This Review is part of a thematic series on the **Role of Mitochondria in Cardiovascular Diseases**, which includes the following articles:

Mitochondrial Dysfunction in Atherosclerosis

Free Radicals, Mitochondria, and Oxidized Lipids: The Emerging Role in Signal Transduction in Vascular Cells

Defective Mitochondrial Biogenesis: A Hallmark of the High Cardiovascular Risk in Metabolic Syndrome?

Mitochondrial Biology and Vascular Biology

Role of Mitochondria in Insulin Resistance

Marshall S. Runge, Guest Editor

Mitochondrial Dysfunction in Atherosclerosis

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Abstract—Increased production of reactive oxygen species in mitochondria, accumulation of mitochondrial DNA damage, and progressive respiratory chain dysfunction are associated with atherosclerosis or cardiomyopathy in human investigations and animal models of oxidative stress. Moreover, major precursors of atherosclerosis—hypercholesterolemia, hyperglycemia, hypertriglyceridemia, and even the process of aging—all induce mitochondrial dysfunction. Chronic overproduction of mitochondrial reactive oxygen species leads to destruction of pancreatic β-cells, increased oxidation of low-density lipoprotein and dysfunction of endothelial cells—factors that promote atherosclerosis. An additional mechanism by which impaired mitochondrial integrity predisposes to clinical manifestations of vascular diseases relates to vascular cell growth. Mitochondrial function is required for normal vascular cell growth and function. Mitochondrial dysfunction can result in apoptosis, favoring plaque rupture. Subclinical episodes of plaque rupture accelerate the progression of hemodynamically significant atherosclerotic lesions. Flow-limiting plaque rupture can result in myocardial infarction, stroke, and ischemic/reperfusion damage. Much of what is known on reactive oxygen species generation and modulation comes from studies in cultured cells and animal models. In this review, we have focused on linking this large body of literature to the clinical syndromes that predispose humans to atherosclerosis and its complications. **(*Circ Res*. 2007;100:460-473.)**

Key Words: oxidative stress ■ DNA damage ■ obesity ■ diabetes ■ aging

Atherosclerotic vascular disease and its clinical sequelae are the leading causes of morbidity and mortality in the Western world. An elevated level of low-density lipoprotein (LDL) is associated with increased risk of coronary artery disease.[1,2] Oxidative modification of LDL, and its transport into the subendothelial space of the arterial wall at the sites of endothelial damage, is considered an initiating event for atherosclerosis.[3–6] Oxidative modification of LDL results from the interaction of reactive oxygen species (ROS) and reactive nitrogen species, produced from vascular wall cells and macrophages,[5] with LDL. The resulting increased oxidative and nitrosooxidative stress induces endothelial dysfunction by impairing the bioactivity of endothelial nitric oxide and promotes leukocyte adhesion, inflammation, thrombosis, smooth muscle cell proliferation—all processes that exacerbate atherosclerosis.

Of the many potential cellular sources of chronic ROS production, mitochondria and nonphagocytic NAD(P)H oxidase are the major sources under physiological conditions.[7,8] Increased mitochondrial ROS generation and dysfunction are associated with cardiovascular and many other diseases.[9–11] Aortic samples from atherosclerotic patients had greater mitochondrial DNA (mtDNA) damage than nonatherosclerotic aortic samples from age-matched transplant donors.[12]

Original received October 3, 2006; revision received November 29, 2006; accepted January 8, 2007.

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Circulation Research **is available at http://circres.ahajournals.org** **DOI: 10.1161/01.RES.0000258450.44413.96**

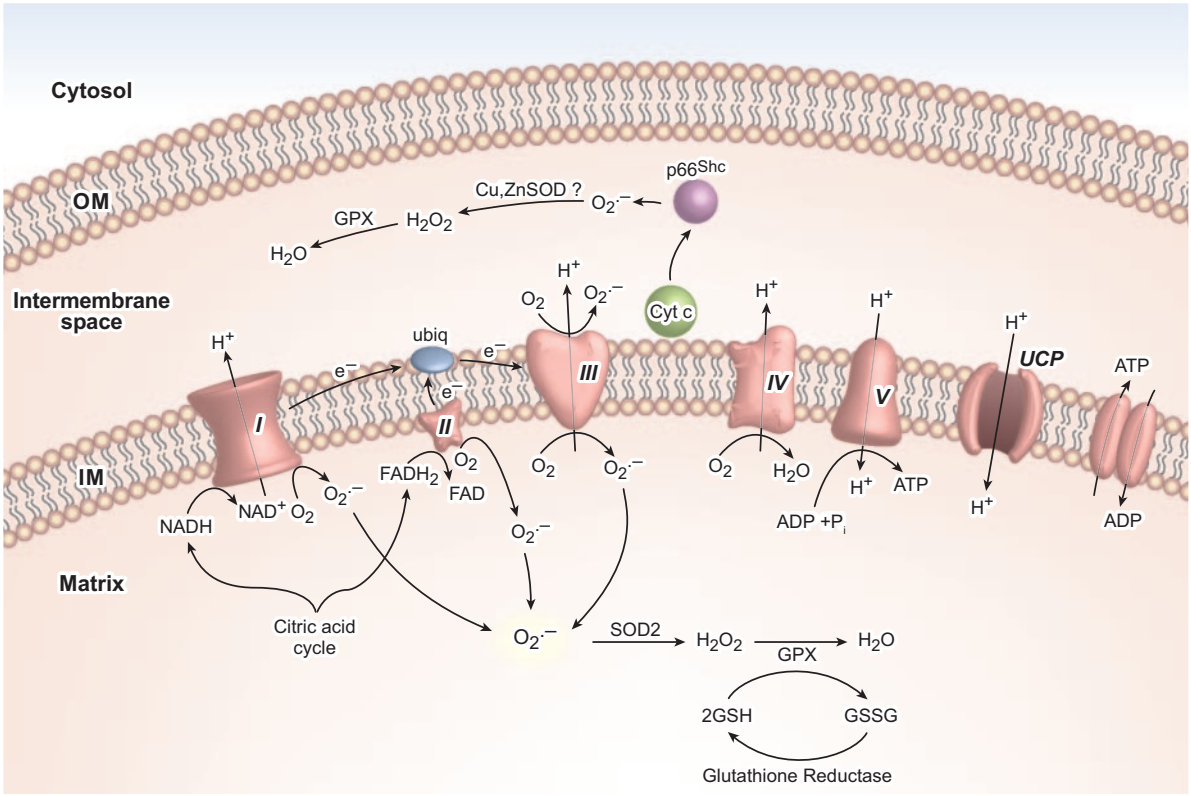

Figure 1. Oxidative phosphorylation, superoxide production, and scavenging pathways in mitochondria. Electrons (e^-) from NADH and $FADH_2$ pass through complex I and complex II, respectively, and then to complex III via ubiquinol. Cytochrome *c* transfers electrons from complex III to complex IV, which reduces O_2 to form H_2O. Flow of electrons is accompanied by proton (H^+) transfer across the inner mitochondrial membrane at complexes I, III, and IV, creating an electrochemical gradient, $\Delta\psi$. Protons reenter the mitochondrial matrix through complex V, which uses the proton-motive force to generate ATP. The proton-motive force also drives ATP–ADP exchange by ANT. UCPs allow protons to return to the matrix, reducing ROS formation. Complex I leaks electrons to generate $O_2^{\cdot-}$ toward the matrix, whereas complex III generates $O_2^{\cdot-}$ toward both matrix and intermembrane space. $P66^{Shc}$ in the intermembrane space subtracts electrons from cytochrome c to produce $O_2^{\cdot-}$. Superoxide is dismutated to H_2O_2 by Cu, ZnSOD in intermembrane space and by SOD2 in the matrix. H_2O_2 is reduced to H_2O by glutathione peroxidase (GPX) using GSH, and the resultant oxidized glutathione (GSSG) is reduced back to GSH by glutathione reductase. P_i stands for inorganic phosphate.

MtDNA damage not only correlates with the extent of atherosclerotic lesions in apolipoprotein E (apoE) knockout mice but precedes atherogenesis in young apoE knockout mice. Mitochondrial dysfunction, resulting from manganese superoxide dismutase (SOD2) deficiency, increased mtDNA damage and accelerated atherosclerosis in apoE knockout mice, consistent with the notion that increased ROS production and DNA damage in mitochondria is an early event in the initiation of atherosclerosis.

Thus, mitochondrial dysfunction may play an important role in the initiation and development of atherosclerosis. In this review, we will discuss (1) mitochondrial oxidative dysfunction, ROS production, regulatory mechanisms and targets of ROS; (2) the mechanisms by which mitochondrial dysfunction and ROS production could lead to vascular dysfunction and atherosclerosis; and (3) the role of mitochondrial dysfunction in various atherosclerotic risk factors.

Oxidative Phosphorylation

ROS are produced by oxidative phosphorylation (OXPHOS) pathway involved in energy production in mitochondria. OXPHOS is composed of 5 multiple subunit complexes embedded in the inner mitochondrial membrane. Electrons are transferred from NADH to molecular oxygen through an electron transport chain (ETC) consisting of complexes I (NADH dehydrogenase), II (succinate·ubiquinone oxidoreductase), III (ubiquinol·cytochrome oxidoreductase), and IV (cytochrome *c* oxidase) (Figure 1). Electrons are donated to complex I from NADH or to complex II via succinate and passed on to ubiquinol via coenzyme Q and then ubisemiquinone. Ubiquinol donates electrons to complex III, which, in turn, transfers electrons to cytochrome *c*. From cytochrome *c*, electrons move to complex IV and in this process molecular oxygen is reduced to H_2O. The transfer of electrons through the ETC leads to pumping of protons across the mitochondrial inner membrane at complexes I, III, and IV, creating transmembrane electrochemical gradient, $\Delta\psi$. The proton-motive force, which drives the reentry of protons into the matrix, is used by complex V (ATP synthase) to condense ADP and inorganic phosphate to synthesize ATP. Matrix ATP is then exchanged for cytosolic ADP by the adenine nucleotide translocase (ANT).

It has been estimated that 0.2 to 2.0% of the molecular oxygen consumed by mitochondria is reduced by a single electron transfer from the ETC to form superoxide anion ($O_2^{\cdot-}$).[13,14] All the subunits of complex II are encoded by

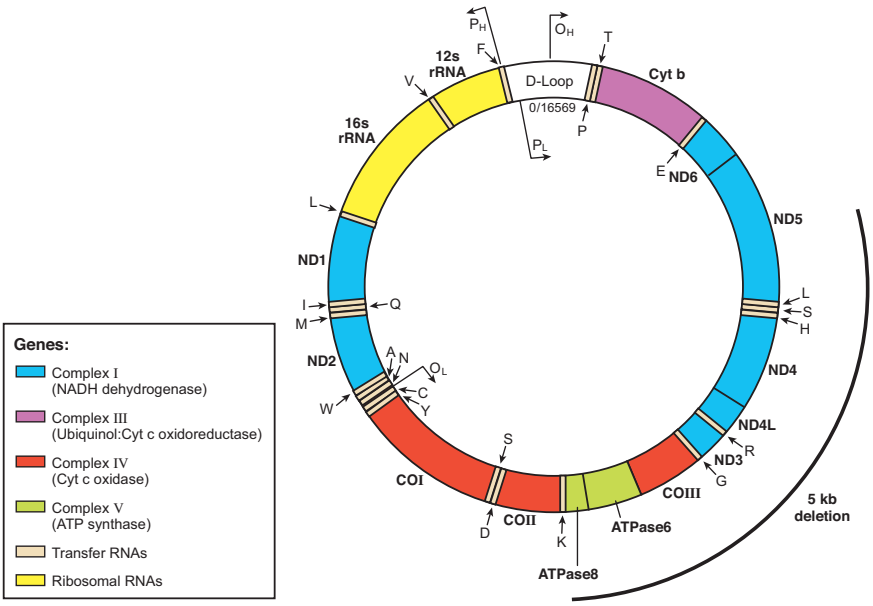

Figure 2. Human mitochondrial genome encodes 37 genes (16 569 bp; 13 polypeptides, 22 tRNAs, and 2 rRNAs). Each mitochondrion contains several copies of double-stranded, circular DNA bound to the inner membrane on the matrix side (D-loop region). Human mtDNA codes for 7 of the 43 subunits of complex I (NADH dehydrogenase), 1 of the 11 subunits of complex III (ubiquinol: cytochrome *c* oxidoreductase), 3 of the 13 subunits of complex IV (cytochrome *c* oxidase), and 2 of 16 subunits of complex V (ATP synthase). The tRNA genes are labeled by letters indicating the cognate amino acids. Mutations in complex I lead to higher ROS production. The common 5-kb deletion is increased several hundred fold in hearts from patients with coronary artery disease. A mutation substituting cytidine for uridine immediately 5′ to the mitochondrial $tRNA^{Ile}$ anticodon causes hypertension and hypercholesterolemia. Cyt b indicates cytochrome *b*.

nuclear genes, whereas the subunits of the other 4 complexes are encoded by both nuclear and mtDNA. The human mtDNA is a 16 569-bp circular, double-stranded molecule attached to the mitochondrial inner membrane. Most cells contain hundreds of mitochondria, and each mitochondrion contains 5 to 10 copies of mtDNA.[11] The mtDNA contains 13 genes coding for polypeptides essential for OXPHOS, 12S and 16S ribosomal RNA (rRNA) genes and 22 transfer RNA (tRNA) genes required for protein synthesis in mitochondria (Figure 2).

Because human mtDNA lacks protective histones and many of the repair mechanisms of the nuclear genome,[15–17] and is located proximal to ROS generation (mitochondrial inner membrane), it is vulnerable to damage by ROS. MtDNA mutations and/or mitochondrial dysfunction are associated with cardiovascular diseases. Atherosclerotic occlusion of coronary arteries and subsequent reperfusion is associated with significant increase in mtDNA damage and concomitant compensatory increase in the expression of OXPHOS genes.[18,19] In fact, hearts from patients with coronary artery disease had 8 to 2000 times more mtDNA deletions than their age-matched controls.[19] MtDNA damage, in turn, leads to increased ROS production and atherogenesis.[20] For example, mutations in complex I genes lead to increased mitochondrial ROS production.[21,22]

ROS Production in Mitochondria

In the mitochondrial electron transport chain, complex IV retains all partially reduced intermediates until full reduction of oxygen is achieved.[23] Other complexes may leak electrons to oxygen, partially reducing this molecule to O_2^-. Complex I and III are the primary source of O_2^- production in mitochondria[23–25] (Figure 1). Superoxide is released into the matrix from complex I, whereas it is released into both the matrix and intermembranous space by complex III.[26,27] ROS production depends on the metabolic state of mitochondria. For example, O_2^- production is greater during state IV (low electron flow and ATP synthesis, low ADP levels, high $NADH/NAD^+$ ratio, and low oxygen consumption) respiration than in state III respiration (high electron flow, fast ATP synthesis, partial depolarization, and a decreased $NADH/NAD^+$ ratio).[28] In the absence of ADP, electrons derived from succinate ($FADH_2$-linked complex II substrate) can reversely flow to complex I generating increased O_2^- production, and, for this reason, complex I is considered the major physiologically and pathologically relevant ROS-generating site in mitochondria.[26,29]

Superoxide anions in the mitochondrial matrix are quickly dismutated to hydrogen peroxide (H_2O_2) by SOD2 (MnSOD), whereas those in the intermembranous space are converted by SOD1 (Cu, ZnSOD)[30] (Figure 1). Hydrogen peroxide can be reduced to highly reactive hydroxyl radical in the presence of reduced transition metals.[31] In mitochondria, H_2O_2 is reduced to water by the enzymes glutathione peroxidase or catalase.[13] Glutathione peroxidase catalyzes the 2-electron reduction of H_2O_2 using reduced glutathione (GSH) as the hydrogen donor. GSH, a tripeptide consisting of glutamate, cysteine, and glycine, is synthesized in cytosol and transported into mitochondria. The process of reducing H_2O_2, to produce oxidized glutathione, results in oxidation of GSH. Oxidized glutathione is reduced to yield GSH by the enzyme glutathione reductase using NADPH as the substrate. However, with the exception of mitochondria from the heart, catalase is not present in mitochondria from other tissues.[32]

Nitric oxide (NO) is produced in mitochondria[33,34] and is an important modulator of O_2^- production, as the ETC contains several $NO^\bullet$ reactive-redox metal centers.[35] At physiological concentrations, $NO^\bullet$ modulates mitochondrial oxygen consumption by inhibiting cytochrome *c* oxidase in a reversible process.[36,37] Also, $NO^\bullet$ undergoes radical–radical reaction with O_2^- at near diffusion-limited rates forming peroxynitrite ($ONOO^-$), an oxidant capable of irreversible nitration of proteins, inactivation of enzymes, DNA damage, and disruption of mitochondrial integrity.[38–42]

A recent report suggested the involvement of $p66^{Shc}$ in mitochondrial ROS production.[43] This protein forms a mo-

lecular complex with cytochrome *c*, subtracting electrons to catalyze the partial reduction of oxygen to form O_2^- (Figure 1). $P66^{Shc}$, partially localized in the intermitochondrial membrane space, is a down stream target of p53 and is indispensable for increase in ROS production, cytochrome *c* release, dissipation of mitochondrial transmembrane potential and apoptosis.[44,45] However, $p66^{Shc}$ does not affect mitochondrial transmembrane potential under steady-state conditions, indicating the existence of 2 distinct functional states: an inactive basal state and an active proapoptotic state. In support of this hypothesis, it has been demonstrated that mitochondrial $p66^{Shc}$ exists as a high-molecular-weight complex that includes mitochondrial heat shock protein (mtHSP) 70, and, following proapoptotic signals, $p66^{Shc}$–mtHSP70 complex is destabilized, releasing monomeric $p66^{Shc}$ to interact with cytochrome *c*.[44] The importance of $p66^{Shc}$ in regulating oxidative stress burden is underlined by the observation that $p66^{Shc-/-}$ mice have increased resistance to paraquat and a 30% increase in life span.[46] Furthermore, $p66^{Shc-/-}$ cells have decreased basal and stress-induced ROS levels,[45,47] and this was attributed to reduced mitochondrial oxidative phosphorylation.[48] That the expression of $p66^{Shc}$ might be relevant in cardiovascular function is evident from the observations that $p66^{Shc-/-}$ mice are protected against ROS-dependent, age-related endothelial dysfunction,[49] high-fat–induced atherosclerosis,[50] as well as susceptibility to hindlimb ischemia.[51]

ROS produced initially in mitochondria or by enzyme sources such as NAD(P)H oxidase in the cell act in a positive feedback, leading to more ROS production from mitochondria in a process termed ROS-induced ROS release.[52,53] In cardiac myocytes, photodynamically triggered ROS production in mitochondria led to subsequent increased mitochondrial ROS generation through induction of mitochondrial permeability transition.[52] Support for ROS-induced ROS release was provided by the observation that angiotensin II–induced cardioprotection against ischemic/reperfusion injury in the rat myocardium is mediated by increased mitochondrial ROS production.[54] The protective effect of angiotensin II was eliminated by pretreatment with 5-hydroxydecanoate (an inhibitor of mitochondrial ATP-sensitive potassium channels) and apocynin (an NAD[P]H oxidase inhibitor), and, also, 5-hydroxydecanoate inhibited angiotensin II–induced ROS formation.

Mitochondria Are Targets of ROS

In addition to being a major site of ROS production, mitochondria are compromised by severe and/or prolonged oxidative stress.[55,56] Oxidative modifications of mitochondrial proteins, lipids, and mtDNA result in loss of function. Mitochondrial enzymes or enzyme complexes that are sensitive to inhibition by ROS include aconitase, α-ketoglutarate dehydrogenase, pyruvate dehydrogenase, and complexes I, II, and III.[57] Oxidative inactivation of mitochondrial DNA polymerase γ could slow mtDNA replication and eventually lead to inhibition of oxidative phosphorylation.[58] Oxidative inactivation of ANT would diminish oxidative phosphorylation and the supply of ATP to the detriment of all energy-dependent processes in the cell.[59] Similarly, nitration of active-site Tyr34 in SOD2 results in the inactivation of SOD2,[60] and, consistent with this, increase in 3-nitrotyrosine adduct levels of this enzyme were correlated with decreased activity in vivo.[61]

Cardiolipin, a phospholipid present almost exclusively within the mitochondrial inner membrane, is an early target of ROS either because of its high content of unsaturated fatty acids or proximity to ETC.[62] This phospholipid plays an important role in mitochondrial bioenergetics, as it optimizes the activity of some ETC complexes and ANT by binding to cytochrome *c*.[63,64] ROS-induced cardiolipin oxidation impairs complex I activity[62] and induces cytochrome *c* release.[65] Thus, oxidative modification of proteins and lipids along with ROS-induced mtDNA damage can alter the cellular energetics impacting the pathophysiology.

Regulation of ROS Production in Mitochondria

ROS generation in mitochondria is regulated by a number of factors, such as oxygen concentration, efficiency of ETC, availability of electron donors including NADH and $FADH_2$, and activity of uncoupling proteins (UCPs) and cytokines.[23,56,66] Mitochondrial O_2^- production increases linearly with increase in oxygen concentration.[67] By this equation, formation of ROS should decrease with hypoxia. Yet, a paradoxical increase in mitochondrial ROS generation was reported under moderately hypoxic conditions.[68,69] Under hypoxic conditions, $NO^\bullet$ produced at low concentration may bind and inhibit cytochrome *c* oxidase, which results in the reduction of upstream electron transport complexes and formation of O_2^- at low oxygen concentrations.[23,70,71] Peroxynitrite can induce O_2^- formation by inhibiting respiratory complexes I and II, enhancing the build-up of semiubiquinone and culminating in apoptosis via the activation of mitochondrial permeability transition.[72] Ramachandran et al demonstrated that chronic exposure to high levels of $NO^\bullet$ decreases activity and protein levels of respiratory complexes I, II, and IV, all of which were accompanied by an increase in cellular *S*-nitrosothiol levels, modification of cysteine residues, and an increase in the labile iron pool.[73] Taken together, the data that oxygen and $NO^\bullet$ are important regulators of mitochondrial respiration and ROS formation are compelling.

UCPs are another set of important physiological regulators of mitochondrial ROS production.[74,75] Activation of these inner mitochondrial membrane anion transporters allows protons to leak back into the mitochondrial matrix, decreasing the mitochondrial membrane potential and ROS generation.[76,77] The regulatory role of UCPs in atherogenesis is inferred from the observation that transplantation of bone marrow from UCP-2–deficient mice to LDL receptor–deficient mice markedly increased atherosclerotic lesion size and increased nitrotyrosine staining in plaques.[78] Consistent with this observation, UCP-2 overexpression inhibited ROS production and apoptosis induced by linoleic acid and lysophosphatidylcholine.[79] Conversely, increased O_2^- production, hypertension, and dietary arthrosclerosis were reported with inducible expression of UCP-1 in aortic smooth muscle cells,[80] indicating tissue-specific function of different UCPs. Superoxide, in turn, activates UCPs, lowering proton motive

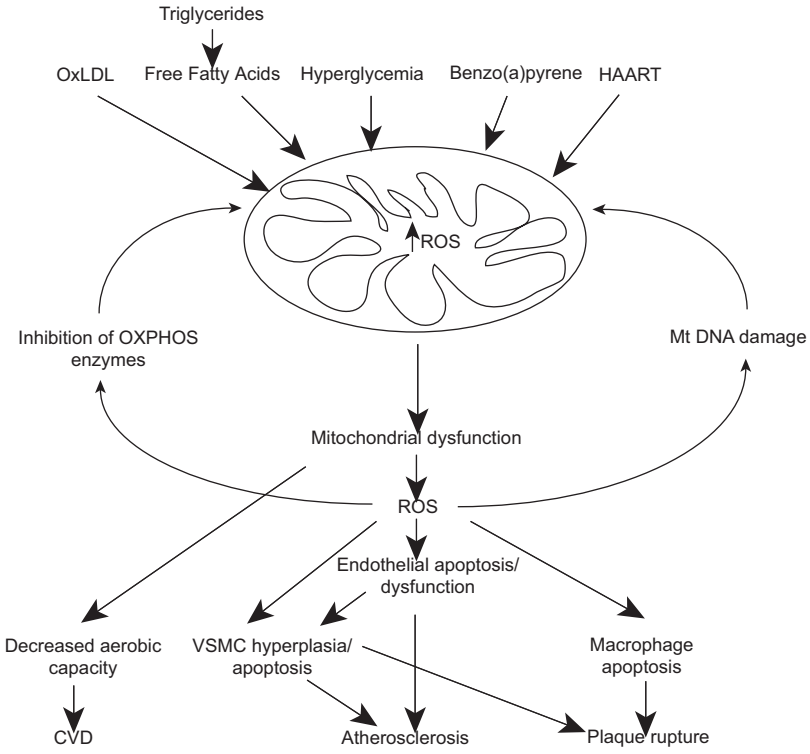

Figure 3. Atherogenic mechanisms of mitochondrial dysfunction. Various agents associated with vascular pathophysiology induce mitochondrial dysfunction and increase ROS production. Mitochondrial dysfunction leads to decreased aerobic capacity, a strong predictor of mortality. Increased mitochondrial ROS production causes endothelial dysfunction/apoptosis and VSMC proliferation/apoptosis, leading to the development of atherosclerosis. Apoptosis of VSMCs and macrophages caused by enhanced ROS production affects atherosclerotic lesion progression and may cause plaque rupture. The figure also depicts the interplay between mitochondrial dysfunction and ROS production. CVD indicates cardiovascular disease.

force and attenuating O_2^- production in a feedback regulation.[74,81]

Mitochondrial Dysfunction and Pathophysiological Mechanisms of Atherosclerosis

Low aerobic capacity is a strong predictor of mortality in subjects with or without cardiovascular diseases.[82–84] On a practical basis, this can be manifested as poor exercise tolerance and/or performance with exercise testing. Regardless of the presence or absence of known cardiac or pulmonary dysfunction, or other comorbidities, this simple finding correlates significantly with increased risk of death within 1 year. Expression of OXPHOS genes is coordinately decreased and is associated with low aerobic capacity (Figure 3).[85]

Increased production of ROS in mitochondria damages lipids, proteins, and mtDNA. Of these, it is likely that mtDNA is the most sensitive to physiologically relevant ROS-mediated damage. Preferential increase in mtDNA damage (compared with transcriptionally inactive nuclear β-globin gene), decrease in steady-state levels of mtDNA-encoded mRNA transcripts, mitochondrial protein synthesis, membrane potential, and total cellular ATP pools were observed in vascular smooth muscle cells (VSMCs) and endothelial cells exposed to ROS in cell cultures.[39] 4-Hydroxynonenal, an end product of membrane lipid peroxidation implicated in the pathogenesis of atherosclerosis, induces VSMC apoptosis through mitochondrial dysfunction and increased production of ROS.[86,87] In contrast, increased ROS production attributable to haploinsufficiency of SOD2 isoform reduced aconitase activity in both basal and agonist-stimulated conditions and increased VSMC proliferation.[88]

Mitochondrial dysfunction is also involved in the increased susceptibility to ischemic injury, certainly, in part, because of opening of the permeability transition pore (PTP)[89–91] (Figure 4). The molecular components of PTP include ANT in the inner mitochondrial membrane, voltage-dependent anion channel in the outer mitochondrial membrane, cyclophilin D in the matrix, and regulatory molecules such as benzodiazepine receptor, hexokinase, and creatine kinase.[92] Transient PTP opening causes depolarization of mitochondrial membrane potential, whereas longer opening leads to matrix swelling and outer mitochondrial membrane rupture. The latter causes the release of proapoptotic molecules within the intermembrane space, leading to cell death via caspase-dependent and caspase-independent mechanisms.[93] Consistent with this, mitochondrial depolarization has been implicated in hyperglycemia-induced apoptosis of human aortic endothelial cells (Figure 3).[94] Decrease in ANT activity associated with ischemia and inhibition of both ANT activity and oxidative phosphorylation evident during reperfusion may contribute to cardiac failure.[95] Chen et al reported that overexpression of SOD2 offered protection against ischemia/reperfusion injury,[96] whereas heterozygous deficiency of this enzyme impaired postischemic recovery of the myocardium[97] in mice. Together these data support the role of mitochondrial function in protection against ischemia/reperfusion injury.

Various pharmacological inhibitors of mitochondrial energy metabolism significantly increase mitochondrial ROS production and impair endothelium-dependent vascular relaxation.[98–100] Rotenone (which inhibits electron transport at flavin mononucleotide) abolished acetylcholine-induced, endothelium-dependent relaxation of rat and mouse carotid arteries[100] and rat

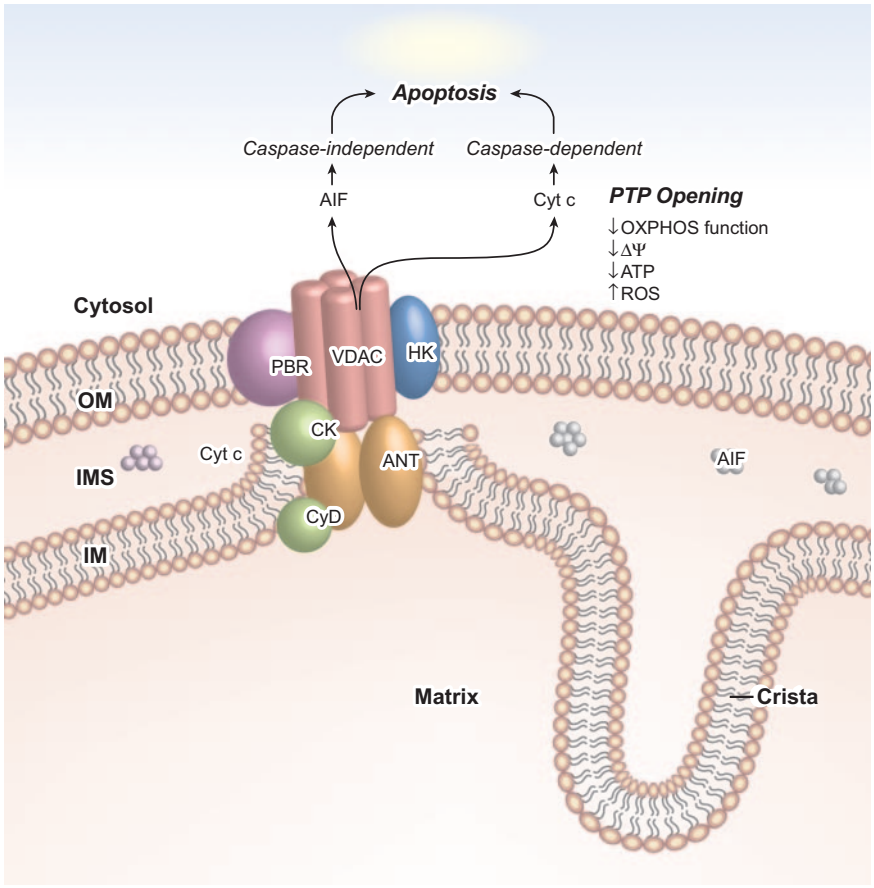

Figure 4. Mitochondrial PTP and ischemia/reperfusion injury. PTP is composed of voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane (OM), ANT in the inner mitochondrial membrane (IM), and cyclophilin D (CyD) in the matrix. Peripheral benzodiazepine receptor (PBR), hexokinase (HK), and creatine kinase (CK) are the other components of PTP. High membrane potential in normally functioning mitochondria keeps the PTP closed. Under pathophysiological conditions such as ischemia/reperfusion, the PTP opens, allowing the entry of H_2O and solutes. The opening of PTP causes mitochondrial swelling and release of apoptosis-initiating factor (AIF) and cytochrome *c* from the intermembrane space (IMS), which ultimately results in apoptosis. PTP opening and cytochrome *c* (Cyt c) release will also impair OXPHOS, lower transmembrane electrochemical gradient ($\Delta\psi$), and increase ROS production.

and rabbit aortas.[101–103] Similarly, antimycin A (which inhibits electron transport at cytochrome b–c_1) and oligomycin (which inhibits mitochondrial F_1–ATPase) inhibit the production of endothelial NO in rabbit aorta.[103] However, rotenone did not affect vascular relaxation induced by NO donors,[102] which suggests that intact mitochondrial function plays an important role in the production of NO in endothelial cells.

Together, these data illustrate that mitochondrial dysfunction impairs aerobic capacity and endothelial function/viability and induces VSMC proliferation or apoptosis, leading to the development of atherosclerosis (Figure 3).

Dyslipidemia

Apoptotic endothelial cells, VSMCs, T lymphocytes, and macrophages are present in atherosclerotic lesions,[104,105] and their number increases significantly with lesion progression, suggesting that apoptosis plays a critical role in plaque erosion and rupture.[106,107] Oxidized LDL (oxLDL) induces apoptosis of all cells involved in atherogenesis[108–110] (Figure 3), and mitochondrial-dependent pathways play a critical role in this process. OxLDL-induced apoptosis of human umbilical vein endothelial cells (HUVECs) is mediated by dysfunction of mitochondrial membrane potential and the release of cytochrome *c* into cytosol and suppression of apoptosis by cyclosporin A, an antiatherogenic agent, correlated with the prevention of mitochondrial dysfunction.[111] Recently, it was shown that oxLDL-induced vascular cell apoptosis involves 2 distinct calcium-dependent mitochondrial pathways.[112] The first is mediated by activation of the cysteine protease calpain; release of tBid, a truncated form of the proapoptotic Bcl-2 family member Bid; opening of the mitochondrial PTP; release of cytochrome *c*; and subsequent activation of caspase-3. The second pathway is mediated by the release of apoptosis-inducing factor, which is cyclosporine insensitive and caspase independent (Figure 4). Interestingly, it was also shown that mitochondrial-derived O_2^- is essential for oxidation of LDL in vitro.[113]

Macrophages in advanced atherosclerotic lesions accumulate excess free cholesterol, which is a potent inducer of their death.[114,115] Free-cholesterol loading of mouse peritoneal macrophages induced apoptosis by decreasing mitochondrial membrane potential, inducing cytochrome *c* release, activating caspase-9, and increasing the levels of the proapoptotic protein Bax.[116] OxLDL also induced lysis of human macrophages by promoting mitochondrial dysfunction and scavengers of peroxide radicals that restored mitochondrial membrane potential and prevented macrophage lysis.[117] Furthermore, increase in oxidative stress in mitochondria is evident from induction of transcription and expression of SOD2 in human macrophages incubated with oxLDL.[118] Consistent with this in vitro observation, SOD2 activity and GSH concentration were higher in atherosclerotic intima compared with the media of the aorta of heritable hyperlipidemic rabbits, but a significant inverse correlation of these 2 with lesion size was also observed. TUNEL-positive nuclei were present in the macrophages of these atherosclerotic aorta and exposure to oxLDL induced increased apoptosis in

human macrophages. Hypercholesterolemia significantly increased mtDNA damage and protein nitration of heart homogenates, indicating that atherosclerotic risk factors induce mitochondrial damage and dysfunction.[61] MtDNA copy number in leukocytes is redox sensitive[119] and low in hyperlipidemic patients.[120] Together, these data suggest that dyslipidemia-induced mitochondrial damage and dysfunction not only induce atherosclerotic lesion formation but also affect lesion composition/progression.

Hypertension

Hypertension is next in importance to dyslipidemia as a risk factor for atherosclerosis, and mitochondrial dysfunction has been implicated in increased arterial blood pressure. Mitochondrial energy deficiency and calcium overload play a role in the pathogenesis of arterial hypertension.[121] Similarly, decrease in mitochondrial energy metabolism and abnormality of calcium metabolism was reported in hypertrophied myocardium of spontaneously hypertensive rats.[122,123] Mitochondrial antioxidant system is also important in protection against hypertension because arterial blood pressure increased with aging or high-salt diet in SOD2-deficient mice.[124] Mild respiratory uncoupling in arterial smooth muscle cells increased oxidative stress, hypertension, and dietary atherosclerosis in mice.[80] Recently, it was shown that a mutation in mitochondrial tRNA results in hypertension, hypercholesterolemia, and hypomagnesemia.[125] Because cholesterol concentration and blood pressure increase at approximately 30 years of age in these patients, it is hypothesized that this gene mutation interacts with environmental or age-related decline in mitochondrial function in the development of hypertension and hypercholesterolemia.[126] Together, these findings indicate a potential role of mitochondrial dysfunction in hypertension.

Diabetes

Diabetes mellitus is a major risk factor for coronary artery disease morbidity and mortality,[127,128] and people with type 2 diabetes experience higher rates of ischemic events and death after a first myocardial infarction.[129,130] Worldwide, the incidence of diabetes mellitus continues to increase at rapid rates because of lifestyle and dietary changes. Understanding mechanisms by which diabetes mellitus contributes to atherosclerotic risk is, thus, of great importance.

Insulin resistance is a common occurrence in patients with type 2 diabetes and in subjects with impaired glucose tolerance.[131] Insulin resistance causes hyperglycemia as lack of insulin signaling decreases transport of glucose into muscle and fat, while increasing glucose production by the liver.[132,133] Hyperglycemia-induced increases in production of O_2^- by the mitochondrial ETC in endothelial cells has been implicated in glucose-mediated vascular damage.[134–136] Normalizing mitochondrial ROS levels by an inhibitor of electron transport complex II, by an uncoupler of oxidative phosphorylation, by overexpression of UCP-1 or SOD2 each prevented glucose-induced activation of protein kinase C (PKC), formation of advanced glycation end products (AGE), and activation of the polyol pathway, which results in sorbitol accumulation and nuclear factor κB activation—all of which have been implicated in hyperglycemia-induced vascular dysfunction, including atherosclerosis[134] (Figure 5). Activation of nuclear factor κB induces expression of vascular cell adhesion molecule-1 and monocyte chemoattractant protein-1 in aortic endothelial cells stimulating atherogenesis.[137]

Hyperglycemia-induced increase in mitochondrial O_2^- production also decreases glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity and increases hexosamine pathway activity in aortic endothelial cells.[138] Activation of the hexosamine pathway causes increased glycosylation and subsequent transactivation of transcription factor Sp1, resulting in increased expression of Sp1-dependent genes such as transforming growth factor-β_1 and plasminogen activator ihibitor-1 (Figure 5). Elevated plasma levels of plasminogen activator ihibitor-1 are strongly associated with increased risk of ischemic heart disease,[139,140] whereas transforming growth factor-β_1 plays a key role in early atherosclerosis and restenosis.[141] Recently it was shown that the activation of the major pathways of hyperglycemic damage in endothelial cells induced by enhanced mitochondrial O_2^- production is mediated via inhibition of glycolytic enzyme, GAPDH.[136] The GAPDH inhibition is caused by poly(ADP-ribosyl)ation by poly(ADP ribose) polymerase, which is activated by nuclear DNA strand breaks produced by mitochondrial O_2^- overproduction. Inhibition of GAPDH increases the entry of upstream glycolytic metabolites into pathways of glucose overuse, including increased flux through AGE and PKC glucotoxic pathways.[135,142]

Central obesity is associated with insulin resistance and is a risk factor for type 2 diabetes and atherosclerosis.[143] Central obesity is characterized by increased cytosolic triglyceride levels in adipose and nonadipose tissues.[144,145] In tissues, triglycerides are the source of long-chain acyl–coenzyme A esters (LCACs), the metabolically active form of fatty acids. In central obesity, LCAC levels are increased because of a steady-state equilibrium with triglycerides.[146] Impairment of glucose utilization by LCACs induces insulin resistance in skeletal muscles.[144] High concentrations of LCACs decrease intramitochondrial ADP concentration, perhaps by inhibiting ANT,[147] leading to increased ROS production.[148,149] Incidentally, long-chain fatty acids not only induce hyperplasia in normal islet β cells but also lower the threshold for glucose-induced insulin secretion.[146,150] Thus, insulin resistance may first lead to hyperinsulinemia.[148] However, increased levels of free fatty acids (FFAs) will cause progressive deterioration of β-cell function/apoptosis, resulting in insulin deficiency. This progression from initial hyperinsulinemia to insulin deficiency is characteristic of the development of type 2 diabetes.[150–152]

Incubation of endothelial cells with high concentrations of FFAs, similar to those found in insulin-resistant subjects, has been shown to increase O_2^- production several fold.[153] The increase in ROS production was inhibited by overexpression of either UCP-1 or SOD2, which indicates that mitochondrial ETC is the source of FFA-induced ROS production. Mitochondrial function is also necessary for the progressive oxidation of LDL in endothelial cultures.[113] In addition, small dense LDL particles associated with central obesity are more prone to oxidation.[143,155] Consistent with this, central obesity

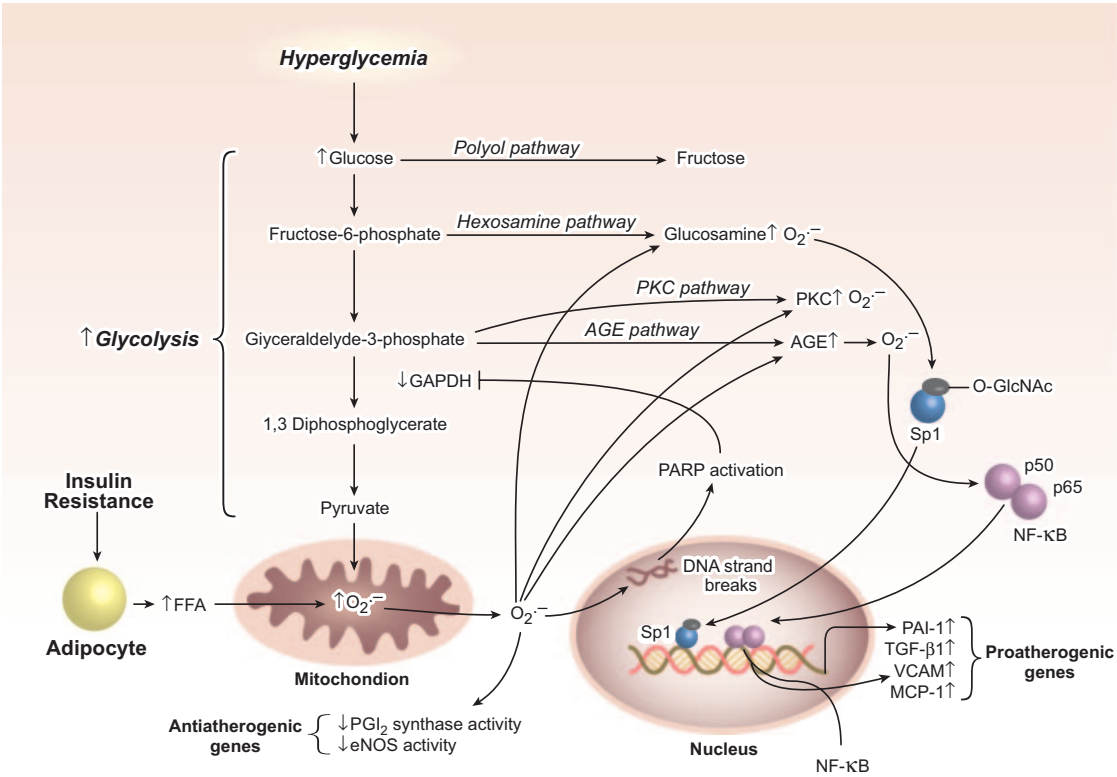

Figure 5. Insulin resistance and hyperglycemia-induced increased mitochondrial superoxide production activates atherogenic signaling pathways. Overproduction of mitochondrial O_2^-, caused by high glucose flux through endothelial cells, results in DNA strand breaks and activation of poly(ADP ribose) polymerase (PARP). Poly(ADP ribose) polymerase ribosylates and inactivates glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The upstream metabolites of disrupted glycolytic pathway are processed through polyol pathway, hexosamine pathway, PKC pathway, or AGE pathway. Glycosylation of transcription factor Sp1, by the addition of N-acetylglucosamine (GlcNAc), causes transactivation of proatherogenic genes such as plasminogen activator inhibitor-1 (PAI-1) and transforming growth factor β_1 (TGF-β1). Activation of transcription factor nuclear factor κB (NF-κB), downstream of AGE pathway, induces transactivation of vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1). Increased flux of FFAs, released from insulin-resistant adipocytes through arterial endothelial cells, also activates hexosamine, PKC, and AGE pathways. In addition, FFA-induced excess mitochondrial O_2^- production inactivates antiatherogenic enzymes such as prostacyclin (PGI_2) synthase and eNOS.

and enhanced FFA levels are strongly correlated with clinical, as well as subclinical, coronary heart disease and atherosclerosis in renal transplant recipients.[156,157] Together, these data indicate that prolonged enhanced mitochondrial ROS production leads to destruction of β-cells, dysfunction of endothelial cells, and increased subendothelial LDL oxidation—all factors that promote atherosclerosis. Atherosclerotic lesions in brain microvessels from Alzheimer's patients and a mouse Alzheimer's model have increased mtDNA deletions and mitochondrial abnormalities, demonstrating that mitochondria in the vascular wall are central targets of oxidative stress–induced damage.[158]

Insulin resistance increases plasma FFA levels because of enhanced lipolytic activity of adipocytes.[159] Increased oxidation of FFAs by aortic endothelial cells would lead to accelerated production of O_2^- by ETC, leading to activation of hexosamine, PKC, and AGE pathways, resulting in the activation of proatherogenic inflammatory pathways[135,153,160] and inactivation of the antiatherogenic enzymes prostacyclin synthase[153,161,162] and endothelial NOS (eNOS)[153,163] (Figure 5). The relevance of these 2 enzymes in atherogenesis is evident from gene knockout models: both apoE$^{-/-}$/prostacyclin receptor$^{-/-}$[164] and apoE$^{-/-}$/eNOS$^{-/-}$[163] mice showed accelerated atherosclerosis compared with apoE$^{-/-}$ mice. Inactivation of prostacyclin synthase and eNOS was prevented by either blocking FFA release from adipocyte tissue or inhibition of mitochondrial fatty acid oxidation and by reduction of O_2^- levels, which supports the role of enhanced mitochondrial metabolism in accelerated atherosclerosis in people with insulin resistance.[153]

Aging

The incidence and prevalence of coronary heart disease and other vascular diseases increases with age, and adults >65 years of age are 4 times more likely to experience coronary heart disease than are those between 40 to 49 years of age.[165] A large body of evidence supports the hypothesis that impaired mitochondrial oxidation is a major contributor to aging.[55,166–168] Several human and animal studies have demonstrated an age-related decline of mitochondrial respiratory function and ATP synthase activity.[169–172] The mitochondrial theory of aging[173] postulates that ROS production, mtDNA damage, and respiratory chain dysfunction are linked to each other to create a "vicious cycle" that leads to progressive decline in mitochondrial function and impairment of cell viability. It is also true that oxidative mtDNA mutations accumulate with age in human and animal organs[167,174] and

that oxidative mtDNA damage is inversely correlated to maximal life span.[175]

Further support for mitochondrial dysfunction in aging is obtained using genetically engineered mice containing a point mutation in mtDNA polymerase-γ (POLG).[176,177] The mutant POLG has normal DNA polymerase activity but lacks 3′→5′ exonucleolytic proofreading activity, and homozygous POLG mutant mice show an increased load (3- to 8-fold) of somatic mtDNA point mutations that accumulate with increasing age. Consistent with this increase in mtDNA mutations, the mutant mice showed decline in respiratory chain enzyme activities and the production of mitochondrial ATP.[176] The mutant mice were found to have reduced life spans and to show symptoms of accelerated aging such as weight loss, hair loss, curvature of the spine, and heart enlargement. However, no increase in ROS levels was observed in these mice, arguing against a direct role of mitochondrial oxidative stress in aging. Instead, it has been suggested that respiratory chain dysfunction per se[178] or increased apoptosis[177] caused by accumulation of mtDNA mutations may play a central role in aging.

One of the several explanations that could account for the lack of increased ROS production in POLG mutant mice is that mutations in POLG may be downstream from mechanisms that generate ROS and the damage to POLG that renders the enzyme error prone might be the result of protein damage by ROS.[179] Consistent with this hypothesis, it was shown that exogenous addition of ROS significantly inhibits the activity of POLG.[58] It was also not clear what effect the respiratory chain dysfunction in these mice had on the vascular aging because pharmacological inhibition of mitochondrial energy metabolism could contribute to endothelial dysfunction and increase susceptibility to atherosclerosis.[100] In contrast to POLG mutant mice, overexpression of human catalase in mitochondria of mice delayed cardiac pathology, reduced oxidative damage and mtDNA deletions and increased median and maximal life span by ≈20%.[181] At present, increases in the incidence of atherosclerosis and mitochondrial damage and dysfunction with increasing age are merely correlative. Detailed cardiovascular phenotyping of the above mice and other accelerated aging mutants to be developed in the future will help ascertain the causal relationship between mitochondrial dysfunction and aging-associated atherosclerosis.

Cigarette Smoking

Cigarette smoking and secondhand smoke significantly increase the risk of early atherosclerosis, the risk burden being cumulative and exceeding the active smoking period.[182,183] Atherogenic effects of cigarette smoking include endothelial injury, platelet activation, oxidation of LDL, and oxidative DNA damage.[183,184] Consistent with the cumulative and residual risk of cigarette smoking on atherosclerosis, a significant increase in mtDNA content was reported in the saliva of smokers and former smokers compared with never smokers[185] and increase in mtDNA content is accompanied by mtDNA deletions, evidence of oxidative damage, decreased transcription of mitochondrial-specific proteins, and apoptosis.[186–188] Significant decrease in complex IV activity of mitochondrial respiratory chain and increase in lipid peroxidation of lymphocyte membranes were observed in circulating lymphocytes from smokers compared with nonsmokers.[189]

Further evidence that cigarette smoke–induced mitochondrial dysfunction is important in the initiation and progression of atherosclerotic lesions was obtained from a number of cell culture and animal studies. Exposure to tobacco smoke filtrate caused loss of mitochondrial membrane potential, apoptosis, or necrosis in human monocytes and endothelial cells.[190] VSMCs isolated from rats treated with benzo-(a)pyrene, a prooxidant in the cigarette smoke, exhibit proliferative phenotype associated with experimentally induced atherogenesis and upregulation of mtDNA transcripts.[191] Rats exposed to low concentrations of passive smoke exhibited impaired mitochondrial oxidative function and increased sensitivity of hearts to ischemia/reperfusion injury.[192] Similarly, exposure to passive cigarette smoke impaired oxidative phosphorylation, diminished cytochrome oxidase activity, increased mitochondrial F_1–ATPase protein levels, and decreased coenzyme Q levels in rabbit cardiomyocytes.[193] Acute tobacco smoke exposure, as might occur in social settings, increases the susceptibility of rat cardiac mitochondria to calcium and promotes mitochondrial permeability transition.[194] Second-hand smoke significantly increased aortic mtDNA damage, decreased ANT activity, and increased nitration and inactivity of SOD2 in mice.[61] Exposure to second-hand smoke in the background of hypercholesterolemia increased atherogenesis and synergistically enhanced mitochondrial damage. Furthermore, prenatal exposure to environmental tobacco smoke significantly enhanced mtDNA damage and atherosclerotic lesion development in adult male apoE$^{-/-}$ mice.[195] Together, these data indicate that cigarette smoking enhances atherogenesis by affecting mitochondrial function, and this effect could be synergistic in the backdrop of other atherosclerotic risk factors.

HIV and Atherosclerosis

Highly active antiretroviral therapy (HAART), which involves a combination of antiretroviral drugs (ie, protease inhibitors [PIs], nucleoside reverse transcriptase inhibitors [NRTIs], nonnucleoside reverse transcriptase inhibitors [NNRTIs], nucleotide reverse transcriptase inhibitors [NtRTIs]) has substantially reduced morbidity and mortality in patients with human immunodeficiency virus type-1 (HIV-1) infection. With the increase in the survival of HIV patients receiving these medications, it is now clear that there is a relationship between either HAART or HIV-1, or both, and accelerated atherosclerotic cardiovascular disease.[196–200] Because mitochondrial toxicity is a common adverse effect of HAART, a commonly accepted theory is that the accelerated atherosclerosis in HAART-treated HIV-1–infected patients is attributable to mitochondrial dysfunction. Phosphorylated NRTIs compete with endogenous deoxyribonucleotides for incorporation into nascent mtDNA chains and ultimately inhibit mtDNA POLG, resulting in mtDNA depletion, altered OXPHOS enzyme activities, and mitochondrial ultrastructural changes.[201–203] Exposure to NRTIs impaired endothelium-dependent vasorelaxation and increased endothelial O_2^-

production in mice.[204] PI therapy can alter lipid metabolism in HIV patients, by either increasing lipid release or inhibiting chylomicron uptake, and the resulting hyperlipidemia can, in turn, affect mitochondrial function and accelerate atherosclerosis.[205] In fact, changes in atherogenic lipid proteins and endothelial dysfunction have been associated with PI therapy in HIV patients.[206] In addition, near-clinical plasma levels of the PI ritonavir caused endothelial mtDNA damage and cell death.[207] The above data indicate that mitochondrial dysfunction is a contributing factor for HIV-associated atherosclerosis.

Conclusions

In conclusion, growing evidence supports the notion that oxidative damage to mitochondrial proteins leads to progressive dysfunction and that dysregulated mitochondrial function is the major unifying mechanism of several risk factors associated with atherosclerosis. Mitochondria play a critical role in atherogenesis by affecting endothelial function, VSMC proliferation, or apoptosis. However, the question of whether abnormalities in mitochondrial function are the cause or response to atherosclerosis and other cardiovascular dysfunctions is far from resolved. A better understanding of the redox-sensitive mitochondrial signal-transduction pathways, availability of pharmacological agents that can manipulate the production and scavenging of mitochondrial ROS and animal models that address the stability of mtDNA, and regenerative therapies that can rescue aerobic respiration in vascular cells with impaired mitochondrial function or enhance myocardial activity could help resolve this issue.

Sources of Funding

Supported by NIH grant HL57352 and NIH/NIA grant P01 AG024282.

Disclosures

None.

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