Review

The emerging role of cardiovascular risk factor-induced mitochondrial dysfunction in atherogenesis

Paolo Puddu, Giovanni M Puddu, Eleonora Cravero, Susanna De Pascalis and Antonio Muscari*

Address: Department of Internal Medicine, Aging and Nephrological Diseases, University of Bologna and S. Orsola-Malpighi Hospital, Bologna, Italy

Email: Paolo Puddu - papuddu@libero.it; Giovanni M Puddu - giovapu@libero.it; Eleonora Cravero - giovapu@libero.it; Susanna De Pascalis - susanna_depascalis@hotmail.com; Antonio Muscari* - antonio.muscari@fastwebnet.it

* Corresponding author

Abstract

An important role in atherogenesis is played by oxidative stress, which may be induced by common risk factors. Mitochondria are both sources and targets of reactive oxygen species, and there is growing evidence that mitochondrial dysfunction may be a relevant intermediate mechanism by which cardiovascular risk factors lead to the formation of vascular lesions. Mitochondrial DNA is probably the most sensitive cellular target of reactive oxygen species. Damage to mitochondrial DNA correlates with the extent of atherosclerosis. Several cardiovascular risk factors are demonstrated causes of mitochondrial damage. Oxidized low density lipoprotein and hyperglycemia may induce the production of reactive oxygen species in mitochondria of macrophages and endothelial cells. Conversely, reactive oxygen species may favor the development of type 2 diabetes mellitus, mainly through the induction of insulin resistance. Similarly - in addition to being a cause of endothelial dysfunction, reactive oxygen species and subsequent mitochondrial dysfunction - hypertension may develop in the presence of mitochondrial DNA mutations. Finally, other risk factors, such as aging, hyperhomocysteinemia and cigarette smoking, are also associated with mitochondrial damage and an increased production of free radicals. So far clinical studies have been unable to demonstrate that antioxidants have any effect on human atherogenesis. Mitochondrial targeted antioxidants might provide more significant results.

Introduction

There is a wide consensus that atherosclerosis (ATS) is an inflammatory disease associated with lipid and protein oxidation in the vascular wall [1-5]. At sites of inflammation, the local cellular environment is enriched with cytokines, chemoactractant chemokines and reactive oxygen species (ROS), such as superoxide anion, mainly produced by the activated leukocytes adhering to the endothelium. Excess ROS and reactive nitrogen species (RNS) generation has been associated with vascular lesion formation and functional defects [6-8]. ROS and RNS free radicals are molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals. The unpaired electrons give the radicals a high degree of reactivity.

ROS, as well as RNS, are products of normal cellular metabolism. When there is an overproduction of ROS/

NSC   The cost of publication in *Journal of Biomedical Science* is bourne by the National Science Council, Taiwan.

Page 1 of 9
(page number not for citation purposes)

RNS or a deficiency of enzymatic or non-enzymatic anti-oxidants, a biological damage to cellular lipids (lipoperoxidation), proteins, glucides and DNA may occur. Moreover, nitric oxide (NO) levels are reduced, due to both decreased production and increased consumption, with possible endothelial dysfunction and vascular impairment [9]. These harmful effects are termed oxidative and nitrosative stress [10].

The toxic effects of free radicals on biomolecules lead to the accumulation of damage in various cellular locations and to the deregulation of redox-sensitive metabolic and signaling pathways, and are also believed to be involved in pathological conditions such as ATS, hypertension, inflammation, diabetes, cancer and other human pathologies. There is evidence that common risk factors for coronary artery disease are associated with increased levels of ROS [11-16].

Recent studies have focused on the role that mitochondria could play in atherogenesis. In fact, mitochondria are both important sources and targets of ROS [14,15]. The mitochondrial dysfunction theory postulates that excess release of ROS and RNS from mitochondria can contribute to the inflammatory vascular reaction leading to the development of atherosclerotic lesions [17,18]. In fact, increased mitochondrial ROS production causes endothelial dysfunction, vascular smooth muscle cell (VSMC) proliferation and apoptosis of VSMCs and macrophages, with ensuing ATS lesion progression and possible plaque rupture [18].

Common cardiovascular risk factors could be involved in this process by adversely affecting the function of endothelial mitochondria, and growing evidence supports the hypothesis that mitochondrial dysfunction may be the most important unifying mechanism explaining the atherogenic action of major cardiovascular risk factors [17-19]. This review will discuss the molecular mechanisms by which atherosclerotic risk factors could lead to mitochondrial dysfunction and subsequent vascular impairment.

ROS and RNS production in mitochondria

ROS include free radicals (mainly superoxide anion and hydroxyl) and normal molecules (such as hydrogen peroxide [H_2O_2] and ozone), some of which can be interconverted enzymatically [20]. For example, superoxide is converted to H_2O_2 by a family of metallo enzymes such as manganese superoxide dismutase (Mn SOD) or copper/zinc superoxide dismutase (Cu/Zn SOD) [21,22]. In turn, in the presence of reduced transition metals, H_2O_2 is mostly transformed in water by glutathione peroxidase or peroxidredoxin III (PRX III) [23].

Superoxide anion is considered the 'primary' ROS. It may be formed in the cytosol by reduction of molecular oxygen by the NADPH oxidase and xanthine oxidase. Other cytosolic or membrane-bound sources of ROS are the uncoupled endothelial nitric oxide (NO) synthase (eNOS) and the arachidonate metabolizing enzymes lipoxygenases and cycloxygenases. However, the mitochondrial respiratory chain is the major source of ROS in most mammalian cells [24-26].

Superoxide anion production can occur at complex I and III in mitochondria [27,28]. Several factors can regulate mitochondrial ROS generation. Zhang and Gutterman [24] have recently reviewed the main molecular pathways of ROS production, focusing on the effects of mitochondrial membrane potential, intracellular Ca2+, electrophilic lipids, and NO.

Nitrosative stress occurs when the generation of RNS in a biological system exceeds its ability to neutralize them [29,30]. Nitric oxide (NO) is a reactive radical acting as an important signaling molecule in several physiological processes. NO and superoxide can react together to produce peroxynitrite anion, which is a potent oxidizing agent capable of causing oxidative damage to biomolecules with subsequent inhibition of their biological function [10].

Mitochondrial ROS and RNS, as well as their metabolic products such as oxidized lipids, can also play a role in signal transduction through specific modifications of cell signaling proteins [31-33]. ROS generation in mitochondria is influenced by multiple factors, including the efficiency of the electron transport chain, oxygen concentration, the availability of electron donors such as NADH and FADH2, the activity of UCPs and cytokines, the activity of antioxidant defenses and the modulation of nuclear factors [17,34,35]. In this respect, a novel area of medical research is now developing.

The role of uncoupling proteins (UCPs) in regulating ROS production

The mitochondrial respiratory chain requires the expression of gene products encoded by both the nuclear and mitochondrial genome [36]. Human mitochondrial genome consists of 37 genes coding for 13 proteins that function as subunits for the respiratory complexes I, III, IV, and V, whereas the genes coding for complex II are entirely nuclear. Nuclear genes play a major role in the biosynthesis of the respiratory chain and the expression of mitochondrial DNA, and all regulatory factors directing the expression of both nuclear and mitochondrial respiratory genes are of nuclear origin [36].

UCPs are mitochondrial anion transporters present in the inner mitochondrial membrane, and their role in the control of energy conversion in mitochondria has recently been demonstrated. The activation of these anion transporters allows protons to leak back into the mitochondrial matrix, thus decreasing mitochondrial membrane potential and ROS generation [37,38]. UCP2 overexpression inhibits ROS production and apoptosis induced by linoleic acid and lysophosphatidylcholine [39]. Superoxide activates UCPs, with subsequent down-regulation of its own production [40,41].

The transcriptional regulation of UCP genes, particularly UCP3 genes, is mediated to a large extent by peroxisome proliferator activated receptors (PPARs) both in normal conditions and in metabolic diseases such as diabetes or obesity [42]. PPARs, as well as liver × receptors, are nuclear receptors significantly involved in the control of lipid metabolism, inflammation, insulin sensitivity and, probably, ATS progression [1,43]. Moreover, PPARs regulate the transcription of mitochondrial and microsomal enzymes [44]. Nunn et al [45] have recently reviewed the involvement of PPARs in modulating mitochondrial proton uncoupling and ROS production.

Mitochondrial oxidative dysfunction
Damage to mitochondria is caused primarily by the ROS generated by mitochondria themselves [34,46], mainly due to the release of electrons by the coenzymes NADH and FADH into the electron transport chain. In addition, a significant amount of ROS can be produced by the enzymes alpha-ketoglutarate dehydrogenase and monoamine oxidase located in the outer membrane of mitochondria [47,48].

The deleterious effects resulting from the formation of ROS in mitochondria are prevented to a large extent by various antioxidant systems. Under normal conditions there is a balance between ROS formation and antioxidants. However, when the antioxidant defenses become insufficient and cannot convert ROS to H_2O_2 fast enough, oxidative damage occurs and accumulates in the mitochondria [49]. Interestingly, free fatty acids (FFA) can decrease the mitochondrial generation of ROS under conditions of reverse electron transport, due to their uncoupling action. However, under conditions of forward electron transport, FFA increase ROS production [50].

Although somewhat controversial [51], an NO production does seem to occur in mitochondria through different pathways [52-55]. The NO produced in mitochondria [52,53] counteracts superoxide at multiple levels. It can rapidly scavenge superoxide via direct radical-radical reaction to form peroxynitrite, a potent oxidant [56-60] capable of decreasing the activity of complex I by forming S-

nitrosothiols [61]. This in turn reduces mitochondrial ROS generation. Moreover, NO can facilitate superoxide scavenging indirectly by stabilizing cytochrome C and preventing its leakage from mitochondria [24].

Asymmetrical dimethyl L-arginine (ADMA), an endogenous NO synthase inhibitor [62], may lead to increased mitochondrial ROS levels [63]. Mitochondrial ROS production can also be increased by the mitochondrial *p66Shc* protein, which also favors cytochrome C release, the dissipation of mitochondrial transmembrane potential and apoptosis [64-67].

Mitochondrial DNA oxidative damage
Mitochondrial DNA (mtDNA) is probably the most sensitive cellular target of ROS since it is located close to the inner mitochondrial membrane, where ROS are produced. Moreover, mtDNA is small in size and is not protected by histone proteins as is the case for nuclear DNA [68,69]. Many different types of oxidative DNA lesions have been described, ranging from base or sugar adduct modifications to single and double-strand breaks [70]. The hydroxyl radical can remove protons from deoxyribose, thus producing a sugar radical and inducing strand breaks and release of the affected DNA base [71]. Moreover, the hydroxyl radical can also abstract a proton from the methyl group of thymine and add it to the C4, C5 and C8 position of purines, thereby generating hydroxy adduct radicals [72].

mtDNA damage correlates with the extent of ATS [16], suggesting that mitochondrial dysfunction may promote atherogenesis [1,18,19]. Many of the DNA modifications can contribute to aging, cancer and neurodegenerative diseases [73], as well as to several other pathophysiological conditions [74]. Damage to mtDNA can have a greater impact on cellular function than damage to nuclear DNA [75].

The accumulation of mtDNA mutations can cause cell dysfunction by altering oxidative phosphorylation and Ca2+ homeostasis, inducing further oxidative stress and a defective turnover of mitochondrial proteins, and affecting the susceptibility to apoptosis [49]. In particular, the mtDNA encoded respiratory enzymes increase electron leak and ROS production, with subsequent enhanced oxidative stress and further damage to mitochondria [76]. A vicious cycle may therefore be generated, leading to progressive accumulation of ROS and oxidative damage to mtDNA [19].

Dyslipidemia
Both cholesterol and oxidized low density lipoprotein (oxLDL) can cause mitochondrial damage [17]. Cholesterol feeding in rabbits is associated with impaired mito-

chondrial function [77]. Similarly, hypercholesterolemia induced mtDNA damage in heart homogenates [78]. Free cholesterol (FC) loading of macrophages [79] is associated with mitochondrial dysfunction, as suggested by decrease in mitochondrial transmembrane potential and activation of the mitochondrial apoptosis pathway, a process playing a key role in atherogenesis. In addition to the involvement of the classic apoptotic Fas pathway, in FC-loaded macrophages there is evidence of mitochondrial cytocrome C release, caspase 9 activation and increased levels of the proapoptotic protein Bax [80].

The role of redox regulation and lipid rafts in macrophages during ox-LDL-mediated foam cell formation has recently been reviewed [81]. Circulating ox-LDL represents an independent risk factor for ATS and acute cardiovascular diseases. Ox-LDL causes the mitochondrial production of ROS in endothelial cells, a process associated with apoptosis [82,83], through the activation of mitochondrial complex II [84], uncoupled eNOS, and the NADPH oxidases [85]. The exposure of endothelial progenitor or mature cells to ox-LDL results in an increased production of mitochondria-derived superoxide, with associated increase in p53 expression and subsequent activation of Bax. The activated Bax translocates into the mitochondria to release cytochrome C, which elicits the apoptotic reaction. Bax is a member of multidomain Bcl-2 proapoptotic proteins, and its activation and translocation into the mitochondria has been shown to cause mitochondrial dysfunction and cell apoptosis [86]. Cheng et al [84] have demonstrated that superoxide anion, but not hydrogen peroxide, can activate p53 and Bax. The superoxide regulation of Bax does not consist in an increase in Bax expression, but rather in an activation through a conformational change.

Vindis et al. [87] have recently shown the involvement of two distinct calcium-dependent mechanisms in ox-LDL-induced apoptosis: the first is mediated by calpain/mitochondrial permeability transitionpore/cytochrome C/caspase, while the second is mediated by a mitochondrial apoptosis inducing factor, which is cyclosporin-insensitive and caspase-independent. Ox-LDL induces apoptosis in all cells involved in ATS: endothelial cells, VSMCs, macrophages, and T lymphocytes [88,89].

Diabetes
Type 2 diabetes mellitus (T2DM) is a multifactorial, heterogeneous, polygenic disease that accounts for > = 90% of all types of diabetes. In T2DM insulin resistance is the major pathologic feature, which often causes a compensatory increase of insulin secretion [90]. ROS are now considered a major factor in the onset and development of T2DM. ROS can induce inactivation of the signaling pathway between the insulin receptor and the glucose trans-

porter system, leading to insulin resistance [91]. On the other hand, in addition to being a possible effect of ROS production, T2DM is also a cause of oxidative stress, with ensuing atherogenic effect [92]. Hyperglycemia induces superoxide generation in endothelial cells, and most of this superoxide may be produced by mitochondria [93]. In diabetes, electron transfer and oxidative phosphorylation are uncoupled, resulting in superoxide formation and inefficient ATP synthesis [23]. Prevention of oxidative damage represents a therapeutic strategy in diabetes [23].

In T2DM the elevation of free fatty acid (FFA) concentrations, with subsequent intramyocellular lipid accumulation, has been proposed as a cause of further insulin resistance and also pancreatic beta-cell death [94,95]. It has been reported [96,97] that both glucose and FFAs may initiate the formation of ROS via mitochondrial and NADPH oxidase mechanisms in muscles, adipocytes, beta cells and other cells. FFAs penetrate cellular organelles including mitochondria, where high ROS levels will result in lipid peroxidation and mitochondrial injury [98].

Interestingly, recent studies revealed that T2DM and insulin resistance are associated with a decreased mitochondrial oxidation function in skeletal muscle [99]. Moreover, in T2DM mitochondria are smaller, round and prone to produce superoxide [100]. Disorders of the mitochondrial transport chain, overgeneration of ROS and lipoperoxides or impairments in antioxidant defenses have been reported in T2DM, as well as in obesity.

Hypertension
Like other risk factor for ATS, human hypertension is a condition associated with endothelial dysfunction and oxidative stress [101-107]. Mitochondrial dysfunction has been potentially implicated in both human and experimental hypertension [108,109]. Deterioration of mitochondrial energy production plays a role in the pathogenesis of hypertension in both spontaneously hypertensive rats (SHRs) [110,111] and mice [112]. Mitochondrial energy deficiency [113] and a decreased activity of complex IV have been observed in the hypertrophied myocardium from SHRs [114]. In these animals there is also an abnormal transport of inorganic phosphate in left ventricular mitochondria [115]. Overall, these data indicate that some defect in the regulation of mitochondrial ATP synthase activity occurs in the cardiomyocites of SHRs. In addition, mitochondrial calcium overload could significantly contribute to the development of hypertensive states [113].

An association of hypertension with mitochondrial uncoupling proteins (UCPs) has been reported both in experimental and human hypertension. In particular, mice with doxycycline-inducible expression of UCP 1 in

arterial walls develop hypertension and dietary ATS [112]. A common polymorphism of the UCP2 gene was associated with hypertension in a Japanese population, and with hypertension and obesity in Caucasians [116].

ROS and RNS can damage mtDNA [117], with decreased energy production, additional generation of ROS, and enhancement of the cellular signals capable of initiating hypertension, as well as ATS [117]. mtDNA mutations have been demonstrated in black Americans with hypertension-associated end-stage renal disease [118]. Moreover, it has been shown that a mutation in mitochondrial tRNA is associated with hypertension, hypercholesterolemia and hypomagnesemia [119]. The putative role of mitochondrial dysfunction in hypertension has been recently reviewed [120].

Aging, hyperhomocysteinemia, cigarette smoking and HIV as risk factors

Aging significantly increases the risk of coronary heart disease and other vascular diseases. Several human and animal studies have shown an age-related impairment of mitochondrial respiratory chain function and ATP synthesis, together with an accumulation of oxidative mtDNA mutations [18]. It has been suggested that mitochondrial dysfunction is a major contributor to aging and aging-associated ATS [18].

Elevated plasma homocysteine is an independent risk factor for ATS. It has been proposed that endothelial dysfunction and ATS can be induced by homocysteine through increased generation of ROS and reduced NO availability [121-124]. Austin e al. [125] have shown that homocysteine promotes mitochondrial damage and alters mitochondrial gene expression and function. Further, homocysteine stimulates the expression of NRF1 and T-fam, two nuclear transcription factors involved in the modulation of mitochondrial biogenesis. This effect was prevented by pre-treatment with antioxidants, suggesting that ROS are important mediators of the effects of homocysteine. In addition, homocysteine induces endothelial cell apoptosis through mitochondrial mechanisms [126,127].

Cigarette smoking may significantly increase the risk of early ATS by affecting mitochondrial function. In fact, in addition to endothelial injury, platelet activation and LDL oxidation, the atherogenic effects of cigarette smoking include oxidative mtDNA damage with mtDNA deletions and loss of mitochondrial membrane potential [13,128-131].

Finally, mitochondrial dysfunction can also be considered a contributing factor for HIV-associated ATS [132-134].

Thus, a range of seemingly unrelated conditions has underlying pathophysiological mechanisms in common, namely ROS production and accumulation of mtDNA damage, resulting in mitochondrial dysfunction and ATS.

Conclusion

Based on experimental evidence and clinical studies, oxidative and nitrosative stress have been shown to be induced by ATS risk factors and to contribute to the onset and development of atherosclerotic vascular damage. Moreover, endogenous risk factors, such as hypertension and diabetes, may be both the cause of vascular ROS generation and a consequence of ROS induced endothelial dysfunction.

Under physiological conditions, the mitochondrial respiratory chain is a major source of superoxide and other ROS [135-139]. This mechanism may be triggered by risk factors, with subsequent endothelial dysfunction and atherogenesis. On the other hand, mitochondria may be not only a relevant source, but also a target of ROS [14,15]. In fact, an excessive production of ROS in mitochondria will damage lipids, carbohydrates, and proteins, as well as mtDNA. Indeed, oxidative mtDNA mutations could represent an important step in the chain of events connecting risk factors to atherogenesis, acting as further causes of ROS generation [78,140]. Ballinger et al. [16] suggested that mitochondrial damage in an early stage can predict ROS and RNS-mediated atherosclerotic lesions. Overall, the pathogenetic role of mitochondrial dysfunction in atherogenesis may now be considered more than a plausible hypothesis [19].

Despite the experimental evidence of the importance of oxidative stress in inducing ATS, clinical studies have been unable to demonstrate that antioxidants have any effect on human atherogenesis [141-143]. Some studies have shown that antioxidants such as alpha tocopherol, ubiquinone and N-acetylcysteine could decrease mitochondrial oxidative damage in different experimental models [144-148]. However, the effectiveness of these compounds is limited, since they do not significantly accumulate within mitochondria [149], and strategies for the targeted delivery of antioxidants to mitochondria are now in the developmental stages [150]. In fact the antioxidant moieties can be bound by covalent attachment to lipophilic triphenylphosphonium cations, which, due to the large mitochondrial membrane potential, do accumulate within the mitochondria [151]. The targeted version of ubiquinol (MitoQ) has been used most extensively [151,152], and is now being tested in man as an oral drug for the treatment of hepatitis C [153] and Parkinson's disease [154]. However, despite these promising data, more pre-clinical and clinical studies are needed in order to

evaluate both the effectiveness and the safety of mitochondria targeted antioxidants.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

PP designed the article and wrote the first draft. GMP specifically prepared the sections concerning mitochondrial oxidative dysfunction and mitochondrial DNA oxidative damage. EC specifically prepared the sections concerning dyslipidemia and diabetes. SDP contributed to literature search and edited the manuscript. AM participated in designing the article, revised critically the manuscript, and re-arranged the final version. All authors read and approved the final manuscript.

References

1. Puddu GM, Cravero E, Arnone G, Muscari A, Puddu P: **Molecular aspects of atherogenesis: new insights and unsolved questions.** *J Biomed Sci* 2005, **12:**839-853.
2. Stocker R, Keaney JF Jr: **Role of oxidative modifications in atherosclerosis.** *Physiol Rev* 2004, **84:**1381-1478.
3. Navab M, Ananthramaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fonarow GC, Vahabzadeh K, Hama S, Hough G, Kamranpour N, Berliner JA, Lusis AJ, Fogelman AM: **The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL.** *J Lipid Res* 2004, **45:**993-1007.
4. Madamanchi NR, Vendrov A, Runge MS: **Oxidative stress and vascular disease.** *Arterioscler Thromb Vasc Biol* 2005, **25:**29-38.
5. Bergt C, Pennathur S, Fu X, Byun J, O'Brien K, McDonald TO, Singh P, Anantharamaiah GM, Chait A, Brunzell J, Geary RL, Oram JF, Heinecke JW: **The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport.** *Proc Natl Acad Sci USA* 2004, **101:**13032-13037.
6. Ross R: **The pathogenesis of atherosclerosis: a perspective for the 1990s.** *Nature* 1993, **362:**801-809.
7. Berliner JA, Heinecke JW: **The role of oxidized lipoproteins in atherogenesis.** *Free Radic Biol Med* 1996, **20:**707-727.
8. Freeman BA, White CR, Gutierrez H, Paler-Martínez A, Tarpey MM, Rubbo H: **Oxygen radical-nitric oxide reactions in vascular diseases.** *Adv Pharmacol* 1995, **34:**45-69.
9. Victor VM, Rocha M, Solá E, Bañuls C, Garcia-Malpartida K, Hernández-Mijares A: **Oxidative stress, endothelial dysfunction and atherosclerosis.** *Curr Pharm Des* 2009, **15:**2988-3002.
10. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J: **Free radicals and antioxidants in normal physiological functions and human disease.** *Int J Biochem Cell Biol* 2007, **39:**44-84.
11. Alexander RW: **Atherosclerosis as disease of redox-sensitive genes.** *Trans Am Clin Climatol Assoc* 1998, **109:**129-145.
12. Ito H, Torii M, Suzuki T: **Decreased superoxide dismutase activity and increased superoxide anion production in cardiac hypertrophy of spontaneously hypertensive rats.** *Clin Exp Hypertens* 1995, **17:**803-816.
13. van Jaarsveld H, Kuyl JM, Alberts DW: **Exposure of rats to low concentration of cigarette smoke increases myocardial sensitivity to ischaemia/reperfusion.** *Basic Res Cardiol* 1992, **87:**393-399.
14. Esposito LA, Melov S, Panov A, Cottrell BA, Wallace DC: **Mitochondrial disease in mouse results in increased oxidative stress.** *Proc Natl Acad Sci USA* 1999, **96:**4820-4825.
15. Wallace DC: **Mitochondrial genetics: a paradigm for aging and degenerative diseases?** *Science* 1992, **256:**628-632.
16. Ballinger SW, Patterson C, Knight-Lozano CA, Burow DL, Conklin CA, Hu Z, Reuf J, Horaist C, Lebovitz R, Hunter GC, McIntyre K, Runge MS: **Mitochondrial integrity and function in atherogenesis.** *Circulation* 2002, **106:**544-349.
17. Ballinger SW: **Mitochondrial dysfunction in cardiovascular disease.** *Free Radic Biol Med* 2005, **38:**1278-1295.
18. Madamanchi NR, Runge MS: **Mitochondrial dysfunction in atherosclerosis.** *Circ Res* 2007, **100:**460-473.
19. Puddu P, Puddu GM, Galletti L, Cravero E, Muscari A: **Mitochondrial dysfunction as an initiating event in atherogenesis: a plausible hypothesis.** *Cardiology* 2005, **103:**137-141.
20. Pourova J, Kottova M, Voprsalova M, Pour M: **Reactive oxygen and nitrogen species in normal physiological processes.** *Acta Physiol (Oxf)* 2009 in press.
21. Pieczenik SR, Neustadt J: **Mitochondrial dysfunction and molecular pathways of disease.** *Exp Mol Pathol* 2007, **83:**84-92.
22. Wallace DC: **A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine.** *Annu Rev Genet* 2005, **39:**359-407.
23. Green K, Brand MD, Murphy MP: **Prevention of mitochondrial oxidative damage as a therapeutic strategy in diabetes.** *Diabetes* 2004, **53(Suppl 1):**S110-S118.
24. Zhang DX, Gutterman DD: **Mitochondrial reactive oxygen species mediated signaling in endothelial cells.** *Am J Physiol Heart Circ Physiol* 2007, **292:**2023-2031.
25. Li JM, Shah AM: **Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology.** *Am J Physiol Regul Integr Comp Physiol* 2004, **287:**1014-1030.
26. Mueller CF, Laude K, McNally JS, Harrison DG: **ATVB in focus: redox mechanisms in blood vessels.** *Arterioscler Thromb Vasc Biol* 2005, **25:**274-278.
27. Davidson SM, Duchen MR: **Endothelial mitochondria: contributing to vascular function and disease.** *Circ Res* 2007, **100:**1128-1141.
28. O'Malley Y, Fink BD, Ross NC, Prisinzano TE, Sivitz WI: **Reactive oxygen and targeted antioxidant administration in endothelial cell mitochondria.** *J Biol Chem* 2006, **281:**39766-39775.
29. Klatt P, Lamas S: **Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress.** *Eur J Biochem* 2000, **267:**4928-4944.
30. Ridnour LA, Thomas DD, Mancardi D, Espey MG, Miranda KM, Paolocci N, Feelisch M, Fukuto J, Wink DA: **The chemistry of nitrosative stress induced by nitric oxide and reactive nitrogen oxide species. Putting perspective on stressful biological situations.** *Biol Chem* 2004, **385:**1-10.
31. Gutierrez J, Ballinger SW, Darley-Usmar VM, Landar A: **Free radicals, mitochondria, and oxidized lipids: the emerging role in signal transduction in vascular cells.** *Circ Res* 2006, **99:**924-932.
32. Cooper CE, Patel RP, Brookes PS, Darley-Usmar VM: **Nanotransducers in cellular redox signaling: modification of thiols by reactive oxygen and nitrogen species.** *Trends Biochem Sci* 2002, **27:**489-492.
33. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H: **Role of oxidative stress in atherosclerosis.** *Am J Cardiol* 2003, **91:**7A-11A.
34. Turrens JF: **Mitochondrial formation of reactive oxygen species.** *J Physiol* 2003, **552:**335-344.
35. Dröge W: **Free radicals in the physiological control of cell function.** *Physiol Rev* 2002, **82:**47-95.
36. Scarpulla RC: **Nuclear control of respiratory gene expression in mammalian cells.** *J Cell Biochem* 2006, **97:**673-683.
37. Teshima Y, Akao M, Jones SP, Marbán E: **Uncoupling protein-2 overexpression inhibits mitochondrial death pathway in cardiomyocytes.** *Circ Res* 2003, **93:**192-200.
38. Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, Couplan E, Alves-Guerra MC, Goubern M, Surwit R, Bouillaud F, Richard D, Collins S, Ricquier D: **Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production.** *Nat Genet* 2000, **26:**435-439.
39. Lee KU, Lee IK, Han J, Song DK, Kim YM, Song HS, Kim HS, Lee WJ, Koh EH, Song KH, Han SM, Kim MS, Park IS, Park JY: **Effects of recombinant adenovirus-mediated uncoupling protein 2 overexpression on endothelial function and apoptosis.** *Circ Res* 2005, **96:**1200-1207.
40. Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA, Harper JA, Roebuck SJ, Morrison A, Pickering S, Clapham JC, Brand MD: **Superoxide activates mitochondrial uncoupling proteins.** *Nature* 2002, **415:**96-99.
41. Brand MD, Esteves TC: **Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3.** *Cell Metab* 2005, **2:**85-93.

42. Villarroya F, Iglesias R, Giralt M: **PPARs in the Control of Uncoupling Proteins Gene Expression.** *PPAR Res* 2007, **2007**:74364.
43. Puddu P, Puddu GM, Muscari A: **Peroxisome proliferator-activated receptors: are they involved in atherosclerosis progression?** *Int J Cardiol* 2003, **90**:133-140.
44. Feige JN, Gelman L, Michalik L, Desvergne B, Wahli W: **From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions.** *Prog Lipid Res* 2006, **45**:120-159.
45. Nunn AV, Bell J, Barter P: **The integration of lipid-sensing and anti-inflammatory effects: how the PPARs play a role in metabolic balance.** *Nucl Recept* 2007, **5**:1.
46. Duchen MR: **Mitochondria in health and disease: perspectives on a new mitochondrial biology.** *Mol Aspects Med* 2004, **25**:365-451.
47. Andreyev AY, Kushnareva YE, Starkov AA: **Mitochondrial metabolism of reactive oxygen species.** *Biochemistry* 2005, **70**:200-214.
48. Adam-Vizi V, Chinopoulos C: **Bioenergetics and the formation of mitochondrial reactive oxygen species.** *Trends Pharmacol Sci* 2006, **27**:639-645.
49. James AM, Murphy MP: **How mitochondrial damage affects cell function.** *J Biomed Sci* 2002, **9**:475-487.
50. Schönfeld P, Wojtczak L: **Fatty acids decrease mitochondrial generation of reactive oxygen species at the reverse electron transport but increase it at the forward transport.** *Biochim Biophys Acta* 2007, **1767**:1032-1040.
51. Lacza Z, Pankotai E, Csordás A, Gero D, Kiss L, Horváth EM, Kollai M, Busija DW, Szabó C: **Mitochondrial NO and reactive nitrogen species production: does mtNOS exist?** *Nitric Oxide* 2006, **14**:162-168.
52. Ghafourifar P, Richter C: **Nitric oxide synthase activity in mitochondria.** *FEBS Lett* 1997, **418**:291-296.
53. Giulivi C, Poderoso JJ, Boveris A: **Production of nitric oxide by mitochondria.** *J Biol Chem* 1998, **273**:11038-11043.
54. Ghafourifar P, Cadenas E: **Mitochondrial nitric oxide synthase.** *Trends Pharmacol Sci* 2005, **26**:190-195.
55. Lacza Z, Lacza Z, Kozlov AV, Pankotai E, Csordás A, Wolf G, Redl H, Kollai M, Szabó C, Busija DW, Horn TF: **Mitochondria produce reactive nitrogen species via an arginine-independent pathway.** *Free Radic Res* 2006, **40**:369-378.
56. Radi R, Beckman JS, Bush KM, Freeman BA: **Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide.** *Arch Biochem Biophys* 1991, **288**:481-487.
57. Ballinger SW, Patterson C, Yan CN, Doan R, Burow DL, Young CG, Yakes FM, Van Houten B, Ballinger CA, Freeman BA, Runge MS: **Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells.** *Circ Res* 2000, **86**:960-966.
58. Beckman JS, Koppenol WH: **Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly.** *Am J Physiol* 1996, **271**:1424-437.
59. Cassina A, Radi R: **Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport.** *Arch Biochem Biophys* 1996, **328**:309-316.
60. Radi R, Cassina A, Hodara R: **Nitric oxide and peroxynitrite interactions with mitochondria.** *Biol Chem* 2002, **383**:401-409.
61. Dahm CC, Moore K, Murphy MP: **Persistent S-nitrosation of complex I and other mitochondrial membrane proteins by S-nitrosothiols but not nitric oxide or peroxynitrite: implications for the interaction of nitric oxide with mitochondria.** *J Biol Chem* 2006, **281**:10056-10065.
62. Scalera F, Borlak J, Beckmann B, Martens-Lobenhoffer J, Thum T, Täger M, Bode-Böger SM: **Endogenous nitric oxide synthesis inhibitor asymmetric dimethyl L-arginine accelerates endothelial cell senescence.** *Arterioscler Thromb Vasc Biol* 2004, **24**:1816-1822.
63. Sud N, Wells SM, Sharma S, Wiseman DA, Wilham J, Black SM: **Asymmetric dimethylarginine inhibits HSP90 activity in pulmonary arterial endothelial cells: role of mitochondrial dysfunction.** *Am J Physiol Cell Physiol* 2008, **294**:C1407-C1418.
64. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, Pelliccia G, Luzi L, Minucci S, Marcaccio M, Pinton P, Rizzuto R, Bernardi P, Paolucci F, Pelicci PG: **Electron transfer between cyto-**

chrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis.** *Cell* 2005, **122**:221-233.
65. Orsini F, Migliaccio E, Moroni M, Contursi C, Raker VA, Piccini D, Martin-Padura I, Pelliccia G, Trinei M, Bono M, Puri C, Tacchetti C, Ferrini M, Mannucci R, Nicoletti I, Lanfrancone L, Giorgio M, Pelicci PG: **The life span determinant p66Shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates trans-membrane potential.** *J Biol Chem* 2004, **279**:25689-5695.
66. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, Pelicci PG: **The p66shc adaptor protein controls oxidative stress response and life span in mammals.** *Nature* 1999, **402**:309-313.
67. Napoli C, Martin-Padura I, de Nigris F, Giorgio M, Mansueto G, Somma P, Condorelli M, Sica G, De Rosa G, Pelicci P: **Deletion of the p66Shc longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet.** *Proc Natl Acad Sci USA* 2003, **100**:2112-2126.
68. Croteau DL, Bohr VA: **Repair of oxidative damage to nuclear and mitochondrial DNA in mammalian cells.** *J Biol Chem* 1997, **272**:25409-25412.
69. Van Remmen H, Hamilton ML, Richardson A: **Oxidative damage to DNA and aging.** *Exerc Sport Sci Rev* 2003, **36**:149-153.
70. Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamasoba T, Tanokura M, Weindruch R, Leeuwenburgh C, Prolla TA: **Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging.** *Science* 2005, **309**:481-484.
71. Halliwell B, Aruoma OI: **DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems.** *FEBS Lett* 1991, **281**:9-19.
72. Grollman AP, Moriya M: **Mutagenesis by 8-oxoguanine: an enemy within.** *Trends Genet* 1993, **9**:246-249.
73. Bohr VA: **Repair of oxidative DNA damage in nuclear and mitochondrial DNA, and some changes with aging in mammalian cells.** *Free Radic Biol Med* 2002, **32**:804-812.
74. Stuart JA, Bourque BM, de Souza-Pinto NC, Bohr VA: **No evidence of mitochondrial respiratory dysfunction in OGG1-null mice deficient in removal of 8-oxodeoxyguanine from mitochondrial DNA.** *Free Radic Biol Med* 2005, **38**:737-745.
75. Stuart JA, Brown MF: **Mitochondrial DNA maintenance and bioenergetics.** *Biochim Biophys Acta* 2006, **1757**:79-89.
76. Wei YH, Lu CY, Lee HC, Pang CY, Ma YS: **Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function.** *Ann N Y Acad Sci* 1998, **854**:155-170.
77. Mikaelian NP, Khalilov EM, Ivanov AS, Fortinskaia ES, Lopukhin IuM: **Mitochondrial enzymes in circulating lymphocytes during hemosorption for experimental hypercholesterolemia.** *Biull Eksp Biol Med* 1983, **96**:35-37.
78. Knight-Lozano CA, Young CG, Burow DL, Hu ZY, Uyeminami D, Pinkerton KE, Ischiropoulos H, Ballinger SW: **Cigarette smoke exposure and hypercholesterolemia increase mitochondrial damage in cardiovascular tissues.** *Circulation* 2002, **105**:849-854.
79. Yao PM, Tabas I: **Free cholesterol loading of macrophages is associated with widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway.** *J Biol Chem* 2001, **276**:42468-42476.
80. Tabas I: **Consequences of cellular cholesterol accumulation: basic concepts and physiological implications.** *J Clin Invest* 2002, **110**:905-911.
81. Schmitz G, Grandl M: **Role of redox regulation and lipid rafts in macrophages during Ox-LDL-mediated foam cell formation.** *Antioxid Redox Signal* 2007, **9**:1499-1518.
82. Raha S, Robinson BH: **Mitochondria, oxygen free radicals, and apoptosis.** *Am J Med Genet* 2001, **106**:62-70.
83. Sato T, Machida T, Takahashi S, Iyama S, Sato Y, Kuribayashi K, Takada K, Oku T, Kawano Y, Okamoto T, Takimoto R, Matsunaga T, Takayama T, Takahashi M, Kato J, Niitsu Y: **Fas-mediated apoptosome formation is dependent on reactive oxygen species derived from mitochondrial permeability transition in Jurkat cells.** *J Immunol* 2004, **173**:285-296.
84. Cheng J, Cui R, Chen CH, Du J: **Oxidized low-density lipoprotein stimulates p53-dependent activation of proapoptotic Bax**

leading to apoptosis of differentiated endothelial progenitor cells. *Endocrinology* 2007, **148**:2085-2094.

85. Fleming I, Mohamed A, Galle J, Turchanowa L, Brandes RP, Fisslthaler B, Busse R: **Oxidized low-density lipoprotein increases superoxide production by endothelial nitric oxide synthase by inhibiting PKCalpha.** *Cardiovasc Res* 2005, **65**:897-906.

86. Wei MC, Zong WX, Cheng EH, Lindsten T, Panoutsakopoulou V, Ross AJ, Roth KA, MacGregor GR, Thompson CB, Korsmeyer SJ: **Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death.** *Science* 2001, **292**:727-730.

87. Vindis C, Elbaz M, Escargueil-Blanc I, Augé N, Heniquez A, Thiers JC, Nègre-Salvayre A, Salvayre R: **Two distinct calcium-dependent mitochondrial pathways are involved in oxidized LDL-induced apoptosis.** *Arterioscler Thromb Vasc Biol* 2005, **25**:639-645.

88. Mallat Z, Tedgui A: **Apoptosis in the vasculature: mechanisms and functional importance.** *Br J Pharmacol* 2000, **130**:947-962.

89. Geng YJ, Libby P: **Progression of atheroma: a struggle between death and procreation.** *Arterioscler Thromb Vasc Biol* 2002, **22**:1370-1380.

90. Hayden MR, Sowers JR: **Redox imbalance in diabetes.** *Antioxid Redox Signal* 2007, **9**:865-867.

91. Newsholme P, Haber EP, Hirabara SM, Rebelato EL, Procopio J, Morgan D, Oliveira-Emilio HC, Carpinelli AR, Curi R: **Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity.** *J Physiol* 2007, **583**:9-24.

92. Mehta JL, Rasouli N, Sinha AK, Molavi B: **Oxidative stress in diabetes: a mechanistic overview of its effects on atherogenesis and myocardial dysfunction.** *Int J Biochem Cell Biol* 2006, **38**:794-803.

93. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M: **Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage.** *Nature* 2000, **404**:787-790.

94. Azevedo-Martins AK, Monteiro AP, Lima CL, Lenzen S, Curi R: **Fatty acid-induced toxicity and neutral lipid accumulation in insulin-producing RINm5F cells.** *Toxicol In Vitro* 2006, **20**:1106-1113.

95. McGarry JD: **Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes.** *Diabetes* 2002, **51**:7-18.

96. Brownlee M: **The pathobiology of diabetic complications: a unifying mechanism.** *Diabetes* 2005, **54**:1615-1625.

97. Haber EP, Procópio J, Carvalho CR, Carpinelli AR, Newsholme P, Curi R: **New insights into fatty acid modulation of pancreatic beta-cell function.** *Int Rev Cytol* 2006, **248**:1-41.

98. Schrauwen P, Hesselink MK: **Oxidative capacity, lipotoxicity, and mitochondrial damage in type 2 diabetes.** *Diabetes* 2004, **53**:1412-1417.

99. Befroy DE, Petersen KF, Dufour S, Mason GF, de Graaf RA, Rothman DL, Shulman GI: **Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients.** *Diabetes* 2007, **56**:1376-1381.

100. McBride HM, Neuspiel M, Wasiak S: **Mitochondria: more than just a powerhouse.** *Curr Biol* 2006, **16**:551-560.

101. Romero JC, Reckelhoff JF: **State-of-the-Art lecture. Role of angiotensin and oxidative stress in essential hypertension.** *Hypertension* 1999, **34**:943-949.

102. Raij L: **Nitric oxide in hypertension: relationship with renal injury and left ventricular hypertrophy.** *Hypertension* 1998, **31**:189-193.

103. Redón J, Oliva MR, Tormos C, Giner V, Chaves J, Iradi A, Sáez GT: **Antioxidant activities and oxidative stress byproducts in human hypertension.** *Hypertension* 2003, **41**:1096-1101.

104. Russo C, Olivieri O, Girelli D, Faccini G, Zenari ML, Lombardi S, Corrocher R: **Anti-oxidant status and lipid peroxidation in patients with essential hypertension.** *J Hypertens* 1998, **16**:1267-1271.

105. Paravicini TM, Touyz RM: **Redox signaling in hypertension.** *Cardiovasc Res* 2006, **71**:247-258.

106. McIntyre M, Bohr DF, Dominiczak AF: **Endothelial function in hypertension: the role of superoxide anion.** *Hypertension* 1999, **34**:539-5345.

107. Ward NC, Croft KD: **Hypertension and oxidative stress.** *Clin Exp Pharmacol Physiol* 2006, **33**:872-876.

108. Kumar KV, Das UN: **Are free radicals involved in the pathobiology of human essential hypertension?** *Free Radic Res Commun* 1993, **19**:59-66.

109. Chan SH, Wu KL, Chang AY, Tai MH, Chan JY: **Oxidative impairment of mitochondrial electron transport chain complexes in rostral ventrolateral medulla contributes to neurogenic hypertension.** *Hypertension* 2009, **53**:217-227.

110. Brookes PS, Levonen AL, Shiva S, Sarti P, Darley-Usmar VMS, Levonen AL, Shiva S, Sarti P, Darley-Usmar VM: **Mitochondria: regulators of signal transduction by reactive oxygen and nitrogen species.** *Free Radic Biol Med* 2002, **33**:755-764.

111. Atlante A, Seccia TM, Pierro P, Vulpis V, Marra E, Pirrelli A, Passarella S: **ATP synthesis and export in heart left ventricle mitochondria from spontaneously hypertensive rat.** *Int J Mol Med* 1998, **1**:709-716.

112. Bernal-Mizrachi C, Gates AC, Weng S, Imamura T, Knutsen RH, DeSantis P, Coleman T, Townsend RR, Muglia LJ, Semenkovich CF: **Vascular respiratory uncoupling increases blood pressure and atherosclerosis.** *Nature* 2005, **435**:502-506.

113. Postnov IuV: **The role of mitochondrial calcium overload and energy deficiency in pathogenesis of arterial hypertension.** *Arkh Patol* 2001, **63**:3-10.

114. Chen L, Tian X, Song L: **Biochemical and biophysical characteristics of mitochondria in the hypertrophic hearts from hypertensive rats.** *Chin Med J (Engl)* 1995, **108**:361-366.

115. Seccia TM, Atlante A, Vulpis V, Marra E, Passarella S, Pirrelli A: **Abnormal transport of inorganic phosphate in left ventricular mitochondria from spontaneously hypertensive rats.** *Cardiologia* 1999, **44**:719-725.

116. Ji Q, Ikegami H, Fujisawa T, Kawabata Y, Ono M, Nishino M, Ohishi M, Katsuya T, Rakugi H, Ogihara T: **A common polymorphism of uncoupling protein 2 gene is associated with hypertension.** *J Hypertens* 2004, **22**:97-102.

117. Rachek LI, Grishko VI, Ledoux SP, Wilson GL: **Role of nitric oxide-induced mtDNA damage in mitochondrial dysfunction and apoptosis.** *Free Radic Biol Med* 2006, **40**:754-762.

118. Watson B Jr, Khan MA, Desmond RA, Bergman S: **Mitochondrial DNA mutations in black Americans with hypertension-associated end-stage renal disease.** *Am J Kidney Dis* 2001, **38**:529-536.

119. Wilson FH, Hariri A, Farhi A, Zhao H, Petersen KF, Toka HR, Nelson-Williams C, Raja KM, Kashgarian M, Shulman GI, Scheinman SJ, Lifton RP: **A cluster of metabolic defects caused by mutation in a mitochondrial tRNA.** *Science* 2004, **306**:1190-1194.

120. Puddu P, Puddu GM, Cravero E, De Pascalis S, Muscari A: **The putative role of mitochondrial dysfunction in hypertension.** *Clin Exp Hypertens* 2007, **29**:427-434.

121. McCully KS: **Homocysteine and vascular disease.** *Nat Med* 1996, **2**:386-389.

122. Duell PB, Malinow MR: **Homocyst(e)ine: an important risk factor for atherosclerotic vascular disease.** *Curr Opin Lipidol* 1997, **8**:28-34.

123. Kanani PM, Sinkey CA, Browning RL, Allaman M, Knapp HR, Haynes WG: **Role of oxidant stress in endothelial dysfunction produced by experimental hyperhomocyst(e)inemia in humans.** *Circulation* 1999, **100**:1161-1168.

124. Zhang X, Li H, Jin H, Ebin Z, Brodsky S, Goligorsky MS: **Effects of homocysteine on endothelial nitric oxide production.** *Am J Physiol Renal Physiol* 2000, **279**:671-678.

125. Austin RC, Sood SK, Dorward AM, Singh G, Shaughnessy SG, Pamidi S, Outinen PA, Weitz JI: **Homocysteine-dependent alterations in mitochondrial gene expression, function and structure. Homocysteine and H2O2 act synergistically to enhance mitochondrial damage.** *J Biol Chem* 1998, **273**:30808-30817.

126. Tyagi N, Ovechkin AV, Lominadze D, Moshal KS, Tyagi SC: **Mitochondrial mechanism of microvascular endothelial cells apoptosis in hyperhomocysteinemia.** *J Cell Biochem* 2006, **98**:1150-1162.

127. Lee SJ, Kim KM, Namkoong S, Kim CK, Kang YC, Lee H, Ha KS, Han JA, Chung HT, Kwon YG, Kim YM: **Nitric oxide inhibition of homocysteine-induced human endothelial cell apoptosis by down-regulation of p53-dependent Noxa expression through the formation of S-nitrosohomocysteine.** *J Biol Chem* 2005, **280**:5781-5788.

128. Masayesva BG, Mambo E, Taylor RJ, Goloubeva OG, Zhou S, Cohen Y, Minhas K, Koch W, Sciubba J, Alberg AJ, Sidransky D, Califano J:

Mitochondrial DNA content increase in response to cigarette smoking. *Cancer Epidemiol Biomarkers Prev* 2006, **15**:19-24.

129. Miró O, Alonso JR, Jarreta D, Casademont J, Urbano-Márquez A, Cardellach F: **Smoking disturbs mitochondrial respiratory chain function and enhances lipid peroxidation on human circulating lymphocytes.** *Carcinogenesis* 1999, **20**:1331-1336.

130. Eaton MM, Gursahani H, Arieli Y, Pinkerton K, Schaefer S: **Acute tobacco smoke exposure promotes mitochondrial permeability transition in rat heart.** *J Toxicol Environ Health A* 2006, **69**:1497-1510.

131. Yang Z, Knight CA, Mamerow MM, Vickers K, Penn A, Postlethwait EM, Ballinger SW: **Prenatal environmental tobacco smoke exposure promotes adult atherogenesis and mitochondrial damage in apolipoprotein E-/- mice fed a chow diet.** *Circulation* 2004, **110**:3715-20.

132. Holmberg SD, Moorman AC, Williamson JM, Tong TC, Ward DJ, Wood KC, Greenberg AE, Janssen RS, HIV Outpatient Study (HOPS) investigators: **Protease inhibitors and cardiovascular outcomes in patients with HIV-1.** *Lancet* 2002, **360**:1747-1748.

133. Friis-Møller N, Weber R, Reiss P, Thiébaut R, Kirk O, d'Arminio Monforte A, Pradier C, Morfeldt L, Mateu S, Law M, El-Sadr W, De Wit S, Sabin CA, Phillips AN, Lundgren JD, DAD study group: **Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study.** *AIDS* 2003, **17**:1179-1193.

134. Jericó C, Knobel H, Calvo N, Sorli ML, Guelar A, Gimeno-Bayón JL, Saballs P, López-Colomés JL, Pedro-Botet J: **Subclinical carotid atherosclerosis in HIV-infected patients: role of combination antiretroviral therapy.** *Stroke* 2006, **37**:812-817.

135. Harrison DG: **Cellular and molecular mechanisms of endothelial cell dysfunction.** *J Clin Invest* 1997, **100**:2153-2157.

136. Luft R, Landau BR: **Mitochondrial medicine.** *J Intern Med* 1995, **238**:405-421.

137. Han D, Williams E, Cadenas E: **Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space.** *Biochem J* 2001, **353**:411-416.

138. Barja G, Herrero A: **Localization at complex I and mechanism of the higher free radical production of brain nonsynaptic mitochondria in the short-lived rat than in the longevous pigeon.** *J Bioenerg Biomembr* 1998, **30**:235-243.

139. Cohen G, Kesler N: **Monoamine oxidase and mitochondrial respiration.** *J Neurochem* 1999, **73**:2310-2315.

140. Ballinger SW, Bouder TG, Davis GS, Judice SA, Nicklas JA, Albertini RJ: **Mitochondrial genome damage associated with cigarette smoking.** *Cancer Res* 1996, **56**:5692-5697.

141. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico: **Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial.** *Lancet* 1999, **354**:447-455.

142. Rapola JM, Virtamo J, Ripatti S, Huttunen JK, Albanes D, Taylor PR, Heinonen OP: **Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infarction.** *Lancet* 1997, **349**:1715-1720.

143. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P: **Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators.** *N Engl J Med* 2000, **342**:154-160.

144. Matthews RT, Yang L, Browne S, Baik M, Beal MF: **Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects.** *Proc Natl Acad Sci USA* 1998, **5**:8892-8897.

145. Lass A, Sohal RS: **Electron transport-linked ubiquinone-dependent recycling of alpha-tocopherol inhibits autooxidation of mitochondrial membranes.** *Arch Biochem Biophys* 1998, **352**:229-236.

146. Victor VM, Rocha M, De la Fuente M: **N-acetylcysteine protects mice from lethal endotoxemia by regulating the redox state of immune cells.** *Free Radic Res* 2003, **37**:919-929.

147. Victor VM, Rocha M, De la Fuente M: **Immune cells: free radicals and antioxidants in sepsis.** *Int Immunopharmacol* 2004, **4**:327-347.

148. Victor VM, Rocha M, Esplugues JV, De la Fuente M: **Role of free radicals in sepsis: antioxidant therapy.** *Curr Pharm Des* 2005, **11**:3141-3158.

149. Kagan VE, Serbinova EA, Stoyanovsky DA, Khwaja S, Packer L: **Assay of ubiquinones and ubiquinols as antioxidants.** *Methods Enzymol* 1994, **234**:343-354.

150. Armstrong JS: **Mitochondrial medicine: pharmacological targeting of mitochondria in disease.** *Br J Pharmacol* 2007, **151**:1154-1165.

151. Murphy MP, Smith RA: **Targeting antioxidants to mitochondria by conjugation to lipophilic cations.** *Annu Rev Pharmacol Toxicol* 2007, **47**:629-656.

152. Graham D, Huynh NN, Hamilton CA, Beattie E, Smith RAJ, Cochemé HM, Murphy MP, Dominiczak AF: **Mitochondria-Targeted Antioxidant MitoQ$_{10}$ Improves Endothelial Function and Attenuates Cardiac Hypertrophy.** *Hypertension* 2009, **54**:322-328.

153. Gane EJ, Orr DW, Weilert F, Keogh GF, Gibson M, Murphy MP, Smith RAJ, Lockhart MM, Frampton CM, Taylor KM: **Phase II study of the mitochondrial antioxidant mitoquinone for hepatitis C [abstract].** *J Hepatol* 2008, **48**:S318.

154. Snow BJ, Rolfe FL, Murphy MP, Smith RA, Lockhart MM, Frampton CM, Taylor KM: **Phase II study of the mitochondrial antioxidant mitoquinone for Parkinson's disease.** *Neurology* 2008, **70**:A483-A484.