their function and increase ROS in diabetes mellitus.\textsuperscript{25}

ROS-induced modifications to mitochondrial components, including mtDNA, proteins, and lipids, may further exacerbate ROS production and set up a vicious cycle that contributes to vascular disease.\textsuperscript{88,91} mtDNA damage may lead to decreased expression of electron transport chain components or expression of defective components that produce more ROS.\textsuperscript{92} Risk factors, including older age, cigarette smoking, hypercholesterolemia, and hyperglycemia, are associated with increased mtDNA damage in cardiovascular tissues.\textsuperscript{93} mtDNA damage correlates with the extent of atherosclerosis in mouse models and human tissues.\textsuperscript{24} It has recently been proposed that glycation of mitochondrial proteins might also alter their function and increase ROS in diabetes mellitus.\textsuperscript{25}

Modified mitochondrial proteins accumulate in disease states. In the early stages of disease, the combination of dynamics, mitophagy, and biogenesis may replace damaged mitochondrial components and maintain normal mitochondrial function. However, impairment of these quality-control mechanisms leads to the retention of dysfunctional mitochondria that produce excess ROS and promote vascular disease. It is interesting to note that methods are available to measure mtDNA damage in human tissues.\textsuperscript{91} Oxidative damage to mtDNA in circulating leukocytes has considerable potential as a reliable marker of mitochondrial dysfunction that can be made in readily available blood cells. However, large-scale application of this approach has not been reported.

Only a few human studies have specifically implicated mitochondria as the source of excess ROS in patients. In patients with diabetes mellitus, circulating leukocytes and arterioles isolated from subcutaneous fat display higher mitochondrial ROS production and membrane hyperpolarization.\textsuperscript{76,77} Furthermore, impaired endothelium-dependent vasodilation in freshly isolated arterioles from diabetic individuals is reversed by mild membrane depolarization or mitochondria-targeted antioxidants.\textsuperscript{78} Interestingly, a recent study showed that low glucose concentrations also stimulate mitochondrial ROS production, membrane hyperpolarization, and endothelial dysfunction in cultured cells and isolated human arterioles.\textsuperscript{97} These findings might explain clinical trials suggesting that tight glucose control actually might be harmful in patients with diabetes mellitus and suggest that mitochondria-derived ROS from any cause can induce endothelial dysfunction.\textsuperscript{97}

Mitochondrial components and hypertension. Polymorphisms in mitochondrial genes, including a subunit of complex IV, correlate with blood pressure in the Framingham Heart Study.\textsuperscript{58} In addition, there is a maternal link in the heritability of blood pressure that may implicate the mitochondrial genome.\textsuperscript{99} However, the consequences of these polymorphisms for ROS production and their specific importance for endothelial function remain unknown.

Mitochondrial Antioxidant Enzymes
The first line of defense against mitochondrial superoxide is MnSOD. MnSOD is located in the mitochondrial matrix and catalyzes the conversion of superoxide anion to hydrogen peroxide. Consistent with a protective role in the endothelium, MnSOD−/− mice exhibit impaired endothelium-dependent vasodilation.\textsuperscript{100} In addition, apolipoprotein E−/− MnSOD−/− mice display earlier mtDNA damage and accelerated atherosclerosis compared with apolipoprotein E−/− MnSOD−/− mice.\textsuperscript{94}

Levels of hydrogen peroxide are regulated by antioxidant enzymes, including catalase and several mitochondrial and cytosolic peroxidases. Catalase is located in cytosolic peroxisomes. Important mitochondrial peroxidases include thioredoxin-2, peroxiredoxin-3, and glutaredoxin-2. Glutathione peroxidase-1 is located both in mitochondria and in the cytosol in endothelial cells.\textsuperscript{4,101} As noted, increased expression of these enzymes is signaled by AMPK and PGC-1α in response to hydrogen peroxide and other forms of oxidative stress in endothelial cells.\textsuperscript{16,17} Studies in experimental models have shown that reduced expression of mitochondrial antioxidant enzymes can induce mitochondrial damage, endothelial dysfunction, and atherogenesis.\textsuperscript{94,100,102} Conversely, overexpression of these proteins is protective against the development of vascular disease.\textsuperscript{103}

Uncoupling Proteins
There is considerable interest in mitochondrial UCPs and their role in the regulation of ROS production.\textsuperscript{104,105} UCPs are localized to the inner mitochondrial membrane and facilitate movement of protons down their concentration gradient into the matrix, thus making membrane potential less negative and uncoupling electron transport chain activity from ATP production. UCP1 is expressed in brown fat and plays a role in adaptive thermogenesis. UCP2, which is the primary isoform in endothelial cells, and UCP3 have high sequence homology with UCP1 but are expressed at lower levels. UCP2 and UCP3 contribute to the regulation of mitochondrial membrane potential when they are activated by superoxide and act in a compensatory fashion to decrease ROS production (Figure 2). There is controversy about how ROS dynamically activate UCP2 and UCP3. In this regard, studies have implicated protein modification by 4-hydroxynonenal and reversible glutathionylation.\textsuperscript{105–107} Although UCPs may function in an adaptive manner in most circumstances, it also has been suggested that activation of UCPs in myocytes under conditions of nutrient excess may increase ROS production in...
the electron transport chain. This effect may lead to increased myocardial oxygen consumption and reduced cardiac efficiency in type 2 diabetes mellitus.106

Experimental studies confirm the importance of UCP2 in endothelial cells. Decreasing expression with antisense oligonucleotides increases mitochondrial membrane potential and ROS production.104 Conversely, UCP2 overexpression reduces ROS production and inflammatory activation in cultured cells and improves endothelium-dependent dilation in isolated rat aorta.109 Expression of UCP2 is dynamically regulated, and exposure to ROS, nutrient excess, and other forms of cellular stress stimulate UCP2 expression as part of a stress response coordinated by AMPK and PGC-1α.16,17 In vivo studies have shown increased atherosclerosis, ischemic stroke, and impaired endothelium-dependent vasodilation in UCP2-null mice.110–112

There is epidemiological evidence that UCP2 may relate to human cardiovascular disease. For example, the 866 G>A variant is associated with increased UCP2 expression and a decrease in the incidence of cardiovascular disease events.113 Polymorphisms at this locus also may be associated with carotid artery atherosclerosis.114 Interprestation of these findings is complicated by the observation that UCP2 and UCP3 gene variants are also linked to incident type 2 diabetes mellitus.115 The direct importance of UCP for the regulation of endothelium-dependent vasodilation or other aspects of endothelial function has not been directly examined in humans.

NO
NO is another important regulator of mitochondrial bioenergetics and oxidant production. The effects of NO are complex and depend on the relative availability of oxygen and NO, as well as the cellular redox state.116 NO inhibits complex IV (cytochrome c oxidase) by competing with oxygen binding at the binuclear heme a/a3 center. In endothelial cells with physiological levels of bioactive NO, this interaction inhibits mitochondrial respiration and facilitates ROS signaling.4,18 When cellular levels of reduced glutathione are relatively low in states of oxidative stress, NO has a chronic inhibitory effect on complex I via S-nitrosylation in a manner that blunts mitochondrial ROS production. High levels of NO, which may occur in acute inflammatory states, reduce mitochondrial reserve capacity in endothelial cells, which may reflect a decreased ability to respond to cellular stress.117 Conditions that reduce endothelial NO bioavailability may promote excess mitochondrial ROS production by withdrawing the inhibitory effects of NO.4,19 Under such conditions, peroxynitrite formed by the reaction of NO and superoxide may modify complexes I and III in a manner that further increases ROS production.4,19 Antioxidant enzymes, including MnSOD, are also inactivated by peroxynitrite, further exacerbating oxidative stress within mitochondria.118

Mitochondria and Calcium Homeostasis in the Endothelium
Cytosolic calcium levels regulate many aspects of endothelial function. For example, receptor-dependent agonists, such as acetylcholine and serotonin, activate eNOS by increasing cytosolic calcium and stimulating the binding of calcium/calmodulin.119 Calcium activates calcium/calmodulin-dependent protein kinase II, which plays a role in eNOS gene expression and phosphorylation state, and regulates actin cytoskeletal elements that influence endothelial cell shape, motility, and barrier function.120 Calcium is also involved in vascular endothelial growth factor signaling and angiogenic functions of the endothelium.121

As has been extensively reviewed,122–125 mitochondria and the endoplasmic reticulum (ER) cooperate in the regulation of calcium trafficking and thereby control key aspects of endothelial function. In brief, mitochondria serve as calcium buffer sites and regulate calcium uptake and release by the ER.123,124 Although the ER is the major storage site, ≈25% of cellular calcium is localized to mitochondria. During cell stimulation, continuous flux of calcium through mitochondria is needed for store-operated entry and ER calcium store refilling.126 Localized calcium uptake as well as the position of mitochondria in relation to the plasma membrane produce calcium microdomains that are important in these processes.124,125,127

Calcium movement in and out of mitochondria is highly regulated.124,125 Generally speaking, the availability of multiple uptake and extrusion channels serves to buffer mitochondrial calcium levels over the physiological range of cytosolic calcium. When calcium load exceeds the buffering threshold, mitochondrial calcium increases rapidly and may trigger mitochondrial-dependent apoptosis. In endothelial cells, hydrogen peroxide–induced increases in mitochondrial calcium may depend, in part, on decreased calcium extrusion via inhibition of the sodium/calcium exchanger.128 Because calcium flux through mitochondria is important for ER calcium stores, inhibition of calcium extrusion by hydrogen peroxide may deplete ER calcium stores and thereby trigger the unfolded protein response and apoptosis.129,130

The ER maintains specific domains for direct interaction with mitochondria known as the mitochondria-associated membrane (MAM). As has been recently reviewed, the MAM tethers the ER to mitochondria and is enriched with proteins relevant to calcium and lipid metabolism.129 MAMs facilitate calcium exchange that is important for ER regulation of mitochondrial energetics and apoptosis.129

A recent study suggests that MAMs are relevant to pulmonary hypertension. In pulmonary arterial vascular smooth muscle cells, disruption of mitochondria–ER interactions by the reticulin protein Nogo-B impairs hypoxia-induced apoptosis, leading to the typical proliferative lesions seen in pulmonary hypertension.130 Interestingly, Nogo-B expression is also increased in endothelial cells in pulmonary hypertension, and it has been suggested that endothelial cells also may assume a proliferative apoptosis-resistant phenotype in this condition. However, the specific importance of Nogo-B and other aspects of MAM biology have not been extensively studied in endothelial cells.

Physiological changes in mitochondrial and cytosolic calcium concentrations have important regulatory effects on many aspects of mitochondrial function, including ROS production, energetics, motility, dynamics, and biogenesis.4,123 Calcium activates Krebs cycle enzymes and oxidative phosphorylation, thus increasing ATP production.124,129 Cytosolic calcium regulates mitochondrial motility through the Miro-Milton protein complex, which interacts with the outer mitochondrial membrane and couples mitochondria to microtubules.125,131 Miro acts as a calcium sensor and is
Mitochondria are implicated in cell death pathways, including apoptosis and programmed necrosis (necroptosis).\(^6\),\(^13\) Necroptosis is initiated by death receptors, such as TNF receptor 1, and involves the activities of receptor-interacting proteins 1 and 3.\(^13\)\(^5\),\(^13\) Studies suggest that mitochondria contribute to necroptosis execution. For example, TNF-α–induced necrosis requires the formation of protein complexes that include receptor-interacting protein 1 (RIP1) and receptor-interacting protein 3 (RIP3), and the mitochondrial phosphoglycerate mutase-5/protein phosphatase (PGAM5) at the mitochondrial outer membrane.\(^13\) Interestingly, phosphoglycerate mutase-5 activates DRP1 by dephosphorylation, thus facilitating mitochondrial fragmentation.\(^13\) Nitration of mitochondrial complex I and membrane depolarization also contribute to necroptosis in endothelial cells.\(^13\) Although myocardial cell necroptosis contributes to ischemic injury and heart failure,\(^13\) relatively little is known about the importance of this process in the endothelium.

Mitochondria are also central mediators of apoptosis.\(^3\),\(^2\),\(^19\),\(^14\) Briefly, intrinsic apoptosis is initiated by cellular stressors, including hypoxia, ROS, oxidized low-density lipoprotein, and DNA damage. Such stimuli activate BH3–only proteins, which inhibit antiapoptotic factors, including B-cell lymphoma 2 (BCL-2), and allow activation of BCL2-associated X protein (BAX) and BAK. It is generally held true that these proapoptotic factors initiate mitochondrial outer membrane permeabilization and depolarization, opening of the MPT pore and the release of mitochondrial proteins into the cytosol.\(^3\),\(^2\),\(^19\) However, recent studies have questioned the role of MPT pore opening in intrinsic apoptosis.\(^14\) The intrinsic apoptosis pathway also involves mitochondrial fission, because DRP1 and BAX/BAK cooperate in the induction of outer membrane permeabilization and fission.\(^3\),\(^2\),\(^14\),\(^2\) Release of cytochrome c, second mitochondria-derived activator of caspases/DIABLO, HtrA2/Omi, and other mitochondrial proteins activate caspases and apoptosis.\(^19\)

The MPT pore has received considerable attention because of its possible role in cell death pathways as well as its role in ischemia-reperfusion injury and ischemic preconditioning. As recently reviewed, the MPT pore is believed to be a multicomponent and relatively large nonspecific channel.\(^14\) Although the essential and nonessential components of the MPT pore remain highly controversial, both adenine nucleotide translocase and cyclophilin D serve regulatory functions, and the roles of the voltage-dependent anion channel and BAX have been established as nonessential.\(^14\),\(^14\) Under physiological conditions, the pore is functionally closed. In vitro experiments with endothelial cells suggest that short MPT openings may serve a protective function, dissipating membrane potential in response to oxidative stress or calcium overload, whereas lengthy MPT openings may trigger apoptosis.\(^3\),\(^12\),\(^13\)\(^14\) However, it remains uncertain whether short or prolonged MPT openings contribute to physiological or pathological processes in vivo.

In the endothelium, cardiovascular risk factors can trigger apoptosis by multiple mechanisms. Apoptosis is observed in cells exposed to high glucose and in animal models of diabetes mellitus, and under these conditions, apoptosis is inhibited by interventions that decrease ROS or inhibit MPT pore opening.\(^14\),\(^14\) Hyperglycemia-induced apoptosis has been shown to involve activation of nuclear factor-κB and protein kinase C, which, as discussed, can activate p66Shc and trigger the MPT pore.\(^5\),\(^7\) Downregulation of connexin 43 in the inner mitochondrial membrane in retinal endothelial cells also contributes to apoptosis under high glucose conditions.\(^14\) In cardiac myocytes, connexin 43 is known to play a role in ischemic preconditioning,\(^14\) possibly by functioning as an uncoupling protein or potassium channel to decrease mitochondrial ROS.\(^15\) Another reported stimulus for apoptosis in endothelial cells and renal podocytes is phosphorylation of DRP1 at serine-600 by rho-associated coiled-coil containing protein kinase 1 (ROCK1).\(^3\),\(^14\) This mechanism may have clinical relevance because rho-associated coiled-coil-containing protein kinase 1 inhibitors are in development as drugs.\(^15\) As discussed, DRP1 is also activated by dephosphorylation at serine-656 by calcineurin in response to increased cytosolic calcium, suggesting multiple regulatory mechanisms that link mitochondrial dynamics and calcium signaling with apoptosis.

In addition to diabetes mellitus, other risk factors may provoke apoptosis in endothelial cells. Senescent endothelial cells are more sensitive to proapoptotic stimuli,\(^15\) which may be attributable in part to decreased expression of BCL-2.\(^15\) Relevant to hypertension, angiotensin II induces apoptosis in cultured endothelial cells.\(^14\) As discussed, angiotensin II–stimulated apoptosis represents an example of ROS–induced ROS signaling and is mediated by NOX, protein kinase C, mitochondrial ATP-sensitive potassium channels, and peroxynitrite formation.\(^8\) The effects of angiotensin II are
blunted by overexpression of the mitochondrial peroxidase thioredoxin 2, further suggesting that mitochondrial ROS play a role in this response. Relevant to dyslipidemia and atherosclerosis, oxidized low-density lipoprotein has been shown to induce apoptosis in endothelial cells by increasing mitochondrial ROS and activating p53, which leads to BAX translocation to the mitochondrial membrane and release of cytochrome c.

Several human studies suggest that endothelial apoptosis may be important in vascular disease. For example, serum from patients with heart failure induced apoptosis in cultured endothelial cells, as reflected by DNA fragmentation and mitochondrial cytochrome c release. In addition, circulating endothelial microparticles from patients with heart failure display markers of apoptosis. Similarly, serum from patients with acute coronary syndrome induces endothelial cell apoptosis, and the effect was blunted by treatment with an angiotensin-converting enzyme inhibitor. Although the circulating factors that induced endothelial cell apoptosis were not identified in those studies, similar changes were induced in isolated endothelial cells by TNF-α, which is known to be increased in patients with heart failure and coronary artery disease. Finally, study of surgical specimens from patients undergoing carotid endarterectomy demonstrated endothelial apoptosis in regions of low shear stress, which are known to be prone to atherosclerosis. Overall, these limited human studies show associations between mitochondria-regulated cell death pathways and cardiovascular disease, and may suggest targets for therapy.

Heme Synthesis and Metabolism in the Endothelium

The mitochondrion is the site of heme synthesis. Heme is essential for the function of electron transport chain complexes and a variety of other proteins, including eNOS. Heme synthesis begins with the formation of amino levulinic acid (ALA) by ALA synthase-1. ALA is then transported from the mitochondrion to the cytoplasm, where it is converted to coproporphyrinogen III. This intermediate is transported back into the mitochondrion and converted to protoporphyrin IX. Ferrous iron (Fe2+) is incorporated into protoporphyrin IX to form heme.

In addition to providing enzyme prosthetic groups, heme synthesis may function to reduce oxidative stress by sequestering redox active iron that would otherwise promote ROS formation. Inhibition of heme synthesis in rats is associated with decreased NO production and impaired endothelium-dependent vasodilation. Interestingly, expression of ALA synthase-1 is regulated by PGC-1α, possibly coordinating heme synthesis with other cytoprotective responses, such as mitochondrial biogenesis and expression of antioxidant enzymes.

The mitochondrial inner membrane transport protein ATP-binding cassette–mitochondrial erythroid (ABC–me) plays a role in heme synthesis and reduction of oxidative stress. ABC–me expression is regulated by heme and was initially recognized because of its role in erythropoiesis. ABC–me is believed to function as an ALA transporter and to stabilize mitoferrin 1, a mitochondrial iron importer. Interestingly, cardiac ischemia-reperfusion injury in mice heterozygous for ABC–me (ABC–me–/–) is associated with increased ROS production, oxidative damage to mitochondria and the sarcoplasmic/ER calcium/ATPase pump, impaired mitochondrial respiration, and more severe cardiac dysfunction. These effects may be attributable to altered heme synthesis and mitochondrial iron homeostasis. A preliminary study demonstrated that ABC–me is expressed in endothelial cells and that high glucose lowers ABC–me expression, which might inhibit heme synthesis and thereby contribute to mitochondrial ROS production and vascular dysfunction.

Heme is metabolized by heme oxygenase-1, which forms biliverdin and carbon monoxide, and releases iron. Biliverdin is subsequently converted to bilirubin. It has been argued that heme oxygenase may have both pro-oxidant and antioxidant effects in the cell. Release of redox active iron may increase the formation of ROS, and carbon monoxide inhibits complex IV in a manner that increases ROS. On the contrary, bilirubin is an antioxidant and carbon dioxide has vasodilator properties that mimic the generally favorable effects of NO via activation of guanylyl cyclase. Heme oxygenase expression is induced by AMPK via the transcription factor nuclear respiratory factor-2. A recent study showed that endothelial-selective constitutive activation of AMPK prevented the development of endothelial dysfunction in a heme oxygenase–dependent manner in a mouse model of diabetes mellitus. Heme oxygenase also has been shown to have antiapoptotic effects in endothelial cells.

Several clinical studies have examined the links between heme metabolism and human cardiovascular disease. For example, patients with diabetes mellitus have reduced red blood cell ALA synthase activity. A large number of observational studies have shown correlations between heme oxygenase-1 expression in circulating blood cells and coronary artery disease and diabetes mellitus. Many of these studies show higher levels of heme oxygenase-1 in patients with risk factors or vascular disease. These findings might implicate heme oxygenase in disease pathogenesis or could represent a compensatory response. It is impossible to distinguish these possibilities or to draw conclusions about the utility of targeting heme oxygenase for therapy, given the observational design of these studies. The specific importance of heme synthesis and heme oxygenase activity for endothelial function has not been directly examined in humans.

Interventions That Improve Mitochondrial Function in the Endothelium

As discussed, a variety of risk factors induce mitochondrial dysfunction in the endothelium, which may contribute to the pathogenesis of atherosclerosis. This observation prompts speculation that interventions that restore mitochondrial function might be protective. A list of potential interventions that might affect vascular disease is provided in Table 1. Also refer to the review by Walters et al that focuses on these and other interventions that affect mitochondrial function in the myocardium.

Mitochondria-Directed Antioxidants

There is considerable interest in the therapeutic potential of mitochondrial-directed antioxidants. Because mitochondrial-derived ROS are important for signaling, a strategy that reduces ROS to physiological levels rather than completely eliminates...
Table 1. Potential Interventions to Improve Mitochondrial Function in the Endothelium

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial-targeted</td>
<td>Scavenge mitochondrial ROS</td>
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<tr>
<td>compounds</td>
<td></td>
</tr>
<tr>
<td>MitoQ</td>
<td></td>
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<tr>
<td>Mito-α tocopherol</td>
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<tr>
<td>Mito-tempol</td>
<td></td>
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<tr>
<td>S-nitroso-TPP</td>
<td>Mitochondrial-targeted NO donor</td>
</tr>
<tr>
<td>Lipoic acid</td>
<td>ROS scavenger, cofactor for Krebs cycle</td>
</tr>
<tr>
<td>Carnitine/acyetyl-γ-carnitine</td>
<td>Fatty acid transport into mitochondria</td>
</tr>
<tr>
<td>Activators of AMPK/PGC-1α</td>
<td>Activate biogenesis</td>
</tr>
<tr>
<td>Calorie restriction</td>
<td>Inhibit apoptosis</td>
</tr>
<tr>
<td>Exercise</td>
<td>Decrease ROS</td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td></td>
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<tr>
<td>Metformin</td>
<td></td>
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<tr>
<td>Thiazolidinediones</td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td></td>
</tr>
<tr>
<td>Sirtuin activators</td>
<td>Activate eNOS</td>
</tr>
<tr>
<td>Mdivi-1</td>
<td>Inhibit DRP1 and mitochondrial fission</td>
</tr>
<tr>
<td>FCCP, dinitrophenol</td>
<td>Membrane depolarizers (current agents are</td>
</tr>
<tr>
<td></td>
<td>limited by toxicity)</td>
</tr>
</tbody>
</table>

**Lipoic Acid and Acetyl-γ-Carnitine**

Lipoic acid is a mitochondrial antioxidant and a coenzyme required for the activity of the Krebs cycle. Acetyl-γ-carnitine plays an important role in energy production by conjugating fatty acids for transport into mitochondria. Oral carnitine supplementation in humans is frequently formulated as acetyl-γ-carnitine, which enters mitochondria and improves mitochondrial function to a greater extent than γ-carnitine. In animal models of aging, diabetes mellitus, and hypertension, the combination of lipoic acid and acetyl-γ-carnitine reverses abnormalities of mitochondrial membrane potential, ATP production, and ROS production in a variety of tissues. In cultured endothelial cells, lipoic acid improves NO production, blunts inflammatory activation, and increases the antiapoptotic protein BCL-2. Dietary supplementation with lipoic acid or acetyl-γ-carnitine improves NO bioavailability and reduces atherosclerosis lesion formation in animal models.

Lipoic acid, γ-carnitine, and acetyl-γ-carnitine are available over-the-counter as dietary supplements, and their effects on vascular function have been examined. Lipoic acid improves endothelial function in patients with metabolic syndrome or diabetes mellitus, and γ-carnitine blunts free fatty acid-induced endothelial dysfunction. It remains unclear, however, whether the beneficial effects of these compounds on endothelial function can be attributed to an effect on mitochondrial function.

**Interventions That Activate AMPK and PGC-1α**

Interventions that increase the activity of AMPK and PGC-1α have been consistently shown to improve endothelial function in animals. For example, short-term calorie restriction reverses endothelial dysfunction and oxidative stress in old mice. Calorie restriction also promotes mitochondrial biogenesis and increases expression of MnSOD. The glycolysis inhibitor 2-deoxyglucose activates AMPK, induces autophagy, and protects against cell death in cultured endothelial cells. The thiazolidinediones, including pioglitazone, also have been reported to activate PGC-1α and increase mitochondrial biogenesis in endothelial cells. Metformin, which activates AMPK, has been shown to inhibit MPT pore opening and endothelial apoptosis, and to prevent the development of endothelial dysfunction in experimental models.

With regard to clinical studies, it is well-established that exercise and weight loss improve endothelial vasodilator function in patients with obesity, diabetes mellitus, and other risk factors. A weight loss and exercise program increased expression of PGC-1α in skeletal muscle of elderly women. Metformin reverses endothelial dysfunction in patients with diabetes mellitus, although it remains unclear whether metformin actually activates AMPK in vivo. Given the many favorable effects of these interventions on risk factors and metabolism, mechanisms beyond improved mitochondrial function may be important.

**Sirtuins**

The sirtuins play important roles in the regulation of lifespan and metabolism, with implications for mitochondrial function, and may be targets for therapy, as was recently reviewed. Sirtuins regulate gene expression and enzyme activity via their activity as NAD+-dependent deacetylases. Sirtuin 1 (SIRT1)
deacetylases and activates PGC-1α, leading to favorable effects on glucose and lipid metabolism and expression of mitochondrial genes.193,195–197 SIRT3 is specifically localized to the mitochondrial matrix and controls mitochondrial energetics by deacetylating mitochondrial enzymes.193,198 Interestingly, SIRT3 also deacetylates and activates MnSOD, and thus may reduce mitochondrial superoxide levels.199

With regard to the cardiovascular system, there is extensive evidence that SIRT1 has protective effects against ischemic injury and hypertrophy in the heart.193 SIRT1 is known to deacetylate and activate eNOS in the endothelium and to regulate angiogenesis.193,200,201 In a study of elderly human subjects, SIRT1 expression was reduced in endothelial cells isolated from the brachial artery and correlated with endothelium-dependent vasodilation.202

There is limited evidence that the mitochondrial SIRT3 may have favorable effects in endothelial cells, although it is unclear whether such effects are mediated through alterations in mitochondrial function. For example, endothelial-specific inhibition of nuclear factor-κB in a mouse model is associated with reduced inflammation, improved insulin sensitivity, enhanced mitochondrial biogenesis, improved blood flow, and increased lifespan.203 In vascular tissue from these animals, there is upregulation of SIRT3.203 In humans, aging is associated with reduced expression of SIRT3 in skeletal muscle of sedentary, but not endurance-trained, individuals. To date, the effects of specific SIRT3 activation on endothelial function have not been examined in experimental models or human subjects. However, there is currently great interest in the possibility that activators of SIRT1 and SIRT3 will have benefits against metabolic and cardiovascular disease.194

Resveratrol
Resveratrol (3,4′,5-trihydroxystilbene) is a polyphenolic compound found in a variety of foods, including grapes and red wine. Initial experimental studies reported that resveratrol activates SIRT1.204 More recent evidence suggests that the beneficial effects of resveratrol also involve activation of AMPK.205 In animal models, resveratrol mimics the atheroprotective effects of SIRT1 and SIRT3, which include reduced expression of SIRT3 in skeletal muscle of sedentary, but not endurance-trained, individuals. To date, the effects of specific SIRT3 activation on endothelial function have not been examined in experimental models or human subjects. However, there is currently great interest in the possibility that activators of SIRT1 and SIRT3 will have benefits against metabolic and cardiovascular disease.194

Inhibition of Mitochondrial Fission
Clinical studies suggest that diabetes mellitus and other pathological states are associated with increased mitochondrial fission and impaired autophagy in the endothelium.39,56 Mitochondrial division inhibitor-1 (Mdivi-1) is an inhibitor of the fission protein DRP1 and has been shown to decrease mitochondrial outer membrane permeabilization, cytochrome c release, and apoptotic cell death.210 Mdivi-1 also has been shown to limit ischemia reperfusion injury in isolated myocytes and to reduce infarct size in a mouse model.211 The effects of this compound on endothelial function are unknown.

Mitochondrial Membrane Depolarizing Agents
UCPs, including UCP2, act to induce mild membrane depolarization and reduce mitochondrial ROS production.104 Overexpression of UCP2 improves endothelial function in a rat model,109 suggesting that an uncoupling agent might have therapeutic potential. In arterioles isolated from patients with diabetes mellitus, the uncoupling agent carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone lowers membrane potential and improves endothelial function.206 2,4-dinitrophenol is another uncoupling agent that has been used in humans for the treatment of obesity.172 Clinical trials with this compound were stopped because of high toxicity, but it remains possible that less potent, self-limiting uncoupling agents might prove useful mitochondria-directed therapeutics.172

MicroRNAs
There is recent appreciation of the importance of microRNAs (miRs) in the coordination of many biological processes.212 In pulmonary endothelial cells, miR-210 has been identified as a repressor of iron–sulfur cluster assembly proteins 1/2, which are important for the expression of components of the electron transport chain and Krebs cycle.213 Under conditions of hypoxia and activation of hypoxia-inducible factor-1α, miR-210 is upregulated, thereby repressing mitochondrial iron–sulfur enzymes and mitochondrial respiration in endothelial cells. This response may be important for the Pasteur effect and the mammalian adaptation to hypoxia.213 Given the recent interest in miRs as therapeutic targets,212 these results suggest that targeting miR-210 might be an approach to broadly regulate mitochondrial respiration and endothelial function.

Conclusions
This article reviews the key aspects of mitochondrial function in the endothelium. Mitochondrial content in endothelial cells is relatively modest, and subcellular localization relative to the nucleus, cytoskeletal elements, calcium channels, and the ER is important for ROS and calcium signaling. In addition to regulating biogenesis, PGC-1α orchestrates a cellular response to oxidative stress by increasing the expression of mitochondrial antioxidant enzymes and UCPs. Mitochondrial quality-control mechanisms, including mitochondrial dynamics and autophagy/mitophagy, are needed for normal angiogenic and vasodilator function of endothelial cells. Mitochondrial ROS are important for signaling physiological responses to nutrient status, hypoxia, and shear stress. An array of antioxidant enzymes, UCPs, and other mechanisms in mitochondria combine to modulate and direct ROS signals. Mitochondria...
interact with the ER to regulate calcium signaling, which is essential for the key aspects of endothelial function, including eNOS activation, motility, barrier function, and angiogenesis. In general, physiological ROS signaling and calcium signaling activate short-term or long-term functional changes in endothelial cells to maintain blood flow to tissues through control of vascular tone and angiogenesis. Physiological ROS signaling that promotes inflammation is important for normal immune function. Such signals are also involved in regulated cell death pathways, which eliminate senescent and irreversibly damaged cells. Excessive ROS and disordered calcium signaling, however, contribute to the pathogenesis of vascular disease. Finally, we reviewed the importance of mitochondria in heme synthesis and metabolism, which, in addition to generating heme for use as an enzymatic cofactor, may be emerging as another aspect of mitochondrial function that regulates cellular redox state in the endothelium.

Although a large body of experimental work has shown that mitochondria are important for endothelial function, only a relatively small number of translational studies have shown the clinical relevance of these mechanisms in humans (Table 2). As described, studies of isolated vascular tissue and endothelial cells from patients with diabetes mellitus and elderly humans indicate the importance of subcellular location, biogenesis, dynamics, autophagy, and pathological ROS levels. Only a few studies have investigated the signaling aspects of ROS and calcium, and little is known about the specific importance of endothelial PGC-1α, regulated cell death, and heme metabolism in humans. Epidemiological studies have shown correlations between human disease and polymorphisms of genes related to mitochondrial function. Blood markers of mitochondrial dysfunction, such as mtDNA damage and mitochondrial oxygen consumption in circulating leukocytes or platelets, may facilitate studies of systemic abnormalities of mitochondrial function and their relation to cardiovascular disease. The presented studies suggest that mitochondria-directed therapies have potential for the prevention and management of cardiovascular disease, in part by improving mitochondrial function in the endothelium.

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**Disclosures**

None.

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**Table 2. Translational Methods for Study of Mitochondria and Endothelial Function in Humans**

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FCCP indicates carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone; FIS1, fission 1; MFN2, mitofusin-2; mtdNA, mitochondrial DNA; mitoKATP, mitochondrial ATP-sensitive potassium channel; NO, nitric oxide; OPA1, optic atrophy protein 1; PGC-1α, peroxisome proliferator–activated receptor–γ coactivator-1α; ROS, reactive oxygen species; UCP2, uncoupling protein-2.
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Mitochondria and Endothelial Function
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