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# OXIDATIVE STRESS IN CARDIOVASCULAR DISEASES

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## 11.1 INTRODUCTION

Cardiovascular diseases comprise several complex conditions, such as inflammatory, cerebrovascular, ischemic, hypertensive, and rheumatic diseases. Collectively, cardiovascular diseases contribute to 30% of all causes of mortality around the world, whereas ischemic heart diseases alone represent ~13% (Fig. 11.1). Besides the invaluable loss of lives, cardiovascular diseases also contribute to economic burden [1].

As for all complex diseases, there is not a single cause for cardiovascular diseases. However, several factors have been described as risk factors for the development of cardiovascular diseases [2]. Among these are high-fat diet, smoking, alcohol consumption, age, sex, and race. Interestingly, despite the broad range of manifestation, atherosclerotic plaque formation could be considered the common starting point for all cardiovascular diseases. It has a chronic inflammatory component, but its effects may manifest acutely, as stroke or acute myocardial infarction.

Reactive oxygen species (ROS), reactive nitrogen species (RNS), and oxidative stress are the basis of many hypotheses about the development of cardiovascular diseases. Both ROS and RNS have important physiological functions as chemical weapons in immune cells, vascular tonus regulators, and signaling molecules [3]. Conversely, ROS and RNS can have roles as bad agents during the onset and progression of cardiovascular diseases. Why does this happen? Mainly because risk factors are supposed to cause imbalance, either favoring ROS/RNS production or impairing antioxidant mechanisms. Thus it is easy to understand why smoking, which can decrease antioxidant defenses and generate reactive aldehydes, represents a risk factor for cardiovascular diseases [4].

The discovery of the imbalance between ROS/RNS generation and its consumption in cardiovascular diseases led to the false belief that the Holy Grail was found and that antioxidant therapy could be life saving. The truth is that researchers have not found a common ground and the evidence is still controversial, as we shall see further in this chapter.

## 11.2 FREE RADICALS—ORIGINS AND FATES

Free radicals are molecules or atoms that are able to have a free existence despite having one unpaired electron, which confers a high reactivity. Among the most-studied free radicals are superoxide ($O_2^{\bullet -}$), hydroxyl ($HO^{\bullet}$), peroxyl ($RO_2^{\bullet}$), and nitric oxide ($NO^{\bullet}$). Reactive oxygen species (ROS) are a wide class of reactive molecules that includes free radical and other nonradical molecules that are also highly reactive. Also considered ROS are molecules such as hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), ozone ($O_3$), and peroxynitrite ($ONOO^-$) [3].

Superoxide is the most abundant free radical in cells. Despite this, it is not highly reactive. Even so, it can act as a signaling molecule or as a substrate for $H_2O_2$ and peroxynitrite generation. Perhaps the main source of superoxide is the electron transport chain in mitochondria, since it has been estimated that 1–3% of total oxygen is leaked as

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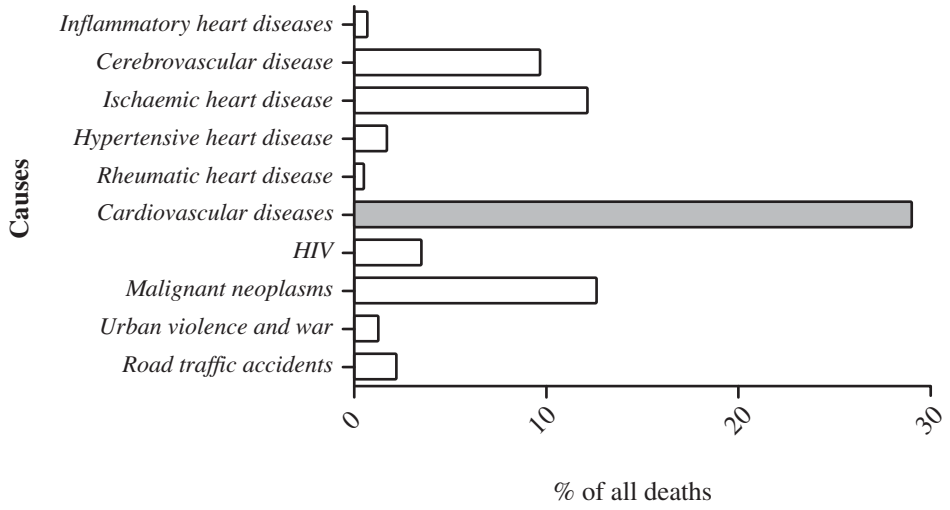


**Fig. 11.1** Estimated deaths by cause worldwide. Data from World Health Organization for 2004.

superoxide in physiological processes. NAD(P)H oxidase oxidizes the reducing agent NAD(P)H and transfers the electron to the molecular oxygen, yielding superoxide. It is mainly employed as a chemical weapon by neutrophils and macrophages, but it is highly important in endothelial and myocardial dysfunction [5]. Xanthine oxidoreductase is also an important source of superoxide that oxidizes xanthine or hypoxanthine in uric acid and transfers the electron to $NAD^+$ rather than to $O_2$. In some cases, such as after myocardial infarction, this enzyme suffers spatial modifications that make it an oxidase that transfers the electron to oxygen rather than to $NAD^+$ (see Fig. 11.2).

Superoxide dismutase is the enzymatic defense against superoxide produced in cells and exists in three isoforms: a manganese-dependent mitochondrial isoform (Mn-SOD), a copper- and zinc-dependent cytosolic isoform (Cu,Zn-SOD), and an extracellular tetrameric Cu,Zn-dependent isoform (EC-SOD). These enzymes act on superoxide, and the product is hydrogen peroxide, according to reaction (1) in Figure 11.3.

Although hydrogen peroxide is not a free radical, it is important as a signaling molecule, since it is highly diffusible. Moreover, the reaction of hydrogen peroxide with transitional metals (e.g. $Cu^+$, $Fe^{2+}$) yields the most reactive and harmful radical in biological systems, hydroxyl. To avoid hydroxyl generation, the antioxidant enzymes such as catalase and glutathione peroxidase (GPx) are able to use hydrogen peroxide to generate water and molecular oxygen, according to reactions (2) and (3) in Figure 11.3, respectively. This chain of events is depicted in Figure 11.2.

NO is a diffusible gas that has important physiological roles. The source of NO can be any of the three isoforms of nitric oxide synthase NOS): neuronal (nNOS), endothelial (eNOS), or inducible (iNOS). NOS converts the amino acid L-arginine into NO and another amino acid, L-citrulline. Certain cofactors are needed, such as FAD, FMN, and tetrahydrobiopterin ($BH_4$). NOS can also produce superoxide in the presence

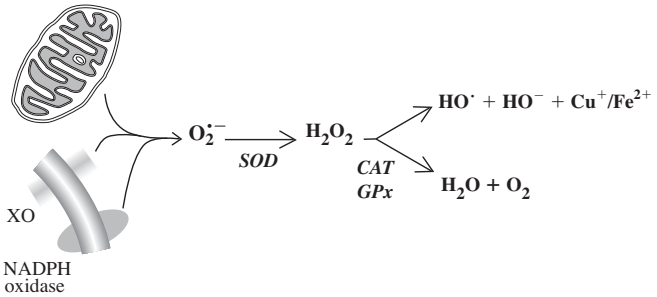


**Fig. 11.2** The three main sources of superoxide radical in cells are mitochondria, xanthine oxidase (XO), and NAD(P)H oxidase. After superoxide production, a chain of coupled enzymatic reactions leads to the consumption of reactive oxygen species to avoid oxidative stress. In the case of hydrogen peroxide accumulation, the reaction with transitional metals generates the most harmful radical, hydroxyl.

(1) $2\ O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$

(2) $2\ H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$

(3) $H_2O_2 + 2\ GSH \xrightarrow{GPx} 2\ H_2O + GSSG$

**Fig. 11.3** The main enzymatic antioxidant system is shown. Equation (1) represents the action of superoxide dismutase (SOD) on superoxide radical. Equation (2) represents the action of enzyme catalase (CAT) on SOD product hydrogen peroxide. Equation (3) represents the action of glutathione peroxidase (GPx) on the same hydrogen peroxide. The difference is the necessity of reduced glutathione (GSH) as cofactor.

of low levels of $BH_4$, which may contribute to atherosclerosis generation, as we shall see. NO exerts its signaling effects binding to iron-heme groups in guanylate cyclase. The signaling effects are best comprehended as antihypertensive, since NO can stimulate cGMP synthesis in smooth muscle of blood vessels and thus induce relaxation and lower blood pressure. Besides its physiological roles, NO can react with superoxide to give peroxynitrite. This nonradical ROS is able to oxidize reduced cysteine, lipids, and DNA. The most-studied damage in proteins is the modification of tyrosine to 3-nitrotyrosine.

To summarize, in case of any disturbance between free radical production and its consumption by the antioxidant system, harmful species can arise. These species are prone to react with any molecule, such as DNA, protein, and lipids, which may lead to errors in transcriptional activity, signaling, and membrane permeability. When this damage overcomes the capability of the organism for repairing it, oxidative stress takes place.

## 11.3 CARDIOVASCULAR DISEASES

Although cardiovascular diseases might vary in their symptoms, endothelial dysfunction, hypertension, and atherosclerosis appear to be at the core of all cardiovascular complications. The formation of atheromatous plaques can increase blood pressure and vice versa. Narrowing of blood vessels may lead to ischemic events that increase free radical production that damages cells, contributing to endothelial and cardiac dysfunction. From development to progression, redox imbalance and subsequent oxidative stress play a crucial role in all these diseases.

### 11.3.1 Atherosclerosis

Atherosclerosis is characterized by thickening of the arterial wall due to macrophage infiltration, smooth muscle migration, and cholesterol deposition. Symptoms can occur late in the course of atherosclerotic plaque formation and are mainly due to the narrowing of vascular lumen or to the rupture of plaque, which leads to the obstruction of a small capillary. Regardless of the type of manifestation, the final event is the decrease of blood supply to the organ. Initiation and progression of atherosclerosis involve complex mechanisms, and many aspects are still not understood.

Again, the participation of free radical in atherosclerotic plaque formation seems to be a unifying theory, since all known risk factors contribute to ROS/RNS generation. For example, $F_2$-isoprostanes, a well-accepted marker of lipid oxidation, nitrated proteins, and lipid hydroperoxides, are all associated with increased risk for developing atherothrombotic diseases. Moreover, several stimuli that in some way participate in atherosclerotic plaque formation also trigger NAD(P)H oxidase-mediated superoxide production [6]. For instance, fluid shear stress, the frictional force generated by blood flow over the vascular endothelium, is a major factor in atherogenesis. Branched and curved arteries are considered prone to lesion, whereas straight arteries are less prone. Interestingly, cultured endothelial cells subjected to shear stress in vitro produce twice as much superoxide and monocyte binding than cells subjected to laminar stress. Also, this effect does not occur in cells deficient in p47 protein, a component of NAD(P)H oxidase complex [7].

NO can be synthesized constitutively by endothelial isoform of NOS (eNOS) or, after an induction, by the inducible isoform (iNOS). The former participates in vascular tonus regulation, antagonism of platelet aggregation, and leukocyte binding, whereas the latter is involved in the proinflammatory process [8]. The inducible isoform is detected in human atheroma as soon as monocyte infiltration and foam cell differentiation starts [9]. Also, it was recently shown that iNOS colocalizes with other proatherosclerotic factors (ACE and $AT_1$) whereas eNOS vanishes in endothelial cells from autopsy [10].

Despite being considered essential as antiatherosclerotic, eNOS deletion in mice is not enough to induce plaque formation because nNOS compensatory activity can keep NO production in a normal range [11]. It is noteworthy that eNOS can undergo a process called uncoupling in which the reduction of molecular oxygen is no longer coupled with L-arginine oxidation. Thus eNOS generates superoxide rather than NO [12]. Among several factors that could lead to eNOS uncoupling, $BH_4$ deficiency and presence of asymmetrical dimethyl arginine (ADMA) appear to be the main factors. $BH_4$ acts by improving L-arginine binding and donating the second electron to oxygen reduction. Thus, in the absence of $BH_4$, eNOS still partially reduces oxygen without oxidizing L-arginine, generating superoxide [12]. ADMA may act as an eNOS inhibitor by competitive inhibition of L-arginine oxidation or may uncouple eNOS activity. In fact, it has been found that coronary artery disease patients carrying the low-activity form of the rate-limiting enzyme in $BH_4$ synthesis (GTP cyclohydrolase I) produce more superoxide and oxidized low-density lipoprotein (oxLDL) and demonstrate diminished response to acetylcholine induced vasorelaxation [13]. Plasma levels of ADMA were inversely related to acetylcholine-induced vasorelaxation in saphenous veins collected from patients undergoing coronary bypass surgery, whereas it was directly related to superoxide production by both saphenous vein and internal mammary artery [14].

Oxidative stress can have two main consequences: First, after oxidizing catalytically active or structural biomolecules, it can lead to a loss or gain of function and thus precipitate some dysfunction. Second, oxidatively modified molecules become a new class of molecules, and some of them can bind to membrane receptors and trigger signals. A good example is the increased level of oxidation of fibrinogen seen in patients with coronary artery disease. Formation of NO-derived species can lead to 3-nitrotyrosine formation in fibrinogen. The consequence is an accelerated and aberrant fibrin polymerization that presents resistance to lysis [15–17].

In addition to oxidative modification, a process called glycation also occurs in diabetes and is believed to contribute to the generation of atherosclerotic plaque. Briefly, glycation consists in a series of processes that involve a nonenzymatic reaction between a reducing sugar (e.g., glucose) and amino groups of proteins to form a reversible Schiff base. This reaction is prone to some arrangements, and free radicals are part of this complex process. After that, a new and heterogeneous class of molecules called collectively advanced glycation end-products (AGEs) arises [18]. Among the epitopes generated are $N^{\varepsilon}$-(carboxymethyl)lysine (CML), pentosidine, and GA-pyridine. CML has been extensively studied and has been associated with a variety of inflammatory processes. Immunohistochemical studies have already found CML and GA-pyridine in human atherosclerotic lesions both intracellularly in foam cells and in the extracellular environment [19, 20].

Whether AGE formation is a consequence of an inflammatory process or it has any active role in atherosclerotic plaque generation seems not to be under debate nowadays. AGEs constitute a class of signaling molecules that exert their effects mainly through receptor for AGE (RAGE). RAGE is a transmembrane receptor belonging to the immunoglobulin superfamily that acts as a multiligand receptor [21]. Among the RAGE ligands are AGEs, S100/calgranulin proteins, high mobility box group 1 protein (HMGB1), and oxLDL [22]. One of the best characterized consequences of RAGE activation is the stimulation of free radical-generating pathways, which in turn will lead to the generation of additional AGEs and oxLDL. Moreover, RAGE stimulation triggers intracellular signaling that culminates with NF-κB translocation to the nucleus, which is largely described as a transcriptional factor associated with proinflammatory response [23]. In line with this, diabetic mice lacking RAGE showed less incidence of atherosclerotic plaque formation in aorta than mice expressing RAGE [24]. The central role of RAGE in atherosclerosis development suggests that this receptor should be seen as a therapeutic target in that condition. In fact, this suggestion has already been tested in animal models with good results [25], but unfortunately there are no current clinical trials testing this approach.

Environmental factors are well-established risk factors for atherosclerosis development, and many of them are linked to oxidative stress generation inside the body or are themselves reactive species. Cigarette smoking is one of the most important and modifiable risk factors associated with the generation and progression of cardiovascular diseases [26]. Cigarette smoke contains high concentrations of free radicals, such as superoxide, NO, carbon-centered radicals, and other toxic compounds, such as acrolein and formaldehyde. Even passive smokers have increased risks for cardiovascular diseases that are mediated by increased oxidative stress [27]. Guinea pigs subjected to cigarette smoking for 28 days develop atherosclerosis, which could be avoided with dietary vitamin C supplementation [28]. This rationale indicates that free radicals are important in the onset and progression of atherosclerotic plaque.

However, antioxidant supplementation in humans (smokers and nonsmokers) has yielded contradictory results. Vitamin E supplementation (800 IU) seems to be protective in a cohort of atherosclerotic patients, decreasing the risk of nonfatal myocardial infarction by half [29]. Despite this, a study with a large cohort followed up for 6 years demonstrated that vitamin E alone has no effect on atherosclerosis progression [30]. Even so, when associated with vitamin C, the antioxidant therapy was able to diminish the intima-media thickening in the common carotid artery of smoker subjects [30].

Diet is another source of toxic compounds that could contribute to atherosclerosis development. AGEs can be generated during the cooking process, and after ingestion they can reach the circulatory system and participate in some mechanisms. Diabetic patients with a poor AGE diet presented decreased levels of circulatory AGE, VCAM, and C-reactive protein compared with a regular diet [31]. The relation between dietary AGEs and atherosclerosis is better understood when looking at preclinical models. For instance, mice subjected to femoral artery injury and fed with low-AGE diet presented less neointima area and larger luminal area than the high-AGE diet group. These data reinforce the role of AGEs in neointima proliferation after vascular lesion [32].

### 11.3.2 Heart Infarction and Heart Failure

The heart is a highly oxidative organ, and fatty acid oxidation is responsible for 60–80% of its energy requirement [33]. Because of this oxidative metabolism, 25–35% of total cardiomyocyte content is occupied by mitochondria [34]. Even under physiological states (normoxia), 1–3% of oxygen is leaked as free radicals by

mitochondria, and this value can be increased several-fold during or after infarction.

Acute myocardial infarction is defined as an interruption in the blood flow in myocardium. The most common cause is the occlusion of coronary artery following the rupture of an atherosclerotic plaque, and the obvious consequence is the death of starved cells. During the infarction, oxidative stress is caused both by inflammatory cells that infiltrate and by cardiomyocytes after the onset of necrosis. There is no doubt that the ischemic process must be stopped and that the reestablishment of blood flow is necessary to avoid acute heart failure. This can be done through pharmacological thrombolytic therapy or surgically, and these interventions help to decrease the mortality rates after acute myocardial infarction [35]. It turns out that this procedure is considered a "double-edged sword" since it is as harmful as it is necessary and results in "ischemia-reperfusion injury" (IRI). The clinical manifestation of IRI is seen as myocardial stunning, arrhythmia, myocyte death, and endothelial/microvascular dysfunction [36]. Several intricate steps occur after IRI, and free radicals act centrally in this process. Chronic treatment with β-blockers, aspirin, angiotensin-converting enzyme (ACE) inhibitors, and statins have improved the survival and the quality of life in infarcted patients. However, myocardial infarction remains a prevalent event with devastating consequences since it cause loss of cardiomyocytes, which are terminally differentiated cells. With few myocytes remaining it is hard to sustain adequate contractile function and heart failure develops.

### 11.3.3 The Ischemic Phase

During the ischemic process low levels of oxygen and nutrients arrive in the heart. The only way to keep the levels of ATP for contractile function is by increasing the glycolysis rate, which will lead to an overload of lactate and acidosis. As the glycogen vanishes, there will be a fall in ATP levels and an increase in its degradation products (hypoxanthine). These changes in energetic balance are the main cause of mitochondrial permeability transition (MPT) pore opening [37]. The MPT pore is a voltage- and $Ca^{2+}$-dependent high-conductance channel, whose opening leads to the increased permeability of inner mitochondrial membrane with consequent dissipation of the electrochemical proton gradient that drives mitochondrial functions. Also, MPT pore opening promotes the release of free radicals and cytochrome *c*, a proapoptotic factor that, once in cytosol, triggers caspase-3 and the apoptotic process [38]. The increase in permeability caused by MPT pore opening causes an influx of water into the mitochondrion, which makes it swell. The final step is the rupture of this organelle. When the insult is severe, there will be neither time nor ATP for the cell to enter the apoptotic process, and necrosis will occur. In fact, it was recently confirmed by atomic force microscopy that mitochondrial swelling happens in infarcted rat hearts [37].

### 11.3.4 The Reperfusion Phase

Prolonged periods of ischemia are related to necrosis, whereas reperfusion is related to the start of apoptosis. The reestablishment of glucose and oxygen restores the ATP levels necessary for apoptosis but also restores the levels of molecular oxygen needed to generate free radicals. Then, as soon as the reestablishment of blood flow takes place, the IRI starts. The ischemic process that precedes reperfusion represents a priming phase in oxidative stress. The ischemic process itself is not free radical generating, since it causes the lack of the main substrate, oxygen. However, as a priming phase, it causes changes in xanthine oxidase (as discussed above), in antioxidant enzymes and in several mitochondrial proteins, which predisposes the cell to an oxidative burst as soon as oxygen enters the cell [39, 40].

All of the mechanisms involved in IRI are not well known, but the overproduction of ROS, mainly by mitochondria and xanthine oxidase, has been related to IRI. During the ischemic process ATP levels fall and the ADP formed is also further oxidized to AMP, adenosine, hypoxanthine, and xanthine. The enzyme responsible for the last two steps is xanthine oxireductase that is present in the organism as the reductase isoform. Xanthine reductase oxidizes xanthine and transfers the electrons to $NAD^+$ instead of to $O_2$. During an insult process, such as heart infarction, the ion gradient disruption leads to $Ca^{2+}$ accumulation and activation of $Ca^{2+}$-stimulated proteinases. In the reoxygenation process, oxidation of –SH groups or limited proteolysis of xanthine reductase converts it to an oxidizing isoform, and now the oxidation of xanthine also leads to the monovalent reduction of molecular oxygen, generating superoxide anion [3].

In fact, since 2003 a clear relationship has been recognized between high serum uric acid and mortality in patients with chronic heart failure [41]. Probably uric acid in serum represents the increased xanthine oxidase activity and, eventually, increased oxidative stress in those patients. In light of these data, some studies have attempted to link the use of allopurinol with decreased mortality in heart failure patients. For instance, allopurinol administration in patients enrolled for revascularization caused less cardiac damage, measured by creatine phosphokinase and troponin I release. Moreover, patients receiving allopurinol showed 13% less incidence of death, infarction, or tachycardia in a 30-day follow-up [42].

Perhaps a major role in IRI is developed by mitochondria. Besides being a source of free radicals in the reperfusion phase, it is also a depository of proapoptotic molecules. Thus any dysfunction would lead to oxidative damage and cell death. Isolated hearts subjected to IRI have provided information about free radical generation in a more confident way, since data from isolated mitochondria may present some bias regarding the isolation process and in vivo data are hard to obtain. In this way, it was evidenced by electron paramagnetic resonance (EPR) that ubisemiquinone, alkyl-peroxy radical, and nitrogen-centered radical were generated after the reperfusion [43]. The mitochondrial source of ubisemiquinone and alkyl-peroxy radical could be confirmed in hearts perfused with sodium amobarbital, an inhibitor of complex I of the mitochondrial electron transport chain. The major consequence was a decreased membrane lipid oxidation and high pressure developed by hearts at the end of reperfusion [43].

An early target of mitochondrial free radicals is cardiolipin, a phospholipid located inside the inner mitochondrial membrane that is responsible for attaching cytochrome *c*. The oxidation of this phospholipid is related to a decreased electron transport activity, increased free radical generation, and cytochrome *c* release with eventual caspase-9 activation [44]. Genetic approaches and pharmacological studies confirm that cardiomyocyte apoptosis plays a crucial role in the pathogenesis of many cardiac syndromes and pathologies. For instance, the inhibition of cardiac myocyte apoptosis reduces infarct size up to 50–70% and decreases cardiac dysfunction after IRI [45, 46].

The impairment of mitochondrial complex I, III, and IV activity due to ROS-induced cardiolipin damage may increase the electron leak from the electron transport chain, generating more superoxide anion radical and perpetuating a cycle of oxygen radical-induced damage, which ultimately leads to a decrease in oxidative phosphorylation and to heart failure on reperfusion. Perhaps a good strategy should be reperfuse with low $pO_2$ in order to try to attenuate free radical generation, as demonstrated in isolated rat hearts. This strategy improved mitochondrial electron transport, decreased $H_2O_2$ production, and improved cardiolipin oxidation compared with normoxic reperfused hearts [47].

Antioxidant enzyme modulation (e.g., SOD, catalase, GPx) seems not to contribute largely to the oxidative damage in IRI. Some studies showed that Mn-SOD decreases after IRI [48], whereas other studies showed no modulation in Mn-SOD, EC-SOD, and Cu,Zn-SOD content [49, 50]. Moreover, IRI also promoted a decrease in catalase and GPx in an in vivo model of transient ischemia [51]. Taken together, these data suggest that the increase in free radical production without a consistent compensation of the antioxidant machinery contributes to oxidative damage in IRI. Artificially increasing antioxidant enzymes by transgenic overexpression or supplementing with exogenous enzymes/vitamins/antioxidants may contribute to limit the damaged area. These data suggest that antioxidant supplementation during reperfusion could avoid IRI and render better results over the long term.

### 11.3.5 Cardiac Remodeling

Cardiac remodeling occurs in response to a variety of stimuli, such as hypertension or myocardial infarction. It represents functional and structural changes, and crucial aspects are involved in remodeling: ventricular hypertrophy, development of interstitial fibrosis, loss of contractility, and chamber dilation. As the signs worsen, heart failure takes place. These changes are associated with an extensive inflammatory process and are, to some extent, necessary to replace the damaged myocardium. As remodeling progresses, it becomes harmful by compromising normal cardiac function [52].

### 11.3.6 Hypertrophy

ROS production is present in a variety of animal models of cardiac hypertrophy (response to $\alpha$-adrenergic agonists, angiotensin II stimulation, and cyclic stretch) [53]. Free radicals have an important part in coordinating the cardiac remodeling, and NAD(P)H oxidase seems to be an important source. A study with mice lacking subunit $gp91^{phox}$ (Nox2) of NAD(P)H oxidase showed that there was no hypertrophic response to angiotensin II in those mice [5]. In contrast, mice lacking $p91^{phox}$ were not protected against hypertrophy by aortic banding (a model of response to pressure overload), which seemed to be compensated by an increase in expression of another related subunit of NAD(P)H oxidase, Nox4. The role of free radicals in hypertrophy was further confirmed by treating these mice with the antioxidant *N*-acetylcysteine, which could prevent the hypertrophic response [54]. Superoxide anion appears to be central in the free radical-guided hypertrophy, since NAD(P)H oxidase modulation can avoid hypertrophic response. The participation of other ROS in cardiac hypertrophy can not be neglected, since *N*-acetylcysteine is a nonspecific antioxidant.

### 11.3.7 Interstitial Fibrosis

The deposition of an excess of extracellular matrix components between cardiomyocytes contributes to decreased cardiac elasticity and electrical conductance (see Section 11.3.10 Arrhythmia). The proliferation of myofibroblasts is another key component of interstitial fibrosis.

NAD(P)H oxidase also participates in collagen I and III deposition in both perivascular space and left

ventricular (LV) tissue. Rats rendered hypertensive by aldosterone treatment showed lower levels of collagen I and III when treated with apocynin, an NAD(P)H oxidase inhibitor [55]. Superoxide from other sources, such as xanthine oxidase, could also participate in this process. Infarcted mice develop extensive cardiac remodeling, but allopurinol, a xanthine oxidase inhibitor, could reverse collagen deposition in myocardium and preserve echocardiographic parameters, such as ejection fraction and chamber diameter [56].

### 11.3.8 Contractility

Multiple mechanisms contribute to contractile dysfunction, including abnormalities of cardiomyocyte excitation-contraction, mitochondrial dysfunction and energetic deficit, loss of myocytes from the ventricular walls, and alterations in the extracellular matrix. Interestingly, NAD(P)H oxidase has a role in contractility of cardiomyocytes independent of other effects that also interfere in contractility (chamber dilation and fibrosis). Cardiomyocytes isolated from mice lacking $gp91^{phox}$ and wild-type mice subjected to aortic banding clearly demonstrated that NAD(P)H oxidase absence improved contractility independent of other effects on the tissue [57]. Contractility has been shown to preserved when another source of superoxide is blocked. Allopurinol, an inhibitor of xanthine oxidase, was able to preserve cardiac contractility in hearts from dogs with dilated cardiomyopathy [58]. The downstream effects of superoxide may involve the generation of other ROS and regulation of sarcolemmal ion channels, sarcoplasmic reticulum calcium release channel (the ryanodine receptor), sarcoplasmic reticulum calcium pump (SERCA 2a), and the contractile proteins themselves [53, 59].

### 11.3.9 Chamber Dilation

Matrix metalloproteinases (MMPs), a family of enzymes that catabolize collagen, elastin, and gelatin, and their tissue inhibitors (TIMPs) are central during cardiac remodeling. Several MMPs become activated in heart failure [60]. In animal models, it has been demonstrated that deletion of MMPs is accompanied by reduced LV [61] dilation and inflammation after myocardial infarction. Conversely, deletion of TIMPs is accompanied by increased LV dilation after myocardial infarction (MI) [62]. Free radical participates in activation of MMPs. Mice subjected to a model of pressure overload by aortic constriction present increased levels of superoxide and MMP-2 activation. Mice lacking iNOS did not present MMP-2 activation or suffer all these adverse remodeling effects on the heart. Such effects could also be avoided by the selective iNOS inhibitor administered in wild-type mice subjected to aortic constriction [63]. In the same line, infarcted hearts from mice that were treated with allopurinol presented attenuated chamber dilation compared with those who were treated with saline [56]. Moreover, other studies have demonstrated that heart-specific deletion of Mn-SOD promoted a progressive dilated cardiomyopathy [64]. Collectively, these data suggest the importance of free radicals in the progression of chamber dilation.

### 11.3.10 Arrhythmia

Arrhythmia is defined as any change in the electrical activity in the heart. It is classified according to the alteration produced. Tachycardia consists of accelerated heartbeat, while bradycardia is characterized by a slower pump rate. Although events of arrhythmia can result in cardiac arrest and sudden death, some arrhythmias occur as sparse events and are not considered as threats. The most important causes of arrhythmia are coronary artery disease and atrial fibrillation (AF), the latter being closely associated with postsurgery inflammation.

### 11.3.11 Atrial Fibrillation

AF is characterized by rapid electrical activity of the atria that leads to abnormal ventricular activity [65]. One general cause of AF is heart failure (HF), although intrinsic mechanisms vary and are not well understood. Besides HF, diabetes mellitus and cardiac surgery increase the risk of AF [66].

Animal models have shown that increased atrial pacing is associated with increased superoxide production. In addition, most of this radical is produced by the enzyme NAD(P)H oxidase [67]. In humans, NAD(P)H oxidase activity is a strong predictor of postoperative AF [68]. The process of atrial fibrosis itself is closely related to the activation of the $AT_1$ receptor by angiotensin II and the subsequent increase in NAD(P)H oxidase activity. Inhibition of $AT_1$ is known to prevent superoxide production [69].

Along with free radical production, inflammation is considered as a risk factor and also a component of AF. Postoperative high levels of interleukin 6 (IL-6) are predictors of AF onset [70]. Moreover, serum levels of hydroperoxides are associated with atrial function and predict AF in patients after radiofrequency catheter ablation [71].

Antioxidants have been proven as efficient in treatment and prevention of AF and arrhythmias, although their role is no more than that of coadjutants. The pool of glutathione (GSH) is a major physiological defense against oxidative stress in the myocardium. Indeed, its precursor *N*-acetylcysteine was shown to decrease the

incidence of postischemia arrhythmias [72, 73]. The use of drugs such as thiazolidinediones might act by reducing the activity of xanthine oxidase or by increasing NO production. This NO is capable of reacting with the overproduced superoxide radical, due to the increased activity of NAD(P)H oxidase. Ascorbic acid also seems to be an important antioxidant, as its levels were found decreased in a dog model of atrial tachypacing [74], and its administration to patients with persistent AF reduced the recurrence of the condition after cardioversion [75]. NO precursors such as L-arginine and sodium nitroprusside have strong potential as therapeutics, since NO acts as an antioxidant and anti-inflammatory [76, 77].

Although their origins might rely on other cardiovascular complications such as heart failure and atherosclerosis, AF and arrhythmias are strong life-threatening conditions with specific mechanisms of development that require specific interventions.

## 11.4 EXERCISE AND PROTECTION AGAINST FREE RADICAL-MEDIATED CARDIOVASCULAR DISEASES

As stated above, environmental factors contribute greatly to increased risk of cardiovascular diseases. Among these are smoking and dietary habits (see above). Another important habit that contributes to cardiovascular diseases is lack of physical activity. Physical activity can either confer resistance to cardiovascular events, such as myocardial infarction, or help during the recovery process.

### 11.4.1 Exercise as a Protector

There is a large amount of epidemiological evidence showing that physical activity habit has a protective effect for several cardiovascular complications, such as atherosclerosis development and hypertension [78]. But does exercise also work for improving life after the myocardial infarction episode? The answer seems to be positive, since men who had physical activity after an episode of myocardial infarction were more prone to survive [79, 80]. Unfortunately, direct evidence of protective effect of physical activity is hard to obtain, thus we can rely on animal models to understand the mechanism involved. From these models we can learn that exercise preconditioning exerts its effects by decreasing the size of infarct [81], a parameter that is closely linked with the mortality rate in humans [82]. But how does exercise decrease mortality of cardiomyocytes after an infarction? The following effects are involved: first, morphological changes concerning myocyte hypertrophy and increased coronary vascularization; second, changes in metabolic efficiency; and third, increase in the myocardium antioxidant potential after a transient release of free radicals.

ROS such as hydrogen peroxide and superoxide were already described as important signaling molecules in a variety of processes, including myocyte hypertrophy and death. Isolated cardiac myocytes subjected to mechanical stretch present increased levels of superoxide and develop hypertrophy [83]. These events are mediated by mitogen kinases, such as ERK1/2, which is implicated in cell growth and can be stimulated by free radicals in cardiac myocytes. When antioxidants are given to these cells, not only superoxide is abolished, but also ERK1/2 activation and cell growth [83]. The participation of ROS in myocyte hypertrophy becomes clearer with the demonstration that hydrogen peroxide delivery to cultured adult cardiomyocytes has a biphasic response, being proliferative in low doses and apoptotic/necrotic at higher doses [84]. Lower doses of $H_2O_2$ (10–30 $\mu$M) induce activation of ERK1/2 and cell growth, whereas higher doses (>100 $\mu$M) induce activation of ERK1/2, JNK, and p38 MAPK, and JNK activation is related to the deleterious effects of $H_2O_2$ treatment [84].

From studies with animals we can learn that high-intensity exercises can increase MnSOD activity in both young and old rat hearts [85, 86], indicating that the heart retains the ability to induce antioxidant defense despite age. Interestingly, the cytoplasmic isoform of superoxide dismutase (Cu,ZnSOD) is, in general, not induced by physical exercise [87]. The chemical product of SOD action is hydrogen peroxide, and it serves as substrate for catalase and glutathione peroxidase [3]. Thus these hydrogen peroxide-consuming enzymes should be kept in balance with the SOD increment to avoid an excess of peroxide and a possible start of oxidative stress. However, it seems that the exercised heart proceeds otherwise, since there is no compensatory increase in catalase or glutathione peroxidase [87]. Thus, taken collectively, these data indicate that the increase in $H_2O_2$ in cardiac myocytes during the exercise can lead to myocyte growth and thus to cardioprotection against future events.

Besides this, free radicals are centrally involved in angiogenesis. Vascular endothelial growth factor (VEGF) is a family of key mediators of angiogenesis after wound or exercise that exert their effects through VEGF receptors (VEGFR1, VEGFR2, and VEGFR3) [88]. A well-conducted study in animals showed that the delivery of low doses of $H_2O_2$ to the wound can increase VEGF mRNA and $CD31^+$ cells and accelerate wound healing [89]. Moreover, either NAD(P)H oxidase deficiency or overexpression of catalase abolished VEGF synthesis and impaired wound healing [89]. These data are consistent with the impaired wound healing seen in chronic granulomatous disease patients, who also present dysfunctional NAD(P)H oxidase [90]. In the same way,

antioxidant therapy is considered antiangiogenic, and some studies have employed it as adjuvant therapy to cancer treatment. Several compounds, such as glutathione, selenium, and resveratrol have been shown to impair angiogenesis [91–93].

But how does hydrogen peroxide induce VEGF synthesis? The pathway is not completely elucidated yet, but some studies employing different cell types have shown that the MAPK pathway, mainly involving MEK, ERK1/2, and p44/42 MAPK, seems to be important [88]. Moreover, VEGF signaling is further stimulated because hydrogen peroxide also triggers NF-κB translocation to the nucleus and stimulates the transcription of VEGF receptors [88].

Finally, physical activity increases the antioxidant potential in the heart in order to counterbalance the permanent increase in free radical generated as a consequence of heart work. Thus, in the case of a myocardial infarction, the heart is more prone to buffer the oxidative burst during the reperfusion process. However, this view is not fully elucidated, as we shall see below. First, there are some studies with chronically exercised animals that do not show any improvement in the heart antioxidant profile [94]. SOD, for instance, appears to be upregulated only after high-intensity exercise [87]. Also, exercise induced MnSOD activity in both young and old rats [86]. Moreover, the systems responsible for consuming hydrogen peroxide do not respond after exercise in most studies [87]. Glutathione increase after exercise is also under debate, and its role in heart adaption is not fully elucidated. In fact, because hydrogen peroxide acts as signaling molecule in heart adaption, we should not to expect an increase in the systems responsible for consuming it.

### 11.4.2 Exercise as a Therapy

After the onset of myocardial infarction a lot of attention is devoted to decreasing the remodeling process that takes place and leads to heart failure. The remodeling process involves cardiomyocyte death, fibroblast proliferation, and extracellular matrix deposition [52], all processes coordinated in some way by free radicals. Besides the classical therapies, exercise training may attenuate cardiac remodeling and may even reverse this process [95]. Unfortunately, few studies have focused on the effect of exercise on free radical after myocardial infarction. In one study, 17 patients were enrolled in a training program for 3 weeks. At the end, the patients presented better hemodynamic performance and lesser hydrogen peroxide production after the physical test [96]. Similar results were found in another study that evaluated antioxidant enzymes in skeletal muscle biopsies from chronic heart failure patients who entered into a physical activity program [97]. Despite these studies, which clearly showed that physical activity performed after a myocardial insult is efficient in restoring free radical/antioxidant balance, there has been no evaluation of the effect of exercise on survival rate in a long-term study.

## 11.5 ANTIOXIDANT THERAPIES FOR CARDIOVASCULAR DISEASES

Atherosclerosis is one of the leading causes of morbidity and mortality in Western countries. Its occurrence is in great part explained by the way of life in a technologically advanced world. Lack of exercise activities and high-fat diet are major risk factors for atherosclerosis. Indeed, evidence suggests that diets low in saturated fat and rich in vegetables contribute against the development and progression of cardiovascular diseases [26]. It is clear that antioxidants present in the diet play an important role by acting against the intrinsic mechanisms of atherosclerosis and other cardiovascular diseases, but clinical trials and follow-up studies are controversial [98].

Studies with animals often support antioxidant therapies to prevent or attenuate atherosclerosis. A low-vitamin C and E diet accelerated atherosclerosis in ApoE-deficient mice [99]. In the same model of atherosclerosis, *N*-acetylcysteine, a precursor of glutathione, reduced plaque formation and superoxide production [100]. Although it may seem fairly obvious that antioxidants can prevent cardiovascular diseases, tests in humans raise many questions about the specificity of each antioxidant and of each disease. Furthermore, some clinical studies with vitamins A and E report an increase in mortality and incidence of cardiovascular disease [101–103].

On the other hand, some antioxidants have been demonstrated to be effective. After infarction, administration of L-arginine has been proved to ameliorate the response to the ischemic event by increasing the levels of NO, which promotes vasodilation and can also neutralize reactive species. Besides NO production, L-arginine also increases the activity of SOD and the levels of thiols, and both are believed to act synergistically with the vasodilation [104]. Potent free radical scavengers like edaravone have also been tested in humans and proven to improve LV ejection fraction and reduce rehospitalization after infarction [105]. However, it is clear that no antioxidant will be found to prevent all cardiovascular diseases or restore cardiovascular function after any deleterious event.

Some success has been achieved using xanthine oxidase inhibitors, such as allopurinol and oxypurinol. Patients with ST segment elevation myocardial infarction who underwent reperfusion showed better recovery markers when treated with allopurinol than those treated with placebo. Allopurinol was able to completely recover

ST elevation in 76% of the patients, against only 58% in placebo-treated subjects. Moreover, markers of myocyte damage, such as cardiac troponin I and CK-MB, were also lower in patients treated with allopurinol [42]. Also, congestive heart failure patients with ejection fraction <40% receiving oxypurinol over a month demonstrated 7% of improvement in ejection fraction compared with the placebo group [106]. Thus xanthine oxidase indeed seems to be important in the course of heart failure, and superoxide may be a therapeutic target in the future.

In this sense, antioxidants are important agents in prophylaxis and treatment, regulating the intra- and extracellular redox status. Fine-tuning of the antioxidant enzymes and production of glutathione is associated with lower incidence of cardiovascular diseases and increase of life span. However, while the specific mechanisms lying behind cardiovascular diseases are still to be elucidated, the use of antioxidants in medicine might remain underrated.

## 11.6 CONCLUSION

Oxidation of biomolecules accelerates cell senescence and is closely associated with neurodegenerative and cardiovascular diseases. As the organism gets older, antioxidant defenses and repair mechanisms become weaker and cannot counteract the production of reactive species and accumulation of oxidative damage. Although this scenario seems cruel, fine regulation of the oxidative status of cells and tissues is a key point in the maintenance of homeostasis. Regarding the cardiovascular system, oxidative stress shows up as a major culprit, participating in endothelial dysfunction, development and progression of atherosclerosis, and cardiac dysfunction. In any case, reducing the production of reactive species or increasing the levels of antioxidants has proven to be important for prevention and treatment. Nevertheless, clinical trials are inconclusive and sometimes controversial. From this perspective, better knowledge of the mechanisms regulating oxidative stress generation, as well as new therapeutic interventions, capable of acting more specifically in each disease, can be foreseen.

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