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Contents

Contributing authors vii

Preface xiii

General concepts about oxidative stress
    *Ulf Landmesser and Helmut Drexler* 1

Lipoproteins and oxidation
    *Sotirios Tsimikas* 17

Pathogenesis of atherosclerosis
    *Juan Viles-Gonzalez, Juan J. Badimon, Valentin Fuster* 49

The antioxidant hypothesis
    *Charlene Bierl, Marc Forgione, and Joseph Loscalzo* 87

Reactive oxygen species as mediators of signal transduction in cardiovascular diseases
    *Charles Kunsch and Xilin Chen* 103

Biomarkers of oxidant stress in vivo: oxidative modifications of lipids, proteins and DNA
    *Ian A. Blair, John A Lawson, Harry Ischiropoulos and Garret A. FitzGerald* 131

Pharmacological compounds with antioxidant activity
    *Sergey Dikalov and David G. Harrison* 167
Antioxidant nutrients and antioxidant nutrient-rich foods against coronary heart disease
   Michel de Lorgeril and Patricia Salen

Antioxidants and chronic vascular disease: animal studies
   Tillman Cyprus and Domenico Pratico

Synthetic antioxidants and atherosclerosis: human studies
   Martial G. Bourassa and Jean-Claude Tardif

Antioxidants and endothelial function: human studies
   Christian Bingelli, Isabella Sudano, Bernd van der Loo, Francesco Cosentino, Georg Noll, and Thomas F. Lüscher

Antioxidant vitamins and cardiovascular disease
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   Jean-Louis Chiasson, Rémi Rabasa-Lhoret and Ashok K. Srivastava

Anti-inflammatory and antioxidant functions of high density lipoproteins
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Oxidative stress in heart failure
   Douglas B. Sawyer and Wilson S. Colucci

Use of antioxidants in patients with congestive heart failure
   Anique Ducharme, Jean Lucien Rouleau, Michel White

Index

vi Antioxidants and Cardiovascular Disease

Antioxidant nutrients and antioxidant nutrient-rich foods against coronary heart disease
   Michel de Lorgeril and Patricia Salen 195

Antioxidants and chronic vascular disease: animal studies
   Tillman Cyprus and Domenico Pratico 227

Synthetic antioxidants and atherosclerosis: human studies
   Martial G. Bourassa and Jean-Claude Tardif 255

Antioxidants and endothelial function: human studies
   Christian Bingelli, Isabella Sudano, Bernd van der Loo, Francesco Cosentino, Georg Noll, and Thomas F. Lüscher 279

Antioxidant vitamins and cardiovascular disease
   Danielle Hollar and Charles H. Hennekens 305

Antioxidants and restenosis after percutaneous coronary intervention: animal studies
   Eric Durand, Ayman Al Haj Zen, Camille Brasselet, Antoine Lafont 327

Antioxidants and restenosis after percutaneous coronary intervention: human studies
   Martial G. Bourassa and Jean-Claude Tardif 337

Oxidative stress in hypertension
   Ernesto L. Schiffrin and Rhian M. Touyz 363

Oxidative stress in the development of diabetes and its complications
   Jean-Louis Chiasson, Rémi Rabasa-Lhoret and Ashok K. Srivastava 381

Anti-inflammatory and antioxidant functions of high density lipoproteins
   Ryan E. Moore and Daniel J. Rader 399

Oxidative stress in heart failure
   Douglas B. Sawyer and Wilson S. Colucci 437

Use of antioxidants in patients with congestive heart failure
   Anique Ducharme, Jean Lucien Rouleau, Michel White 451

Index 477
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Preface

The role and mechanisms of oxidative stress and of antioxidant molecules in patients with cardiovascular disease have been the subject of intense experimental and clinical research recently. Rapid accumulation of new knowledge in this field since the beginning of the 21st century amply justifies this second edition of the book *Antioxidants & Cardiovascular Disease.*

The generation of reactive oxygen species (ROS) is an unavoidable consequence of life in an aerobic environment. Cells produce ROS as part of their general metabolic activity. ROS are a family of molecules derived from oxygen, and characterized by their high chemical reactivity and ability to act as oxidants. ROS encompass free radicals (species containing highly reactive unpaired electrons) such as superoxide (O2-) and hydroxyl radicals (OH), as well as other molecules such as hydrogen peroxide (H2O2) and peroxynitrite (ONOO), which are not free radicals, but can also act as oxidizing agents in biological systems. Under physiological conditions, there is a balance between ROS generation and the activity of enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (glutathione, alpha-tocopherol, ascorbate, thioredoxin) antioxidant defences that decrease ROS concentrations. ROS are normally produced in low concentrations and exert important physiological functions in the vessel wall. However, increased production of ROS or decreased antioxidant defences result in excess production of ROS, a condition referred to as oxidative stress. Oxidative stress can lead to free radical-induced oxidation and damage to bio-molecules such as lipids, DNA and proteins. ROS-mediated cellular damage has been associated with the pathogenesis of many diseases.
including Alzheimer’s disease, rheumatoid arthritis, asthma, diabetes and especially cardiovascular disease.

Major cardiovascular risk factors, such as hypertension, dyslipidemia, diabetes and smoking, are associated with a marked increase in vascular ROS production. Increased ROS induce significant tissue damage and modification of lipids and proteins in the vessel wall. Over two decades ago, the antioxidant hypothesis focused mainly on the oxidative modification of LDL rendering it more atherogenic to promote foam cell formation in the intima. Although the exact mechanisms leading to LDL oxidation in vivo are still not entirely understood, it appears to be one of the earliest atherogenic changes leading to progression of atherosclerosis. In addition, oxidized LDL is intimately involved in the transition of stable atherosclerotic lesions to vulnerable plaques and plaque disruption. A variety of lipid and protein modifications of LDL, which are generated from lipid peroxidation, make it atherogenic. However, LDL oxidation alone may not explain the complex relation of oxidative stress and atherosclerosis.

Atherosclerosis originates from endothelial dysfunction and inflammation. Increased ROS production is a major cause of endothelial dysfunction in experimental and clinical atherosclerosis. Endothelial dysfunction leads to a rapid decrease in nitric oxide (NO) production or availability, due in part to inactivation of NO by superoxide. In addition to its vasodilator effect, NO protects against vascular injury, inflammation and thrombosis. Endothelial dysfunction is a strong independent predictor of future cardiovascular events in patients with cardiovascular risk factors, coronary artery disease and acute coronary syndromes. ROS are involved in endothelial and vascular smooth muscle cell pro-inflammatory signaling, particularly in the regulation of endothelial adhesion molecules (VCAM-1) and chemokine (MCP-1) expression. Moreover, ROS are involved in signaling cascades (redox signaling) leading to vascular pro-inflammatory and pro-thrombotic gene expression involving the transcription factor NF-kappa B. Finally, ROS activate matrix metallo-proteinases (MMPs), contributing to plaque instability and rupture.

One of the most convincing arguments for a major role of oxidative stress in the pathogenesis of atherosclerosis and cardiovascular disease has been the documentation, in numerous experimental and clinical studies, that antioxidant molecules can reverse the atherosclerotic process and can reduce subsequent cardiovascular events. There is a consensus, based on several recent negative clinical trials, that supplementation with natural antioxidants such as vitamins (vitamin A, C, and E) and minerals (zinc and selenium) should not be recommended routinely. The reasons underlying the lack of efficacy of these natural antioxidants in patients with cardiovascular disease or cardiovascular risk factors are still poorly understood. On the other hand,
a diet rich in antioxidant-macro-nutrients, particularly fruits and vegetables, is recommended for all individuals, and some types of diets such as the Mediterranean diet, have been shown to be highly beneficial in the prevention of cardiovascular events in patients with coronary heart disease. Some of the beneficial effects of aspirin, beta-blockers, calcium channel blockers, statins and ACE inhibitors (or angiotensin receptor blockers) in patients with cardiovascular disease are potentially related to their known antioxidant properties. These relationships must be more clearly delineated, however. Other potentially beneficial candidates also deserve to be investigated further. For example, acarbose has been shown to be beneficial in patients with diabetes mellitus. Probucol, a potent antioxidant, has been shown in numerous experimental and clinical studies to prevent atherosclerosis and restenosis after percutaneous coronary interventions. This agent is no longer in clinical use because of unacceptable side effects. An analog of probucol, AGI-1067, has recently been shown by our group to possess antioxidant properties which are comparable to those of probucol, but without the undesirable side effects of the latter. AGI-1067 has been shown to have similar beneficial effects on prevention of coronary atherosclerosis and coronary restenosis in humans and it is currently being investigated for its ability to reduce long-term clinical events in patients with coronary heart disease. Finally, this still represents a very novel approach, which may ultimately lead to major prevention of atherosclerosis and its vascular complications.

In summary, this book addresses a complex but very timely and fascinating problem in cardiovascular medicine. It is written by recognized experts in the fields of atherosclerosis and antioxidants. It should be of interest not only to academicians but also to practicing physicians. The first five chapters review the general concepts of oxidative stress and their relationship to lipid metabolism, endothelial dysfunction, genetics and transcriptional factors. The next seven chapters describe recently defined markers of oxidative stress, pharmacological compounds with antioxidant activity, natural antioxidants found in micronutrients and in nutrient-rich diets, and reviews the recent evidence for their efficacy or lack of efficacy in patients with cardiovascular disease or cardiovascular risk factors. The last seven chapters discuss the potential therapeutic benefits of antioxidants in a number of cardiovascular conditions which include atherosclerosis, restenosis after percutaneous coronary intervention, major cardiovascular risk factors such as hypertension, diabetes mellitus and dyslipidemia, and left ventricular dysfunction and congestive heart failure.
The editors are grateful to the authors and co-authors of the different chapters of the book, and wish to thank them for their excellent contributions.

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Chapter 1

GENERAL CONCEPTS ABOUT OXIDATIVE STRESS

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Introduction

This chapter focuses on general concepts about the role and mechanisms of oxidative stress in atherosclerosis and its resultant cardiovascular events. There is convincing evidence, from both experimental and clinical studies, that the major cardiovascular risk factors are associated with a marked increase of vascular production of reactive oxygen species (ROS) and lipid oxidation. To what extent, however, ROS contribute causally to the pathophysiology of human cardiovascular disease is an area of intense ongoing research.

Whereas initially the oxidative modification hypothesis of atherosclerosis was focused on the oxidative modification of low-density lipoprotein (LDL), rendering it more atherogenic to promote foam cell formation in the intimal space, a large body of evidence has now underscored numerous additional, likely important, oxidative events in cardiovascular disease.

Increased ROS production has been identified as a major cause of endothelial dysfunction in experimental and clinical atherosclerosis, that is associated with a rapid loss of anti-atherogenic and anti-inflammatory properties of endothelium-derived nitric oxide (NO*), in part due to increased inactivation of NO* by superoxide. Moreover, ROS have been shown to be critically involved in signaling cascades leading to vascular pro-inflammatory and pro-thrombotic gene expression, in part involving the transcription factor nuclear factor(NF)-kappaB. Redox signaling may represent a highly localized and specific role of ROS.
In addition, ROS are potent activators of matrix metallo-proteinases (MMPs) that may represent a mechanism whereby ROS could contribute to plaque destabilization and rupture.

The refined understanding of the complexity of oxidative events, that have different cellular localization and involve different ROS as well as potential physiological functions of ROS need to be taken into account when antioxidative treatment strategies are considered.

**Reactive oxygen species (ROS)**

ROS encompass a variety of diverse chemical species, including both free radicals (containing highly reactive unpaired electrons), such as superoxide (O$_2^*$) and hydroxyl radicals (OH$^*$), and other molecular species, such as hydrogen peroxide (H$_2$O$_2$) and peroxynitrite (ONOO$^-$). Accordingly, some of these species, such as superoxide and hydroxyl radicals, are extremely unstable, whereas others, like hydrogen peroxide, are freely diffusible and relatively long-lived$^{(1)}$.

Of note, besides the suggested pathological role of increased ROS production in cardiovascular diseases as discussed below and in other diseases, such as neurodegenerative disease$^{(2)}$, there are likely also physiologically important functions of ROS. For example, ROS play a role in cellular proliferation and host defense. Increased vascular production of ROS, however, may contribute to important processes in the pathophysiology of cardiovascular disease.

**Evidence for increased oxidative stress in cardiovascular disease**

Over the past decade, accumulating data from both experimental studies and studies in patients with coronary disease or cardiovascular risk factors, such as hypercholesterolemia, hypertension, diabetes and smoking, have convincingly demonstrated that there is an association of cardiovascular risk factors with an increased vascular production of ROS. In animal studies, an increased vascular production of ROS, in particular of superoxide, has been shown directly by chemiluminescence and electron spin resonance spectroscopy measurements$^{(3-7)}$. In humans, increased oxidative stress has been demonstrated in patients with cardiovascular risk factors or coronary disease by increased levels of F$_2$ isoprostanes, stable, free radical-catalyzed products of arachidonic acid reflecting lipid peroxidation in vivo$^{(8-11)}$. In addition, the urinary excretion of the F$_2$-isoprostane 8-iso-prostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) was correlated with the number of cardiovascular risk factors$^{(12)}$. 
Figure 1. Reactive oxygen species (ROS) and oxidant and antioxidant enzyme systems involved in the production and detoxification of ROS are shown. Cardiovascular risk factors, such as hypercholesterolemia, hypertension, diabetes and smoking, increase the vascular production of ROS, in particular superoxide. This may be mediated by some of the oxidant enzyme systems shown. Superoxide reacts then rapidly with nitric oxide (NO·) resulting in reduced NO· bioactivity with loss of its vasculoprotective functions and formation of peroxynitrite (ONOO·), that may contribute to lipid oxidation. Superoxide dismutase converts superoxide to hydrogen peroxide. Myeloperoxidase and lipoxygenase are enzyme systems that are likely involved in lipid oxidation. Myeloperoxidase produces hypochlorous acid by using hydrogen peroxide. ROS, in particular hydrogen peroxide, have been suggested to play a critical role in pro-inflammatory signaling.

Moreover, in human atherosclerotic coronary arteries, an intense staining of superoxide has been shown in the plaque shoulder\(^{(13)}\), that is rich in macrophages and prone to rupture, that is thought to underly a majority of clinical cardiovascular events.

Furthermore, increased oxidant stress in human cardiovascular disease has been suggested by several studies analyzing the effect of antioxidants on endothelial dysfunction in patients with coronary disease or cardiovascular risk factors. In these studies structurally different antioxidants, in particular a high local dose of the antioxidant vitamin C, could improve endothelium-dependent vasodilation\(^{(14-21)}\). It is important to note, however, that most of
these studies have used a high-local dose of vitamin C. The local concentration of vitamin C in these studies may exceed up to 100-fold the plasma concentrations achieved by oral treatment with vitamin C. It is therefore questionable whether vitamin C as administered in large scale clinical trials (i.e. 250 mg vitamin C/day in the Heart Protection Study\textsuperscript{22}, can achieve similar effects on endothelial function. In fact, recent studies have suggested that a high local dose of vitamin C is required to impact on endothelial function\textsuperscript{23} and long-term oral treatment with 800 IE of vitamin E and 1000 mg of vitamin C per day had no effect on endothelial function in patients with coronary disease\textsuperscript{24}.

Lipid oxidation

Brown and Goldstein have originally put forward the concept that circulating low-density lipoprotein (LDL) must undergo some kind of structural modification before it becomes fully proatherogenic\textsuperscript{25}. Several different modifications of LDL have been described, including oxidation, aggregation, enzymatic modification, and possibly others, that convert LDL to a form that is recognized by one or more of the macrophage scavenger receptors. The best studied of these and the one for which there is good in vivo evidence is oxidative modification\textsuperscript{26}. Oxidation of LDL modifies its bioactivity extensively in vitro, conferring properties associated with disease pathogenesis. The oxidative modification hypothesis will be discussed in more detail in chapter 4.

This concept alone, however, may not explain the complexity of oxidative stress and atherosclerosis. For example, Witting et al.\textsuperscript{27} observed that the antioxidant probucol and its metabolite bisphenol had a similar effect on vascular lipid oxidation, but the effect of the antioxidant probucol on atherosclerotic lesion formation was more pronounced. Although this study has several limitations\textsuperscript{28}, it may point to the notion that other oxidant mechanisms are also important in atherosclerosis.

Endothelial dysfunction

Originally, oxidative stress was primarily implicated in atherosclerosis by damaging lipids. Whereas oxidized LDL may contribute to endothelial dysfunction, it has now been recognized that oxygen radicals may directly cause endothelial dysfunction, i.e. by reducing endothelial NO\textsuperscript{*} bioavailability\textsuperscript{29,30}. In particular, superoxide (O$_2^-$) reacts rapidly with NO\textsuperscript{*}, resulting in formation of peroxynitrite and loss of NO\textsuperscript{*}’s bioactivity. Endothelial dysfunction in experimental atherosclerosis could be reversed by administration of superoxide scavengers, suggesting that increased vascular superoxide production represents a major cause of endothelial dysfunction\textsuperscript{3,4}. Recently it has been recognized that ROS, and especially
peroxynitrite, can oxidize tetrahydrobiopterin, a critical co-factor for endothelial NO\(^+\) synthase\(^{4,31}\), that leads to dysfunction ("uncoupling") of the enzyme.

**Proposed pathophysiological mechanisms of oxidative stress and cardiovascular disease**

*Figure 2. Proposed mechanisms of how increased vascular ROS production, as stimulated by cardiovascular risk factors, may contribute to cardiovascular disease. Initially, the oxidative modification hypothesis of atherosclerosis was focused on the oxidative modification of LDL cholesterol rendering it fully pro-atherogenic to promote foam cell formation and vascular inflammation. Increased ROS production has now also been identified as a major cause of endothelial dysfunction, in part resulting from increased inactivation of endothelial NO\(^+\) by superoxide. In addition, accumulating data indicate ROS as critical signaling molecules involved in vascular pro-inflammatory and pro-thrombotic gene expression, i.e. endothelial leukocyte adhesion molecule and chemokine expression. ROS are potent activators of matrix metallo-proteinases that are expressed in the shoulder region of atherosclerotic plaques, that could importantly contribute to plaque destabilisation and rupture, thought to underlie a large number of clinical cardiovascular events.*
NO* not only produces vasodilation, but also has anti-atherogenic properties\(^{32-34}\). These include inhibition of leukocyte adhesion molecule expression, inhibition of platelet aggregation and prevention of smooth muscle cell proliferation. Thus, the loss of NO* not only alters vascular tone, but also likely contributes importantly to the development, progression and clinical complications of atherosclerosis. This concept is supported by a growing number of clinical studies indicating that the degree of endothelial dysfunction, measured as impaired endothelium-dependent vasomotion, represents a strong and independent predictor of future cardiovascular events in patients with cardiovascular risk factors, coronary disease, acute coronary syndromes and peripheral artery disease\(^{35-40}\). In fact, the effect of a high local dose of the antioxidant vitamin C on endothelium-dependent vasodilation has been shown to predict future cardiovascular events in a study following 179 patients with coronary disease\(^{41}\), suggesting that oxidative stress-induced endothelial dysfunction has prognostic implications.

**ROS and vascular inflammation**

There is accumulating evidence supporting the concept that, both development of atherosclerotic lesions and clinical cardiovascular complications of atherosclerotic disease, are related to vascular inflammation\(^{42,43}\). In experimental studies, it has been shown that inhibition of leukocyte adhesion and infiltration, regulated by leukocyte adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), and chemokines, such as monocyte chemoattractant protein (MCP-1), prevents atherosclerotic lesion development\(^{44-46}\). Notably, it has been suggested that ROS are importantly involved in endothelial and vascular smooth muscle cell (VSMC) pro-inflammatory signaling, i.e. the regulation of endothelial adhesion molecules and chemokine expression, that may represent an important link of oxygen radicals and vascular disease\(^{47}\). The stimulating effect of cytokines, such as TNF-alpha and interleukin 1, or angiotensin II on endothelial expression of the adhesion molecule VCAM-1\(^{48,49}\) or the chemokine MCP-1\(^{50,51}\) was suppressed by different ROS scavengers, suggesting that ROS are critical mediators of pro-inflammatory signaling in the endothelium. Some of these redox-sensitive pro-inflammatory signaling pathways may involve the transcription factor NF-kappaB\(^{52}\).

In contrast, endothelial NO* production has been shown to exert important anti-inflammatory effects. NO* has been shown to reduce endothelial adhesion molecule and chemokine expression in vitro\(^ {53,54}\). Moreover, NO* synthase gene therapy rapidly reduces hypercholesterolemia-induced leukocyte adhesion molecule expression, i.e. VCAM-1, and ameliorates monocyte infiltration into the arterial wall of cholesterol-fed rabbits\(^ {55}\). The loss of endothelial NO* as a result of increased ROS
production may therefore represent an important mechanism whereby oxidative stress promotes a pro-inflammatory phenotype of the endothelium.

Of note, recent evidence suggests that "redox signaling", i.e. via kinase signaling pathways may be distinct from "oxidative stress," and could be mediated by discrete, localized redox circuitry\(^{(56)}\). Taken together, there are several important links between increased endothelial oxidative stress and ROS production with vascular inflammation. Furthermore, inflammation per se may augment vascular oxidative stress\(^{(57)}\). Therefore, the observed association of vascular oxidative stress and inflammatory markers in patients with coronary disease\(^{(58)}\) may indicate that oxidative stress promotes vascular inflammation, but also that inflammation augments oxidant stress, a potential vicious cycle.

**ROS and thrombosis**

Increased ROS production has been shown to be critically involved in the up-regulation of tissue factor in VSMCs in response to activated platelets\(^{(59)}\). Tissue factor (TF) initiates the extrinsic coagulation cascade leading to thrombin formation. Thrombin induces tissue factor mRNA in human VSMCs by a redox-sensitive, NAD(P)H oxidase dependent mechanism, that may contribute to prolonged procoagulant activity and enhanced thrombogenicity at sites of vascular injury\(^{(60)}\). These findings suggest that vascular pro-thrombotic gene expression is redox-sensitive that may link increased oxidant stress to vascular thrombotic events.

In addition, endothelial NO\(^{\ast}\) has several important anti-thrombotic effects and inhibits platelet adhesion to the endothelium, an effect that is lost after oxidative inactivation of NO\(^{\ast}\). Taken together, ROS have been identified as important mediators of vascular pro-inflammatory and pro-thrombotic gene expression that together with oxidative inactivation of endothelial NO\(^{\ast}\) may promote a pro-inflammatory and pro-thrombotic phenotype of the endothelium.

**ROS activate matrix metallo-proteinases: relevance to plaque instability?**

Plaque rupture is the most common type of plaque complication, and is thought to account for \(\approx 70\%\) of fatal acute myocardial infarctions and/or sudden coronary deaths\(^{(61,62)}\). The expression of MMPs, i.e. MMP-2 (gelatinase A, which degrades collagen IV) and MMP-9 (gelatinase B, which acts on collagen I fibers) that are secreted by macrophages and vascular myocytes, is increased in the rupture-prone shoulders of atherosclerotic plaques\(^{(63)}\). Notably, ROS have been shown to importantly modulate MMPs, that could contribute to lesion instability\(^{(64)}\). It has been demonstrated that pro-MMP-9 and pro-MMP-2 from VSMCs are activated in vitro by ROS\(^{(64)}\). Furthermore, cyclic strain-induced MMP-2 expression in VSMCs was
dependent on activation of the oxidant enzyme NAD(P)H-oxidase\textsuperscript{65}. Sorescu et al. have recently demonstrated particularly high levels of superoxide in the shoulder region of human coronary atherosclerotic plaques\textsuperscript{13}. Thus, MMP activation by ROS could contribute to plaque rupture.

**Other mechanisms linking ROS and cardiovascular disease**

There are additional mechanisms that may link increased oxidant stress and cardiovascular disease. ROS have been suggested to play a major role in mediating VSMC and cardiomyocyte hypertrophy in response to stimuli such as angiotensin II or mechanical stretch, that may contribute to vascular and cardiac remodeling processes.

Another interesting novel concept that needs to be further explored suggests a link between increased oxidative stress and insulin resistance\textsuperscript{66}. In rats over-expressing angiotensin II, superoxide scavenging could improve skeletal muscle insulin-dependent glucose uptake and whole body insulin resistance\textsuperscript{67}, indicating that oxidative stress plays an important role in angiotensin II mediated insulin resistance.

**Sources of ROS in cardiovascular disease**

There are numerous potential sources of ROS that have been studied intensely over the past years (figure 1), and may play a different role for several cardiovascular risk factors. With respect to lipid oxidation, it is still not entirely understood what are the exact mechanisms leading to LDL oxidation in vivo. There is, however, evidence to suggest that 12/15-lipoxygenase may initiate lipid peroxidation\textsuperscript{68,69}. Notably, when 12/15-lipoxygenase deficient mice are crossed with animals deficient in ApoE, atherosclerotic lesion formation is dramatically inhibited\textsuperscript{68,69}. Myeloperoxidase (MPO)-generated ROS, i.e. HOCl, may represent a plausible pathway for converting LDL into an atherogenic form\textsuperscript{70,71}. Notably, increased MPO serum levels could identify patients at risk for cardiac events who presented with chest pain in the absence of myocardial necrosis\textsuperscript{72} or an acute coronary syndrome\textsuperscript{73}.

With respect to ROS-induced impairment of endothelial function, that may have important prognostic implications, the following three superoxide producing oxidant enzyme systems have received most attention, the vascular NAD(P)H oxidase, xanthine oxidase and uncoupled endothelial nitric oxide synthase (figure 3)\textsuperscript{29,74}. Increased vascular activity of the NADPH oxidase and xanthine oxidase have been demonstrated in experimental and clinical atherosclerosis\textsuperscript{6,13,75,76}. Of note, a deficiency of the cytosolic NAD(P)H oxidase component p47phox was associated with a
markedly reduced atherosclerotic lesion formation in the apoE-deficiency mouse model of atherosclerosis(77).

Figure 3. Oxidant and antioxidant enzyme systems are shown that have been implicated as important sources of increased vascular superoxide production in atherosclerosis leading to rapid inactivation of NO causing endothelial dysfunction. Experimental and clinical evidence suggests an activation of the vascular NAD(P)H oxidase system. This may further promote endothelial oxidant stress by increasing endothelial xanthine oxidase levels(61) and by causing uncoupling of the endothelial nitric oxide synthase (eNOS) due to oxidative inactivation of the eNOS cofactor tetrahydrobiopterin (H4B)(31). In advanced atherosclerosis the vascular activity of the superoxide scavenging enzyme extracellular superoxide dismutase (ccSOD) has been shown to be reduced(20).

More recent studies suggest, that intracellular ROS production may also be derived from the mitochondria. The production of mitochondrial superoxide radicals occurs primarily at two discrete points in the electron transport chain, namely at complex I (NADH dehydrogenase) and at complex III (ubiquinone–cytochrome c reductase)(3). This could play a role in atherosclerosis and hyperglycemia(78,79).

Besides increased activation of oxidant enzyme systems in atherosclerosis, a reduced activity of several antioxidant scavenging enzyme systems has been observed in advanced human atherosclerotic disease. In
particular, extracellular superoxide dismutase<sup>20</sup> and glutathione peroxidase activities<sup>60</sup> have been shown to display reduced activities in human atherosclerotic arteries.

**Summary and conclusion**

In summary, there is convincing evidence of an association of increased oxidant stress and cardiovascular risk factors or atherosclerosis in experimental studies and in humans. There is an increasing understanding of the complexity of oxidant mechanisms that may importantly contribute to key pathophysiological processes such as vascular inflammation, thrombosis and plaque rupture, far beyond oxidative modification of lipids. To what extent these mechanisms play a causal role for the development, progression and the complications of human atherosclerosis is an exciting and important area of ongoing intense research.
General concepts about oxidative stress

References

General concepts about oxidative stress


