# Role of Oxidative Stress in the Pathophysiology of Arterial Hypertension and Heart Failure

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## 23.1 Introduction

### 23.1.1 Reactive Oxygen Species (ROS) and Oxidative Stress: General Considerations

Oxygen is essential for cellular respiration and energy production by aerobic organisms. However, the partial reduction of oxygen by several metabolic pathways will inevitably result in the production of reactive oxygen species (ROS). In turn, these short-lived and highly reactive oxygen metabolites have the ability to attack cellular macromolecules, including lipids, proteins and nucleic acids, causing oxidative damage. Under normal conditions, intracellular ROS concentrations are maintained within a balanced, steady-state range, by integrated enzymatic and nonenzymatic antioxidant systems, which not only protect cells from the detrimental effects of

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ROS but also allow the activation of redox signalling pathways that regulate physiological functions. Disruption of redox equilibrium by persistently elevated ROS concentrations leads to oxidative stress and subsequent dysfunctional signalling and macromolecular damage [1–3]. Oxidative stress is widely recognized as an important contributor to ageing, which is characterized by a progressive decline in biological functions and in the organism's ability to adapt to metabolic stress over time, being also aetiologically involved in the pathogenesis of a wide variety of disease processes, namely arterial hypertension, atherosclerosis, heart failure (HF), diabetic neuropathy, renal diseases, neurological diseases, as well as cancer [4, 5]. In contrast to the well documented role of oxidative stress in cardiac diseases, less is known regarding the role of reductive stress in these processes. Reductive stress is the counterpart of oxidative stress and is defined as an aberrant increase in reducing equivalents, leading to decreased ROS levels. Nevertheless, it is becoming increasingly clear that the biological extremes of the redox spectrum play critical roles in disease pathogenesis [6]. In addition to ROS, there is another class of chemically reactive molecules collectively designated as reactive nitrogen species (RNS), which include various nitric oxide ($^{\cdot}NO$)-derived compounds. RNS have been recognized as playing important functions in diverse physiological and pathological redox signalling processes [7, 8]. Similarly to ROS, excessive amounts of RNS have been implicated in cell injury and death by inducing nitrosative stress.

### 23.1.2 Main Types of ROS and RNS

ROS can be divided in two main groups: i) free radicals [e.g. superoxide anion ($O_2^{\cdot -}$), hydroxyl radical ($HO^{\cdot}$), peroxyl radical ($ROO^{\bullet}$)], which are unstable and highly reactive species due to the presence of one or more unpaired electrons; and ii) non-radical oxidants [e.g. singlet oxygen ($^1O_2$), hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl)], that have generally more specific reactivity and higher stability. RNS include $^{\cdot}NO$ and nitrogen dioxide radicals ($^{\cdot}NO_2$) and also non-radicals such as peroxynitrite ($ONOO^-$), nitrous acid ($HNO_2$), peroxynitrous acid (ONOOH) and alkyl peroxynitrites (ROONO) (Table 23.1). Among biological ROS and RNS, $O_2^{\cdot -}$, $H_2O_2$, $^{\cdot}NO$ and $ONOO^-$ appear to play a prominent role in vascular, cardiac, renal and neuronal regulation and dysregulation, thus representing important targets for strategies aiming to reduce redox dysfunction in cardiovascular diseases [9, 10].

### 23.1.3 Mechanisms of ROS Generation in Cardiovascular Diseases

A key consideration for ROS/RNS chemistry and biology is the subcellular compartment where a particular species is generated, as discrete microenvironments can determine which targets will be preferentially attacked. ROS are derived from both endogenous and exogenous sources (Fig. 23.1). Intracellular compartments capable of ROS generation include mitochondria, the endoplasmic reticulum, peroxisomes, nuclei, the cytosol, and plasma membrane enzymatic systems. ROS can also be

**Table 23.1** Major types of ROS and RNS in living systems and corresponding properties

| | Symbol | Half-life (s) | Properties |
|---|---|---|---|
| **ROS – *free radicals*** | | | |
| Superoxide anion | $O_2^{\cdot -}$ | $10^{-6}$ | Low reactivity in aqueous solution; signalling function |
| Hydroxyl | $HO^{\cdot}$ | $10^{-9}$ | Most reactive oxygen radical; reacts almost immediately with every molecule in living cells; diffuses a short distance |
| Peroxyl | $ROO^{\bullet}$ | $10^{-2}$ | Weak oxidant; high diffusibility |
| **ROS – *non-radical oxidants*** | | | |
| Singlet oxygen | $^1O_2$ | $10^{-6}$ | Powerful oxidizing agent |
| Hydrogen peroxide | $H_2O_2$ | $10^{-5}$ | Weak oxidizing and reducing agent; diffuses across membranes; signalling role |
| Hypochlorous acid | HOCl | n/a | Strong reactive species; released by neutrophils |
| **RNS – *free radicals*** | | | |
| Nitric oxide | $^{\cdot}NO$ | $10^{-3}$ | Can yield potent oxidants during pathological states; endogenous signalling molecule |
| **RNS – *non-radical oxidants*** | | | |
| Peroxynitrite | $ONOO^-$ | $10^{-2}$ | Highly reactive intermediate of $O_2^{\cdot -}$ and $^{\cdot}NO$; permeates cell membranes |

*n/a* no data available

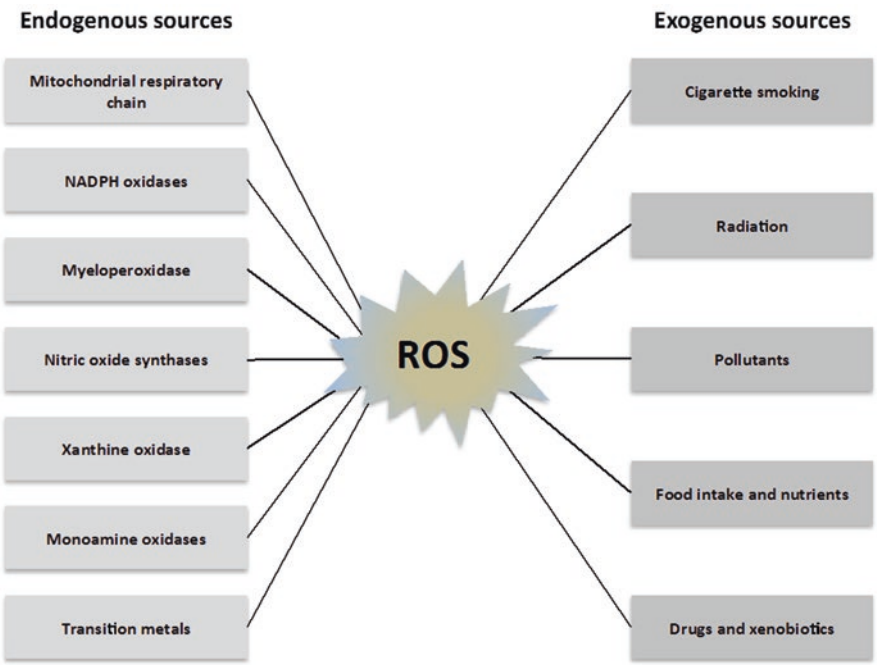


**Fig. 23.1** Major endogenous and exogenous sources leading to ROS production

produced in response to external sources, including pollution, alcohol, tobacco smoke, heavy metals, UV radiation.

#### 23.1.3.1 Mitochondria

Mitochondria play a key role in energy metabolism in many tissues. More than 90% of the oxygen consumed by aerobic organisms is utilized by the mitochondrial electron transport chain (ETC), which generates ATP in a process coupled to the reduction of cellular oxygen to water. The mitochondrial respiratory chain complexes are also an important source of ROS within most mammalian cells [11–13]. In fact, about 1–4% of the oxygen used in these reactions is converted to $O_2^{\cdot-}$ and $H_2O_2$, which may have deleterious consequences to mitochondria if not adequately detoxified [14]. ROS formation in the mitochondria is regulated by the respiratory rate and by the antioxidant enzyme manganese superoxide dismutase (MnSOD) [12]. The mitochondrial respiratory chain appears to be a major source of oxidative stress in some experimental forms of arterial hypertension (e.g. mineralocorticoid hypertension, angiotensin II-induced hypertension) and the inhibition of mitochondrial ROS production has a significant blood pressure-lowering effect in these models [15, 16]. In HF there is also evidence of abnormal ROS production from mitochondrial respiratory chain. Furthermore, the scavenging of mitochondrial ROS has been shown to prevent or reverse HF and to eliminate sudden cardiac death in an animal model of non-ischemic HF that displays important features of human HF (e.g. prolonged QT interval, high incidence of spontaneous sudden cardiac death due to ventricular tachycardia/fibrillation) [17–19].

#### 23.1.3.2 Other Prooxidant Enzymatic Systems

Besides mitochondrial oxidases, there are other important enzymatic sources of ROS, such as NADPH oxidases, myeloperoxidase, $^{\cdot}NO$ synthases, xanthine oxidase and monoamine oxidases (Fig. 23.1).

##### Nicotinamide Adenine Nucleotide Phosphate (NADPH) Oxidases

NADPH oxidases (NOX) are multi-subunit transmembrane enzymes complexes that catalyze the one-electron reduction of molecular oxygen using NADPH as an electron donor. In general, the product of the electron transfer reaction is $O_2^{\cdot-}$, but $H_2O_2$ is also rapidly formed from dismutation of NOX-derived $O_2^{\cdot-}$ due to the presence of superoxide dismutase in the cells or by spontaneous reaction. NOX-derived ROS play a role in host defence and also in various signalling pathways [20]. The NOX family contains seven members (NOX1-5 and Duox1-2) with distinct tissue distribution and roles [20]. NOX1, NOX2 and NOX4 isoforms enzymes appear to be particularly relevant in the pathophysiology of hypertension, being expressed in major sites of blood pressure regulation [20, 21]. For example, NOX1, NOX2 and NOX4 can be found in the central nervous system, where they contribute to sympathetic nerve activity control [21]. In the kidney, NOX2 and NOX4 appear to be the main isoforms regulating renal function and contributing to end-organ damage [21, 22]. These isoforms are also important determinants of vascular tone in several vascular beds, including the renal afferent arteriole, which is critical for the regulation

of renal haemodynamics [23–25]. Endothelial function can be regulated by NOX2, which contributes to impaired vasodilation, or by NOX4, which improves endothelial-dependent vasodilation. NOX1 and NOX4 are also involved in vascular smooth muscle cell growth and migration [20, 23, 24]. Of note, recent studies suggest that NOX5, an isoform that is found in humans but absent in rodents, is also implicated in the pathogenesis of cardiovascular diseases, such as hypertension and atherosclerosis [26]. For example, renal proximal tubular cells from human hypertensive subjects appear to express NOX5 in a greater extent than the other isoforms [27]. Furthermore, in mice expressing human NOX5 in podocytes, the renal function becomes impaired and blood pressure increases [26]. NOX5 expression was also shown in human carotid artery atherosclerotic plaques and to be induced in macrophages exposed to a proinflammatory and prooxidant environment [28].

NOX2 and NOX4, the two isoforms expressed in the heart, appear to be especially relevant in HF [29, 30]. NOX2 contributes to angiotensin II-induced cardiac hypertrophy, atrial fibrillation, myocyte death under stress conditions and post-myocardial infarction remodelling. The inactivation of NOX2 was shown to attenuate ventricular dilatation and contractile dysfunction in experimental models of myocardial infarction. NOX2 deletion also abolished angiotensin II-induced cardiac hypertrophy but was not able to prevent the development of HF caused by severe pressure overload [29, 30]. The role of NOX4 in the heart is more controversial, with both protective and detrimental effects reported. For example, mice lacking cardiac NOX4 display either reduced or aggravated maladaptive remodelling in different models of pressure-overload-induced HF [29, 30]. In what concerns to ischemia-reperfusion injury, it appears that both NOX2 and NOX4 contribute to increased ROS production and damage, as evidenced by the reduced myocardial infarct size/area at risk and lower $O_2^{\cdot-}$ production in NOX2 knockout or NOX4 knockout mice subjected to ischemia-reperfusion injury. However, double knockout of NOX2 and NOX4 exacerbates ischemia-reperfusion injury, probably because low levels of ROS generated by these enzymes are necessary to activate adaptive mechanisms that protect the heart against ischemia-reperfusion injury [31].

**Myeloperoxidase (MPO)**

MPO, a haem-containing enzyme secreted by activated neutrophils and monocytes under inflammatory conditions, produces several oxidizing molecules that can affect lipids and proteins [32]. MPO uses $H_2O_2$ to produce other ROS/RNS, such as HOCl, chloramines, tyrosyl radicals and nitrogen dioxides [32]. Although MPO-derived ROS have a major role as bactericidal agents, they can also cause tissue damage in the heart, vessels, kidney and brain. Vascular tone and endothelial bioavailability of $^{\cdot}$NO appear to be significantly affected by MPO. Interestingly, the MPO G463A polymorphism was associated with an increased risk of hypertension [33]. MPO contributes to vascular and myocardial dysfunction, being significantly increased in acute coronary syndromes and HF [34–36]. Higher MPO values were reported to be associated with increasing likelihood of more advanced HF in chronic systolic HF patients and to predict future adverse clinical events [37].

### NO Synthases

The ·NO synthases (NOS) are a family composed of three enzyme isoforms (neuronal NOS, nNOS; inducible NOS, iNOS; endothelial NOS, eNOS) [38]. NOS are the endogenous sources of ·NO in mammalian cells, in a reaction that converts L-arginine to L-citrulline [38]. ·NO exerts a wide array of regulatory functions on the cardiovascular system, including regulation of vascular tone, blood pressure, cardiomyocyte contractility, sympathetic outflow, smooth muscle cell proliferation, renal renin release and natriuresis [39–41]. However, under conditions of limited bioavailability of the cofactor tetrahydrobiopterin ($BH_4$) or the substrate L-arginine, NOS become unstable and reduces molecular oxygen to $O_2^{\cdot-}$ instead of producing ·NO. This NOS uncoupling is more often described for eNOS and is triggered by oxidative/nitrosative stress [42]. There is evidence that eNOS dysregulation and consequent endothelial dysfunction occur both in hypertension and HF [43, 44]. Treatment with $BH_4$, which contributes to eNOS recoupling, prevented or attenuated hypertension in spontaneously hypertensive rats [37]. It was also shown to reverse cardiac hypertrophy and fibrosis and to improve chamber and myocyte function in mice with heart disease induced by pressure overload [10, 45].

### Xanthine Oxidase

The enzyme xanthine oxidoreductase displays two interchangeable forms, xanthine dehydrogenase (XDH) and xanthine oxidase (XO), that participate in the metabolism of purines by catalyzing the conversion of hypoxanthine to xanthine and xanthine to uric acid [38, 46]. XDH uses $NAD^+$ as the preferential electron acceptor while XO reduces molecular oxygen in a reaction that generates $O_2^{\cdot-}$ and $H_2O_2$ [38, 46]. The XO form predominates in oxidative stress conditions and may contribute to endothelial dysfunction due to its localization in the luminal surface of vascular endothelium [38, 46]. Although XO is capable of generating ROS, both XDH and XO generate uric acid which has antioxidant properties, such as the ability to scavenge $ONOO^-$ and $HO^{\bullet}$, to prevent oxidative inactivation of endothelium enzymes and to stabilize vitamin C [47, 48]. In contrast, uric acid may also exhibit prooxidant and proinflammatory effects. Indeed, increased uric acid levels have been associated with cardiovascular disease [49, 50]. However, it is still unclear whether these effects reflect direct deleterious actions of uric acid or, alternatively, oxidative damage caused by XO-derived ROS.

XO appears to contribute to the pathophysiology of arterial hypertension in SHR, as evidenced by the significant reduction of blood pressure induced by the treatment with XO inhibitors [10, 51]. In humans, some studies have shown a blood pressure-lowering effect of XO inhibition in adolescents with newly diagnosed essential hypertension and an improvement of cardiovascular outcomes in adults with hypertension [52, 53].

In what concerns to heart diseases, XO inhibition was reported to improve left ventricle contractility and myocardial efficiency in an animal model of HF and to attenuate adverse left ventricular remodelling in experimental myocardial infarction

[19]. XO expression and activity was also shown to be increased in coronary arteries from patients with coronary artery disease, contributing to the augmented production of $O_2^{\cdot -}$ [54]. The inhibition of XO with oxypurinol also improved myocardial contractility in patients with ischemic cardiomyopathy [55]. However, other studies failed to demonstrate clinical benefits of oxypurinol treatment in unselected patients with moderate-to-severe HF or in high-risk HF patients with reduced left ventricular ejection fraction and hyperuricemia [56, 57].

**Monoamine Oxidases (MAO)**

MAO-A and MAO-B are flavoenzymes predominantly located at the outer membrane of mitochondria, being responsible for the oxidative degradation of neurotransmitters (catecholamines, serotonin) and biogenic amines in a process that generates $H_2O_2$, ammonia and an aldehyde intermediate. All of these products are potentially deleterious, especially for mitochondria. Pathological stimuli such as neurohormonal and/or chronic hemodynamical stress, inflammation and ischemia-reperfusion can increase the availability of MAO substrates, thus augmenting $H_2O_2$-induced mitochondrial dysfunction in cardiovascular tissues/organs and leading to endothelial dysfunction and HF [9, 58, 59]. In experimental models of hypertension (induced by angiotensin II) and inflammation (induced by lipopolysaccharide), the expression of both MAO isoforms increased in endothelial cells and MAO inhibition attenuated ROS production and restored endothelial-dependent vasodilation [59]. MAO are also important sources of ROS in the heart. There are several important cardiac targets for MAO-derived ROS, besides mitochondria. These include sphingosine kinase-1, an enzyme involved in cell survival, whose inhibition may contribute to cardiomyocyte apoptosis, as well as the contractile proteins, actin and tropomyosin, whose oxidation correlates with ventricular dysfunction, and matrix metalloproteinases, whose activation induces extracellular matrix remodelling. The signalling pathways activated by MAO-derived $H_2O_2$ depend on the availability of MAO substrates and $H_2O_2$ concentration in tissues. Lower amounts of $H_2O_2$ trigger hypertrophy, cell proliferation and matrix remodelling, while higher concentrations lead to mitochondrial dysfunction, apoptosis or necrosis. MAO inhibition appears to be protective in ischemia-reperfusion injury and pressure overload-induced HF [59, 60].

### 23.1.4 Major Endogenous Antioxidant Systems

All living organisms have adapted and developed an endogenous antioxidant defence system, composed of enzymatic and nonenzymatic antioxidants, that is usually effective in neutralizing deleterious effects of ROS (Fig. 23.2). However, when the antioxidant systems are overwhelmed, as observed in most pathological conditions, oxidative stress ensues. Below we provide an overview of the major antioxidant systems with relevance to cardiovascular diseases.

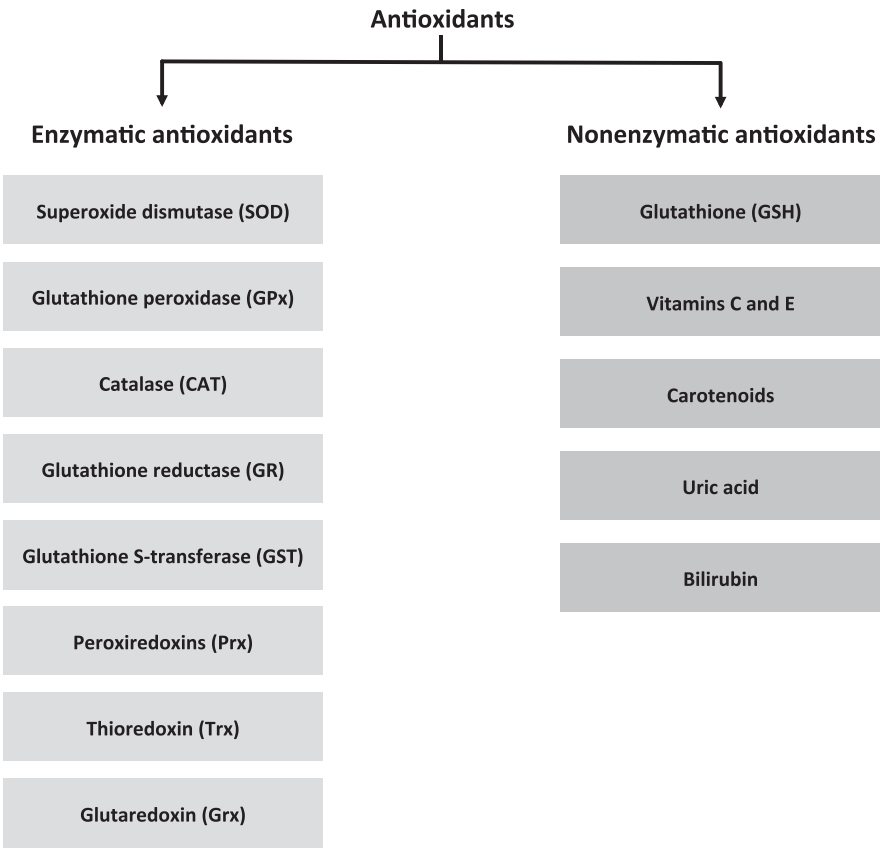


**Fig. 23.2** Major enzymatic and nonenzymatic antioxidants

### 23.1.4.1 Enzymatic Antioxidants

#### Superoxide Dismutases

Superoxide dismutase (SOD) enzymes consist of three isoforms in mammals: the cytoplasmic Cu/ZnSOD (SOD1), the mitochondrial MnSOD (SOD2), and the extracellular Cu/ZnSOD (SOD3), all of which require catalytic metals (Cu or Mn) for their activity [61]. They are considered the major antioxidant defences against $O_2^{\cdot -}$, being responsible for its dismutation to $H_2O_2$ and molecular oxygen, which limits the potentially harmful effects of this radical species [61].

#### Catalase and Glutathione Peroxidase

$H_2O_2$ produced by the action of SODs or oxidases, such as XO, can be further decomposed to water and oxygen. This is achieved primarily by catalase in the peroxisomes and by glutathione peroxidase (GPx) enzymes in the cytosol and

mitochondria. Catalase exists as a tetramer composed of 4 identical monomers, each of which contains a haem group at the active site. Degradation of $H_2O_2$ is accomplished via the conversion between 2 conformations of catalase-ferricatalase and compound I. GPx are selenium-containing enzymes whose activity is dependent on the amount of reduced glutathione (GSH) available [62]. Besides neutralizing $H_2O_2$, GPx also degrades lipid hydroperoxides to lipid alcohols. These reactions lead to the oxidation of GSH to oxidized glutathione (GSSG). Catalase and GPx are differentially required for the clearance of high-levels or low-levels of $H_2O_2$, respectively [63].

**Other Enzymatic Defences**

In addition to the antioxidant enzymatic systems mentioned above, cells also express other specialized enzymes with direct and/or indirect antioxidant functions. Glutathione reductase (GR) regenerates GSH from GSSG in the presence of NADPH. Glutathione-S-transferase (GST) catalyzes the conjugation of GSH with reactive electrophiles and detoxifies some carbonyl-, peroxide- and epoxide-containing metabolites produced within the cell in oxidative stress conditions. Peroxiredoxins (Prx) are selenium-independent enzymes that decompose $H_2O_2$, organic hydroperoxides and peroxynitrite, and thioredoxin (Trx) and glutaredoxin (Grx) systems include various enzymes that regulate the thiol-disulphide state of proteins and modulate their structure and activity [10].

### 23.1.4.2 Nonenzymatic Antioxidants

Nonenzymatic antioxidants, such as GSH, ascorbic acid (vitamin C) and α-tocopherol (vitamin E) play a key role in protecting the cells from oxidative damage and are considered as the second line of defence against active radicals. GSH is termed the master antioxidant given its electron-donating capacity that renders GSH a potent antioxidant *per se*, besides acting as an important cofactor for GPx and other enzymes. Vitamins E and C are among the major dietary antioxidants. Vitamin E, found in lipoproteins, cell membranes and extracellular fluids, terminates lipid peroxidation processes and converts $O_2^{\cdot-}$ and $HO^{\bullet}$ to less reactive forms. Vitamin C is a water-soluble antioxidant that can directly scavenge ROS and lipid hydroperoxides. Carotenoids, such as β-carotene, are lipid soluble antioxidants that function as efficient quenchers of $^1O_2$ but may also scavenge $ROO^{\bullet}$ radicals. Uric acid is a highly abundant aqueous antioxidant, considered to be the main contributor for the antioxidant capacity in the plasma. It has the ability to quench $HO^{\bullet}$ and $ONOO^-$ and may prevent lipid peroxidation, but may also exert prooxidant effects once inside the cells. Bilirubin, the end-product of haem catabolism, has chainbreaking antioxidant properties. Plasma albumin, the predominant plasma protein, is also an antioxidant and can scavenge MPO-derived chlorinated reactive species and $ROO^{\bullet}$ radicals [10].

### 23.1.5 The Dual "Faces" of ROS

It has long been accepted that elevated ROS levels can cause damage to macromolecules and have been implicated in a vast array of pathologies. More recently, it has become apparent that ROS also serve as signalling molecules to regulate biological and physiological processes and that dysregulated ROS signalling may contribute to a host of human diseases [3]. Downstream of ROS production, several signalling pathways are activated, including protein kinases [mitogen activated protein kinases (MAPKs), protein tyrosine kinases (PTKs), protein kinases B and C] and transcription factors (NF-κB, Nrf2) [64]. Nevertheless, our understanding of the signalling "face" of ROS is still in its infancy, as ROS can often act upstream and/or downstream within a given pathway and sometimes in opposing ways (i.e. inhibitory or stimulatory).

## 23.2 Evidence for Redox Changes in Experimental and Human Hypertension

### 23.2.1 Links Between Oxidative Stress and Hypertension

Arterial hypertension, currently defined as systolic blood pressure values ≥140 mmHg and/or diastolic blood pressure ≥90 mm Hg, is a multifactorial, complex disorder, involving many organ systems and constitutes a major risk factor for cardiovascular disease and premature mortality throughout the world [65]. Major pathophysiological mechanisms implicated in the development of hypertension include central nervous system dysregulation and increased activity of sympathetic nervous system, altered renal function with increased renal sodium and water retention and increased peripheral vascular resistance (Fig. 23.3) [51, 66]. The renin-angiotensin-aldosterone system (RAAS) also plays a central role in the regulation of arterial pressure by renal and extrarenal mechanisms (e.g. regulation of sodium homeostasis, autopotentiation of vasoconstrictor responses, vascular hypertrophy, regulation of sympathetic output, facilitation of sympathetic neurotransmitter release, promotion of oxidative stress and inflammation), being intimately involved in hypertension pathophysiology [67–71].

Oxidative stress has emerged as a unifying hypothesis for explaining these diverse mechanisms. Evidence gathered over the last two decades in both experimental models and humans suggests that hypertension arises from increased production of ROS and/or reduced antioxidant capacity in the cardiovascular, renal and central nervous systems [21, 42, 51].

By using animal models of genetic and drug-induced hypertension, we and others have demonstrated increased ROS levels and prooxidant activity, altered antioxidant defences and increased ROS-mediated damage, both at peripheral and central sites of cardiovascular regulation [72–78]. These studies have also underlined the

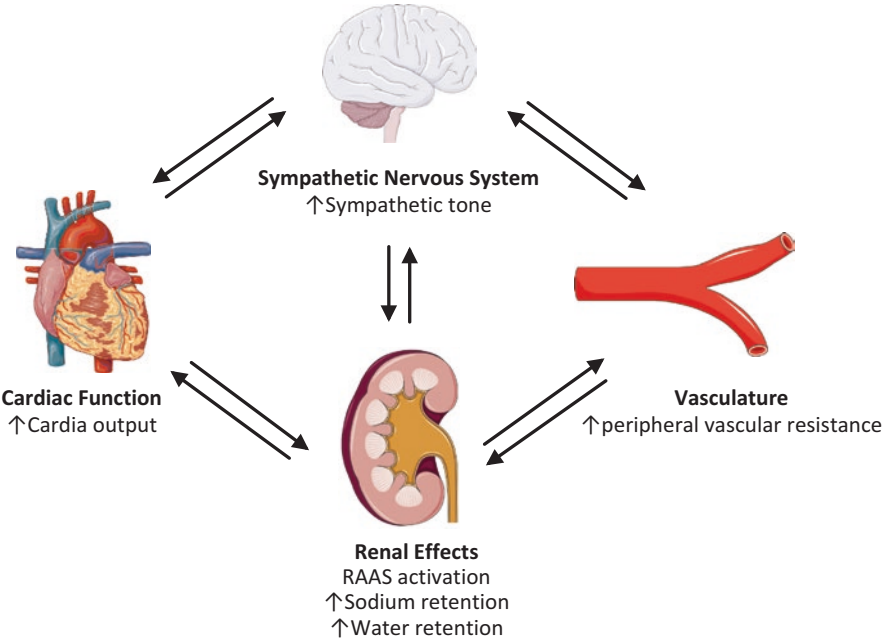


**Fig. 23.3** Organs and mechanisms involved in the development and maintenance of arterial hypertension

importance of the kidney in the pathogenesis of hypertension and identified the renal medulla as a major target for angiotensin II-induced redox dysfunction in hypertension [72, 73]. Similarly to what happens in animals, there is also evidence of redox dysfunction in human hypertensive patients, although the association is less consistent and results vary depending on the biological marker of oxidative stress being investigated. The release of $O_2^{\cdot -}$ from peripheral polymorphonuclear leucocytes is increased in hypertensive patients in comparison with normotensive subjects [79]. Plasma $H_2O_2$ production is augmented in hypertensive patients and, among normotensive subjects, those with a family history of hypertension also exhibit a higher $H_2O_2$ production [80]. Increased levels of byproducts of protein, lipid and DNA oxidative damage, such as malondialdehyde, 8-isoprostanes, 8-oxo-2′-deoxyguanosine, oxidized low density lipoproteins, carbonyl groups and nitrotyrosine, have also been found in biofluids (i.e., plasma, serum and urine) and blood cells of hypertensive patients [81–83]. Furthermore, both enzymatic and nonenzymatic antioxidant defences appear to be reduced in human hypertension [81, 82, 84, 85]. Despite the vast number of studies reporting a close association between oxidative stress and hypertension, there is still an ongoing debate whether oxidative stress is a cause or a consequence of the disorder [86–88].

### 23.2.2 Oxidative Stress as Either a Cause or a Consequence of Hypertension

A large body of literature supports the hypothesis that oxidative stress is a major driver of arterial hypertension. In rats, the induction of oxidative stress through the administration of a common environmental heavy metal pollutant (lead), a glutathione synthesis inhibitor (buthionine sulfoximine-BSO) or a SOD inhibitor (sodium diethyldithiocarbamate-DETC), as well as the intrarenal or intrathecal infusion of $H_2O_2$, lead to increases in blood pressure [72, 89, 90]. The genetic manipulation of enzymes involved in ROS production or metabolism also modifies blood pressure in mice [91–93]. In addition, the exposure of cells and tissues to exogenous oxidants recapitulates molecular events implicated in the pathogenesis of hypertension [72, 94]. Also of importance are the facts that experimental hypertension can be prevented or attenuated by the administration of some antioxidants or inhibitors of ROS production [95–98] and that redox dysregulation, both at systemic and tissue level, precedes the rise in blood pressure [99, 100]. Collectively, these observations in preclinical models of hypertension suggest that oxidative stress plays a causal role in the development of hypertension.

Nevertheless, other authors have failed to demonstrate a direct involvement of oxidative stress in the pathogenesis of hypertension since the administration of antioxidants or inhibitors of ROS generation did not prevent or attenuate experimental hypertension [10]. Indeed, if oxidative stress is causally related to human hypertension, then antioxidants should be able to reduce blood pressure and oxidative damage. However, the majority of clinical trials did not find any blood pressure-lowering effects of antioxidants. One of the largest studies observed no improvement in blood pressure after a 5-year treatment with a combination of vitamin C, vitamin E, and β-carotene versus placebo in subjects thought to be at high risk of cardiovascular disease [101]. Likewise, a recent study found no beneficial effects against major cardiovascular events, including hypertension, after more than a decade of treatment with a multivitamin supplement versus placebo in a population of US male physicians [102]. Furthermore, a meta-analysis failed to reveal a clear benefit after antioxidant supplementation in cardiovascular mortality [103].

There is also evidence that lowering blood pressure *per se* leads to a reduction in oxidative stress and improvement in vascular function [10, 88]. Several antihypertensive drugs with different mechanisms of action, such as angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, beta-blockers and calcium channel blockers, have been shown to attenuate oxidative stress markers in experimental and human hypertension [104, 105]. In light of these observations, some authors suggest that oxidative stress may be rather a consequence than a cause of hypertension. However, some of these antihypertensive agents have direct antioxidant properties and others block the RAAS, whose downstream effects are known to be mediated by ROS [10].

### 23.2.3 Pharmacological Interventions Aimed to Reduce Blood Pressure with Antioxidant Therapies

The rationale for reducing oxidative stress as a therapeutic strategy against hypertension stems from population-based observational studies showing an inverse correlation between plasma antioxidant concentrations, obtained by dietary intake, with blood pressure and cardiovascular risk factors [106]. However, in contrast with preclinical data, no significant improvement in blood pressure has been observed in the vast majority of studies after treatment with single or combination antioxidant therapy in subjects thought to be at high risk of cardiovascular disease (as discussed above in Sect. 23.2.2). A number of potential explanations for the failure of antioxidant supplementation in the chronic suppression of cardiovascular disease in humans have been put forward, including errors in trial design, choice of antioxidants, patient cohorts included in trials, the pathophysiological complexity of ROS/RNS signalling in humans with comorbidities, among others [107]. In what concerns the antioxidants, it is possible that the dose administered and duration of clinical trials were insufficient or agents examined were ineffective and nonspecific. Most antioxidant therapies that have been tested were not chosen because they were proved to be the best antioxidants, but rather because of their easy availability. It is also conceivable that the antioxidants administered failed to target the source of free radicals, particularly if ROS are generated in intracellular organelles and compartments, due to relatively poor uptake of antioxidants by target organs or the interference with other substances that, in some cases, reduce the antihypertensive effects. It is critical to remember that the lack of benefits seen in clinical trials to date does not rule out the essential role of oxidative stress in hypertension and other cardiovascular disorders. Rather, these results highlight the importance of evaluating optimal antioxidant therapies, the ideal cohort of patients to study, and the appropriate trial duration for the future improvement of antioxidant therapy.

## 23.3 Oxidative Stress in Heart Failure

### 23.3.1 The Heart, Metabolic Demand and ROS Production

The mammalian heart is the organ with the highest metabolic demand, consuming a large amount of cellular ATP to maintain the contraction-relaxation cycle. Under physiological conditions, this tremendous energy requirement is fulfilled by the high mitochondrial content of cardiomyocytes [19, 108–110]. Mitochondria ensure the production of more ATP through oxidative phosphorylation, whereby the mitochondrial ETC generates a proton gradient that drives ATP synthesis by ATP synthase. Since this process is sustained by $O_2$, which functions as the final electron acceptor in the ETC, it is not surprising that the heart needs a continuous, as well as adjustable, high supply of $O_2$ to maintain its function and viability [19, 108, 109]. Normally, most of the $O_2$ consumed in oxidative phosphorylation is reduced to

water. However, electron leakage from the ETC also occurs, thus resulting in the formation of a small amount of ROS, namely $O_2^{\cdot-}$ and $H_2O_2$, which can be detoxified by endogenous antioxidant enzymes [19, 108–110]. There are several other ROS-producing enzymes in the heart, namely NOXs, XO, uncoupled NOS, MAOs and MPO, that are present in several cell types such as cardiomyocytes, endothelial cells, vascular smooth muscle cells, fibroblasts, neutrophils, monocytes and macrophages [9, 19, 109–111].

Although large amounts of ROS are markedly detrimental, there is evidence that low-to-moderate ROS concentrations in the heart are involved in physiological processes and beneficial adaptive signalling in response to acute changes in workload or brief ischemic episodes [108, 110, 112]. For example, ROS contribute to cardiomyogenesis of embryonic stem cells and proliferation of neonatal cardiac cells [113, 114]. It has also been reported that $H_2O_2$ derived from dismutation of $O_2^{\cdot-}$ generated by myocardial ETC is involved in coronary dilation, thus linking myocardial oxygen consumption to coronary blood flow [115, 116]. In addition, an increase in mitochondrial-derived ROS appears to mediate the acute inotropic response of cardiomyocytes to β-adrenergic receptor stimulation, being part of the homeostatic physiological signalling in the heart [117]. Importantly, mitochondrial and NOX-derived ROS seem to participate in the protective adaptive responses to moderate hypoxia, through the redox regulation of cardiomyocyte hypoxia-inducible factor activation, and in myocardial ischemic preconditioning, a protective phenomenon triggered by transient ischemic episodes and responsible for enhanced heart resistance to prolonged ischemia-reperfusion scenarios [108, 112, 118, 119].

### 23.3.2 Role of Oxidative Stress in the Pathophysiology of Heart Failure

HF is a complex clinical syndrome derived from structural and/or functional abnormalities in the heart, leading to impaired ventricular filling or ejection [9, 120]. Cardiac dysfunction triggers compensatory haemodynamic and neurohormonal responses attempting to maintain proper tissue perfusion, but these ultimately become maladaptive and deleterious [121]. Typical symptoms of this syndrome include shortness of breath, ankle swelling, fatigue, tiredness and reduced tolerance to exercise [120]. HF is usually a chronic, progressive and terminal illness, associated to poor quality of life for the patient due to the increase in symptoms frequency, severity and distress along disease course. Its prevalence in developed countries ranges from 1–2% in adults but can increase to values equal or higher than 10% in people with more than 70 years old, posing an enormous economic burden on healthcare systems. Of note, HF is the most frequent diagnosis responsible for hospitalization among patients aged 65 years or older [122, 123]. HF aetiologies include those related with diseased myocardium (e.g. ischemic heart disease; toxic damage due to alcoholism, drugs of abuse, medications such as cytostatics, heavy metals or radiation; immune-mediated and inflammatory damage caused by infections or auto-immune conditions; metabolic derangements such as thyroid diseases and

pheocromocytoma; infiltration related with malignancy or other diseases such as amyloidosis; genetic disorders), with abnormal loading conditions (e.g. arterial hypertension; valve and myocardial structural defects; pericardial and endomyocardial pathologies; high output states such as severe anaemia; volume overload caused by renal failure) or with arrhythmias [120].

Despite therapeutic advances, chronic HF often decompensates, leading to the rapid aggravation of symptoms and/or signs of HF and thus requiring hospitalization [120, 124]. The term acute HF frequently refers to this state of acute decompensation of chronic HF but may also represent new-onset HF ("*de novo*" HF) resulting, for example, from acute myocardial dysfunction due to ischemic, inflammatory or toxic insults, acute valve insufficiency or cardiac tamponade (a condition characterized by heart compression and dysfunction as a consequence of pericardial accumulation of fluid, pus, blood, clots or gas due to blunt or penetrating trauma, accidental cardiac perforation following catheterization, infection, cancer and aortic aneurysm rupture) [120, 125].

As mentioned previously, low-to-moderate amounts of ROS contribute to physiological and beneficial adaptive responses in the heart. However, when prooxidant and antioxidant systems are imbalanced, leading to a prevailing prooxidant status, macromolecular damage and harmful signalling may occur and contribute to the genesis and progression of HF [9, 19, 112]. In the heart, there are many processes or targets that can be adversely affected by ROS (Fig. 23.4), namely cardiac contractility, myocardial remodelling, cardiomyocyte apoptosis, mitochondria and

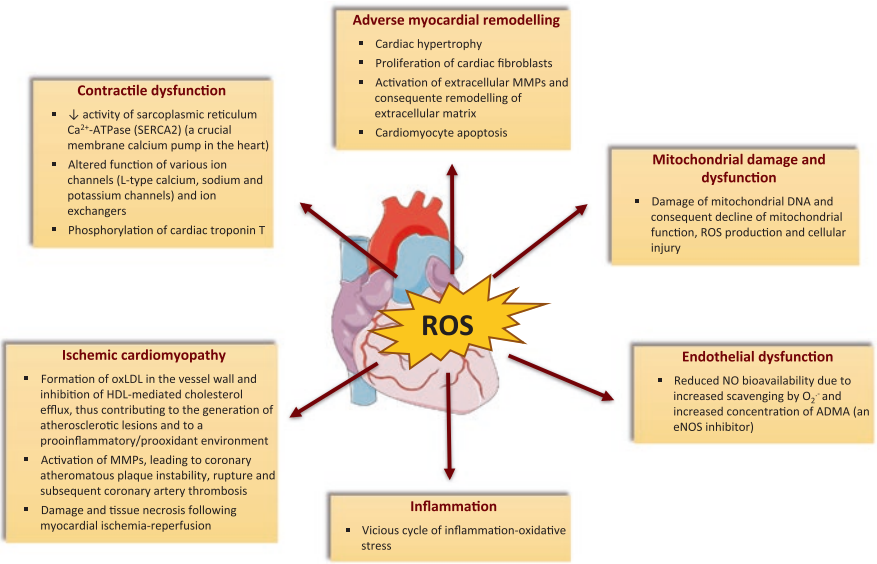


**Fig. 23.4** Adverse effects of ROS in the heart. *ADMA* asymmetric dimethylarginine, *eNOS* endothelial nitric oxide synthase, *DNA* deoxyribonucleic acid, *HDL* high-density lipoprotein, *MMPs* matrix metalloproteinases, *oxLDL* oxidized low-density lipoprotein, *ROS* reactive oxygen species

endothelium [9, 19, 109, 112, 126]. ROS also contribute to ischemic cardiomyopathy by promoting the formation of oxidized low-density lipoprotein (oxLDL), which plays a central role in the pathogenesis of atherosclerosis [9, 109]. Furthermore, the redox sensitive alteration of apolipoprotein A-I, the major protein constituent in high-density lipoproteins (HDL), inhibits the efflux of cholesterol, contributing to atherosclerotic lesions formation and to a prooxidant and proinflammatory environment [127]. The activation of matrix metalloproteinases by ROS is also involved in coronary atheromatous plaque instability, rupture and subsequent coronary artery thrombosis [9, 109]. Of note, after a significant myocardial ischemic insult, the restoration of oxygen supply during the reperfusion phase is responsible for the generation of high amounts of ROS, which contribute to extensive damage and tissue necrosis in the heart [9, 109, 127].

Inflammation plays a central role in the development and progression of chronic HF, regardless of aetiologies [128, 129]. It is also considered an important precipitator and prognostic factor in acute HF [130]. Oxidative stress and inflammation are closely interconnected, contributing to the pathophysiology of HF [9, 109, 131]. Several transcription factors that regulate the expression of proinflammatory cytokines are activated under oxidative stress conditions [9, 109, 131]. In turn, proinflammatory cytokines induce the generation of ROS, thus creating a potential vicious cycle of oxidation and inflammation [9, 131, 132]. Moreover, the production of large amounts of ROS is a feature of activated inflammatory cells, and MPO, a major effector enzyme of neutrophils that is released into the extracellular space during leukocyte activation, also functions as a link between oxidative stress and inflammation [9, 34, 36]. This enzyme uses $H_2O_2$ as a substrate to produce HOCl, which is a potent prooxidant and proinflammatory molecule. Importantly, MPO has the ability to bind and infiltrate in the vascular wall and to utilize $H_2O_2$ derived not only from leukocyte oxidative burst but also from vascular NOX, thus amplifying vascular injury in conditions associated with higher than normal ROS production [9, 36, 133].

Our recent studies have demonstrated the interplay between oxidative stress and inflammatory processes in human HF. In a study involving patients with mild-to-moderate and severe chronic HF, we observed that severe patients had increased values of systemic MPO activity and lower concentrations of lipoxin $A_4$ ($LXA_4$), a specialized proresolving lipid mediator (SPM) that stimulates the resolution of inflammation and tissue regeneration [121, 134]. Furthermore, we found an inverse correlation between $LXA_4$ with proinflammatory/prooxidant markers, such as C-reactive protein (CRP), uric acid and MPO activity, and with markers of heart dysfunction and/or injury, like B-type natriuretic peptide (BNP), troponin I and myoglobin [134]. In addition, in another study evaluating patients with acute HF, cardiogenic shock (the most severe form of HF) and healthy controls, we showed that patients with cardiogenic shock exhibited the highest values of endocan, a marker of endothelial dysfunction, which was significantly associated with inflammatory status [135, 136]. Among the controls and patients evaluated, serum nitrotyrosine, a marker of oxidative/nitrosative stress, was significantly and positively correlated with CRP and high-sensitivity-troponin I, which are markers of

inflammation and myocardial damage, respectively [135]. We also observed that resolvin E1 (RvE1), another mediator of inflammation resolution, increased in line with acute HF severity and was significantly associated with inflammatory/oxidant status and endothelial dysfunction [136].

Noteworthy, LXs and Rvs, besides possessing proresolving and anti-inflammatory properties, have also been shown to exert several protective effects on redox status that may be particularly relevant in the context of HF. These include the blockade of NOX enzymes in endothelial cells and macrophages, inhibition of ROS generation by leukocytes and vascular smooth muscle cells, blockade of angiotensin II-, thrombin- or tumor necrosis factor-α (TNF-α)-induced ROS production in endothelial cells, increased SOD activity and reduced malondialdehyde (MDA) content in the heart, induction of haem oxygenase-1 in endothelial cells and cardiomyocytes and upregulation of nuclear factor erythroid-2 related factor 2 (Nrf2) in cardiomyocytes [121]. Thus, strategies targeting inflammation or promoting its resolution will likely attenuate oxidative stress, and vice-versa, in patients with HF.

### 23.3.3 Biomarkers of Oxidative Stress in Human Heart Failure

Human HF was recently divided into 3 categories according to left ventricular ejection fraction (LVEF): reduced (HFrEF), preserved (HFpEF) or mid-range (HFmrEF) [120]. This definition only comprises the clinical manifestation of an underlying structural and/or functional cardiac abnormality resulting from a myriad of insults, of which the ischemic is the most prevalent in HFrEF. Thus, in clinical trials it is difficult to understand oxidative stress as a cause or consequence of the disease because HF prevails in older ages and it remains underdiagnosed and untreated [137, 138]. It is well established that ageing is associated with increased ROS accumulation, lipid peroxidation and mitophagy as well as atherosclerosis, diabetes and obesity, major risk factors for ischemic heart disease and HF. Despite the association between oxidative stress with clinical outcome in patients with coronary artery disease, no redox biomarker is currently in routine clinical use, in part because they are not specific for individual disease processes [139, 140]. The question remains whether plasma oxidation products reflect systemic or vascular redox state or other biological processes, as well as what is their value for independent stratification and therapeutic management, critical issues to consider them as “biomarkers”. Nevertheless, and surmounting the difficulties associated with the short half-life, limited diffusion and requirement of invasive biopsies to quantify ROS in human tissues, indirect indexes of oxidative stress are gaining increasing acceptance among established biomarkers of HF [141]. These biomarkers can be grouped into three main categories, namely prooxidant enzymes, products of oxidized macromolecules and antioxidant defences.

#### 23.3.3.1 Prooxidant Enzymes

Myeloperoxidase contributes to endothelial dysfunction and mediates dysregulation of vascular tone [10]. The plasma concentration of MPO is elevated in HF

patients compared to controls and its systemic activity is also increased in severe chronic HF compared to mild to moderate HF [134, 142]. Treatment with the inodilator levosimendan seems to reduce the concentration of plasma MPO by decreasing its release from neutrophils in patients with acute decompensation of chronic HF [143]. MPO was selected among others as an incremental prognostic biomarker in a multimarker risk strategy of stratification for cardiovascular death or HF in patients with acute myocardial infarction [144] and it also seems to differentiate forms of acute HF with cardiorenal syndrome [145]. These results, along with its predictive value for cardiovascular morbidity and mortality observed in other relatively large prospective studies, its therapeutic implications and the feasibility of its commercial assays, make MPO one of the most promising redox biomarkers for clinical application [146, 147].

Although NOXs are important cardiovascular ROS sources, available data about the involvement of NOXs in human HF is scarce. One study has described increased NOX2 expression and raised NOX activity in myocardial tissue from human failing hearts compared with non-failing controls, but information is lacking regarding NOX2 association with prognosis or treatment, with the exception of a study reporting a downregulatory effect of mediterranean diet on soluble NOX2-derived peptide values in patients with atrial fibrillation [148]. Nevertheless, compelling evidence suggests that redox protective effects of RAAS inhibitors, which are part of HF pharmacological treatment, are due to the prevention of vascular and phagocytic NOX activation [24, 120, 137].

The $^{\cdot}$NO-generating enzyme eNOS has some limitations as a redox biomarker in humans, not only for its localization (in the vessel wall and cardiomyocytes) but also because the complex regulation of the biosynthesis of its cofactor, $BH_4$, makes hard to estimate the ratio of reduced to oxidized forms ($BH_4/BH_2$) and consequently to calculate eNOS uncoupling, which is responsible for generating $O_2^{\cdot-}$ instead of NO. The administration of $BH_4$ does not improve vascular oxidative stress in patients with coronary disease [149] but indirect strategies like folates [150], statins [9] or polyphenols [151] could do so, thus reinforcing the interest of this pathway for future research in HF. Of note, in high-risk diabetic patients, the cardioprotection and reduction of risk of re-infarction and all-cause mortality afforded by metformin, which is no longer contra-indicated in HF, seems to be related, at least in part, with increased $^{\cdot}$NO bioavailability [152]. Also, the superiority of ticagrelor vs. clopidogrel in reducing cardiovascular events can be explained by the higher $^{\cdot}$NO concentrations triggered by ticagrelor, compared to clopidogrel, through an adenosine-mediated pathway that activates eNOS [153, 154].

#### 23.3.3.2 Products of Oxidized Macromolecules

Lipid peroxidation results from ROS attack to polyunsaturated fatty acids (PUFA) in cell membranes. End-products of lipid peroxidation, including isoprostanes and MDA, affect membrane fluidity, inactivate receptors and enzymes attached to it, and even threaten cell viability. This lipid susceptibility to ROS has attracted considerable attention to the evaluation of lipid peroxides as biomarkers of oxidative stress.

Isoprostanes are produced by ROS-induced peroxidation of arachidonic acid and then released by phospholipases [155]. The most stable and thus most commonly quantified are $F_2$-isoprostanes, which can be assessed in tissues and biological fluids. In HF, isoprostane levels in plasma, urine and pericardial fluid correlate with disease severity and ventricular dilatation [156, 157]. Recent works are hypothesizing that they could be used in a precocious strategy to identify populations with sub-clinical increased cardiovascular risk. In addition, they could also be used to monitor the protective effect of diets (e.g. low-sodium diet), as well as dietary adequacy, in patients with HF [158, 159].

MDA, another product of lipid peroxidation, is routinely evaluated by the thiobarbituric acid-reactive substances (TBARS) assay. There is evidence of increased systemic and intraplatelet production of TBARS in patients with acute or chronic HF [160]. Furthermore, a reduction in TBARS levels was observed in HF patients after treatment with a beta-blocker, short-term inotropic support and vitamin C, but not with the addition of an angiotensin II receptor antagonist to angiotensin converting enzyme inhibitor therapy [161, 162]. MDA appears to contribute to the formation of OxLDL [163] which have been proposed to be an useful predictor of mortality in patients with CHF [164]. MDA or MDA-modified LDL are being evaluated in device studies in advanced HF to monitor oxidative stress in patients under device therapy (implantable cardioverter defibrillator, continuous-flow left ventricular assist device) [165, 166].

Oxidative posttranslational modifications of cellular proteins by means of tyrosine nitration, protein carbonylation, and S-glutathionylation, can accurately reflect oxidative stress in HF patients. One of the most emblematic examples of protein oxidation in HF is myocardial sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA) inactivation by nitration, which may contribute to reduced contractility and progression of HF [167]. Furthermore, ceruloplasmin tyrosine nitration with consequent antioxidant reduced activity is associated with reduced survival in patients with HF [168]. Protein nitration by peroxynitrite and haem peroxidase can result in gain of function or inactivation of different proteins in plasma, vessel wall and myocardium that link nitrosative stress to cardiovascular disease and, for that reason, nitrotyrosine is also emerging as a good candidate for a marker of cardiovascular risk [169].

Protein carbonyls can result from oxidation of amino acid side chains, reaction with lipid peroxidation products and glycation/glycoxidation of Lys amino groups. They are very stable and represent a good mirror of protein oxidation Increased carbonyls were found in diaphragm biopsies from patients with end-stage HF, probably resulting from increased Nox2-derived ROS and imbalanced antioxidant enzymes [170]. The inodilator levosimendan prevented the increase in MDA, protein carbonyls and nitrotyrosine in hospitalized patients with worsening HF. These results point to a cardioprotective effect of this drug and thus its wider use in advanced CHF patients has been hypothesized [171]. Additionally, there is evidence that a polymorphism in angiotensin II type 1 receptor can predict the formation of carbonyls in HF patients, suggesting that angiotensin signalling contributes to oxidative stress in HF [172].

8-hydroxy-2′-deoxyguanosine (8-OHdG) results from oxidative DNA damage and its levels can be quantified in urine. In fact, 8-OHdG was demonstrated to be higher with increasing HF severity and correlated with left ventricular ejection fraction in patients with chronic HF [173]. It also seems to be a tool to evaluate beta-blocker responsiveness in chronic HF patients or even to diagnose subclinical left ventricular diastolic dysfunction in hypertensive patients [174, 175], but more data is needed and/or combination with other biomarkers in a multipanel strategy.

#### 23.3.3.3 Antioxidant Defences

Antioxidant enzymes (e.g. catalase, GPx, SOD) can be measured in blood samples but their values are hard to interpret and these studies have low reproducibility or therapeutic/prognostic implications [176–178] On the other hand, there has been an enthusiastic exploration of non-enzymatic antioxidants, such as biopyrrins (oxidative metabolites of bilirubin) and albumin since urinary levels of biopyrrins have been shown to be associated with HF severity [179] and oxidative stress has been proposed as a cause for the development of hypoalbuminemia in ischemic HF [180]. Nevertheless, the disappointing results of studies evaluating the effects of antioxidant administration in HF patients, particularly the failure of vitamins C and E to improve prognosis and the deleterious effects observed in HOPE and HOPE TOO trials, restrained the enthusiasm in this area [181–183].

#### 23.3.3.4 Other Oxidative Stress Markers

Uric acid is an end-product of purine metabolism in humans derived from XO that catalyses its conversion from hypoxanthine. Although it is one of the most abundant aqueous antioxidants in plasma, it can also exert prooxidant effects [10]. Uric acid is frequently accepted as a biomarker of HF [141] but remains a controversial issue because causality in its relationship with cardiovascular disease remains uncertain. Although affected by renal function and diuretic use, there is enough evidence demonstrating that it can work as an independent and simple, albeit nonspecific, predictor of excessive oxidative stress and of adverse prognosis in HF [184].

## 23.4 Concluding Remarks

A vast body of literature accumulated over the past decades has firmly implicated oxidative stress in the pathogenesis and progression of cardiovascular diseases, including hypertension and HF, as well as associated risk factors and comorbidities. Key molecular events in hypertension and HF, such as oxidative modification of lipids and proteins, endothelial cell activation and inflammation, are facilitated by oxidative stress. More recently, the role of redox signalling and specific molecular targets have also been appreciated. Despite the significant progress in understanding the pathophysiology of these conditions and the promising results in pre-clinical animal models, clinical trials of antioxidant approaches to prevent cardiovascular mortality and morbidity have been, so far, disappointing. Several hypotheses have been put forward, including the failure to appreciate the complexity of the effects of

ROS or inappropriate antioxidant selection or dosage, which warrants future research on new compounds with improved properties. Finally, more human data is required to provide clinical relevance and determine the potential for clinical translation. Nevertheless, several studies indicate that oxidative stress biomarkers may be useful for risk stratification and to monitor the protective effects of pharmacological treatment, diets or devices in human HF.

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