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Chapter
Oxidative Stress in Cardiovascular Diseases
Laura Mourino-Alvarez, Tamara Sastre-Oliva, Nerea Corbacho-Alonso and Maria G. Barderas

Abstract
Cardiovascular diseases encompass a range of pathologies that affect the heart or blood vessels. Oxidative stress is an important factor that contributes to the development of these pathologies. Adverse effects due to oxidative stress manifest when there is an imbalance between the production and elimination of reactive oxygen species (ROS), or when physiological mechanisms of repair for oxidative injury are overburdened. This chapter focuses on ROS accumulation and antioxidant system deficiencies in the context of their influence on cardiovascular disease. We also discuss the importance of high throughput approaches, such as proteomics, with regard to their role in advancing the field of precision medicine for cardiovascular diseases, while keeping in mind the ultimate goal of improving patient care and quality of life.

Keywords: cardiovascular disease, endothelial dysfunction, reactive oxygen species, thiol compounds, oxidative stress

1. Introduction
Cardiovascular diseases (CVDs) constitute a major cause of global mortality and are an important source of rising healthcare costs; furthermore, they result in significant decreases in the quality of life (QoL) of those who suffer from these conditions [1, 2]. The great majority of CVDs are chronic diseases, and hence, rising incidence translates into higher prevalence, which leads to a healthcare burden that may persist over decades. Prevalence has long been on the rise in almost all countries, with reported increases from 271 million in 1990 to 523 million in 2019, while CVD-associated deaths have also followed suite, increasing from 12.1 million to 18.6 million in the same period [3]. CVDs encompass a range of pathologies that affect the heart or blood vessels, including ischemic heart disease, cerebrovascular disease (stroke), peripheral arterial disease, heart failure, and heart valve disease. Hypercholesterolemia, hypertension, and diabetes, which are associated with oxidative stress (OS) and inflammatory activation, are well-known risk factors for CVD [4–7].

OS originates in cells and tissues when the balance between oxidative and antioxidant compounds (or mechanisms) is disrupted in favor of oxidation. This imbalance between ROS production and antioxidant defenses may be due to increased ROS.
formation (e.g., superoxide, hydroxyl, nitric oxide, or hydrogen peroxide) and/or insufficiency in antioxidants (e.g., ascorbate, alpha-tocopherol, or thiol-based redox compounds) [8]. The consequences of OS include lipid peroxidation, membrane damage, and the activation of proteases, nucleases, and protein kinases. With respect to vascular functions, excess ROS regulates the release of factors that drive vasoconstriction or vasodilation, leading to endothelial cell (EC) damage, vascular smooth muscle cell (VSMC) hyperplasia, and structural remodeling (Figure 1) [9, 10].

Regulation of vascular tone is critical for the maintenance of cardiovascular health. In this sense, OS plays an important role as an initiator of the EC dysfunction. In physiological conditions, maintenance of appropriate endothelial function provides vasorelaxant properties through the release of vasoactive substances. Nevertheless, under OS conditions, there exists an imbalance between the production of vasoprotective and vasorelaxant factors and vasoconstrictor substances by the endothelium [11]. In addition, oxidative stress could induce vascular inflammation and injury through activation of the transcription factors, upregulation of adhesion molecules, stimulation of chemokine production, and recruitment of inflammatory cells [9, 10].

In the following sections, we focus primarily on the OS-related mechanisms that have been associated with CVD development. Firstly, we discuss how ROS accumulate and the importance of this accumulation with regard to endothelial dysfunction and

Figure 1.
Cardiovascular risk factors disrupt oxidative metabolism and negatively affect the vascular system through lipid peroxidation, endothelial damage, immune cell activation, structural remodeling, or inflammation.
the vascular system. Next, we concentrate on the role of thiols in the antioxidant system and the consequences of the suppression of this defense system. Finally, we highlight the importance of high throughput approaches, such as proteomics, with respect to their contributions to the advances in the field of precision medicine related to CVD, while keeping in mind the ultimate goal of improving patient care and QoL.

2. ROS accumulation: mitochondrial and ER-related stress

Endothelial damage, vascular dysfunction, cardiac remodeling, immune cell activation, and systemic inflammation are key processes implicated in CVDs, all of which are affected by ROS accumulation [12–15]. The primary oxidase system underlying OS in vascular disease is the NADPH oxidase (NOX) system, the main function of which is to produce ROS [16]. Importantly, while ROS production was originally considered to incite harmful effects, and low levels of ROS are necessary for physiological processes, including cell proliferation, migration, differentiation, and cytoskeletal organization [17]. However, excessive activation of NOX also activates other oxidase systems to maintain OS (Figure 2). These secondary mechanisms include, but are not limited to, endothelial nitric oxide synthase (eNOS) uncoupling, mitochondrial stress, and endoplasmic reticulum (ER) stress, which also contribute to redox changes in CVD [18–20]. eNOS is primarily responsible for the generation of vasoprotective nitric oxide (NO). Endothelial-derived NO is an important vasodilator and a potent inhibitor of platelet aggregation and leukocyte adhesion. As such, NO is one of the most important anti-atherogenic factors in vasculature [21]. Under normal conditions, eNOS exists as a dimer that is stabilized by the essential cofactor, tetrahydrobiopterin (BH4). However, when BH4 is inactivated due to excess ROS, the

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Figure 2.
Excessive activation of NADPH oxidase (NOX) increases the production of ROS to harmful levels. Subsequently, secondary mechanisms, such as endothelial nitric oxide synthase (eNOS) uncoupling, mitochondrial stress, and endoplasmic reticulum (ER) stress, are triggered, perpetuating oxidative stress and injurious effects in a vicious cycle.
dimer breaks down, resulting in the production of vaso-injurious superoxide (O$_2^-$) as opposed to NO [22].

The cross-talk between the NOX system and mitochondria may cause ROS-induced release of ROS, a positive feed-forward mechanism of ROS production. As a result, NOX-derived ROS increases the production of mitochondrial ROS, which in turn stimulates NOX activation [23–25]. One consequence of normal mitochondrial metabolism and homeostasis is the production of controlled levels of ROS caused by the reduction of O$_2$ to O$_2^-$ through the respiratory chain [26]. Under physiological conditions, these levels are safe and normal cellular activity is maintained through the protection of mitochondria by a multilayer network of antioxidant systems. One particular example is the manganese-dependent superoxide dismutase (SOD2) enzyme, which converts the O$_2^-$ anion to hydrogen peroxide (H$_2$O$_2$), which is subsequently transformed to water by protective mechanisms, such as catalase, glutathione peroxidase, and peroxiredoxins [27]. Nevertheless, if ROS levels exceed the normal range, the resulting oxidative environment is harmful and cannot be compensated for by these protective mechanisms [23, 27, 28]. In relation to this, mitochondrial dysfunction also has important implications for CVD. The protective role of different antioxidant systems against atherosclerosis is related to H$_2$O$_2$ metabolisms, such as that driven by the catalase or paraoxonase family, as seen in mouse models [29–31]. Mitochondrial dysfunction is associated with the redox inactivation of the mitochondrial deacetylase Sirtuin 3 (also named Silent acting type information regulation 2 homolog 3, SIRT3) and the mitochondrial antioxidant SOD2 also contributes to the development of hypertension [32, 33]. These discoveries brought forth the use of mitochondrial-targeted interventions as therapeutic agents. Mitoquinone (MitoQ), a mitochondrial ROS scavenger, has been shown to reduce macrophage infiltration into atherosclerotic plaques in a mouse model of metabolic syndrome [34]. Moreover, MitoQ protects against the development of hypertension by improving endothelial NO bioavailability and reduces cardiac hypertrophy in models of hypertension [35]. Similar favorable vascular responses have been demonstrated in humans treated with MitoQ. After oral supplementation, MitoQ was associated with a decrease in plasma oxidized low-density lipoprotein (LDL), a circulating marker of OS, and the amelioration of endothelial function [36].

The ER is increasingly being recognized as an important player in the redox pathophysiology of the cardiovascular system [37–39]. Protein folding contributes to the generation of ROS since ER oxidoreductases serve as terminal electron acceptors with oxygen. When ER stress develops, demands on protein folding are excessively increased and oxygen is not completely reduced, leading to the generation of O$_2^-$ anions with the consequent production of H$_2$O$_2$ or other ROS [40, 41]. The ER and mitochondria are closely associated as the protein folding process is energy-dependent. As a consequence, ATP depletion may occur during ER stress, which can stimulate oxidative phosphorylation in the mitochondria due to increased ATP need, subsequently resulting in greater ROS generation. In addition, mitochondrial ROS production will also increase if unfolded proteins accumulate in the ER via the release of Ca$^{2+}$ or interactions with SOD [42, 43]. Of note, ER stress has been shown to play an important role in vascular cell phenotypic switching, de-differentiation, calcification, and apoptosis, contributing to endothelial dysfunction and vascular remodeling in hypertension and in atherosclerosis [44, 45].

With regard to these data, it has been inferred that compounds, which can alleviate ER stress could be pharmacological options to elicit antioxidant effects. One example is the antihypertensive drug guanabenz, which is demonstrated to confer
protection against the detrimental accumulation of misfolded proteins in cardiac myocytes [46]. However, there is much controversy about this compound, since it has been reported to induce β-cell dysfunction in vitro and in vivo (in rodents) and it may lead to impaired glucose tolerance [47]. Other ER stress inhibitors, such as tauroursodeoxycholic acid (TUDCA) or 4-phenylbutyrate (4-PBA), also have beneficial effects on the cardiovascular system. Administration of TUDCA has been shown to alleviate myocardial contractile dysfunction and reduce blood pressure in rodents [48-50]. Likewise, 4-PBA protects against atherosclerotic lesion growth [51], prevents cardiac rupture and remodeling by inhibiting cardiac apoptotic and fibrotic signaling pathways [52], and attenuates interstitial fibrosis and cardiac hypertrophy caused by pressure overload [53]. These studies are among the studies that provide support to the proof of concept that modulation of ER stress can improve cardiovascular function.

3. Thiol-based redox compounds in CVD

Thiol-based redox compounds, such as thioredoxins (Trxs), glutaredoxins (Grxs), and peroxiredoxins (Prxs), are primary contributors to the intracellular redox state, thereby modulating metabolism, signaling, and cell survival pathways [54]. In proteins, the thiol groups of cysteine side chains are highly susceptible to reversible or irreversible oxidative modifications. Besides forming disulfide bonds between two different proteins, these protein thiols can also form disulfide bridges with low-molecular-weight thiols like glutathione, they can be oxidized to sulfenic, sulfinic, and sulfonic, or suffer S-nitrosylation [55]. These modifications can alter the activity of numerous proteins that contain cysteines (Cys) as their anionic form. As such, thiolate (RS-) plays critical role in protein structure, function, and regulation. Although the Trxs and Grxs systems function differently, they both maintain a reduced intracellular redox state in mammalian cells by reducing protein thiols. Oxidized Grx is reduced by reduced glutathione (GSH), and the oxidized glutathione (GSSG) is then recycled by glutathione reductase (GR) at the expense of NADPH. The Grx system involves the GR, Grx, and the glutathione redox pair (GSH/GSSG). Under oxidative conditions, in which the GSH concentration decreases and GSSG increases, Grx is more likely to be oxidized [55]. Thus, the GSH-to-GSSG ratio (GSH/GSSG) in the cell is an important marker of the redox environment and a major determinant of the cellular redox potential. Meanwhile, Prxs are a family of conserved abundant Cys-based peroxidases that consist of six Prx isoforms (Prx1–6) in mammalian cells. This family plays an essential role in the detoxification of hydrogen peroxide, aliphatic and aromatic hydroperoxides, and peroxynitrite [56, 57].

Currently, the implications of these thiol-based redox compounds on CVDs are being widely studied [55, 58, 59]. Endogenous Trx has the potential to decrease reperfusion-induced arrhythmia [60] and is a central mediator of cardiomyocyte growth [61]. Upregulation of Trxs has been detected in ischemia [62, 63] and cardiac failure [64, 65], probably as a compensatory mechanism in response to myocardial injury. In fact, the role of Trx1, the cytosolic isoform of the Trx family, in myocardial hypertrophy is identified to be fundamental as it acts as a negative regulator under normal conditions but initiates hypertrophic signaling in the myocardium when it is oxidized due to stress [66, 67]. Moreover, the important role of Trx in hypertension has been demonstrated in mice, through experiments involving injection of human Trx and other studies in which Trx-overexpressing transgenic mice were assessed [68]. In these studies, Trx decreased age-related hypertension through different
mechanisms, such as preservation of functional eNOS and NO release, and maintenance of relaxation responses. These researchers also found decreased arterial stiffness and improved vascular flow as a result of both Trx injection and overexpression. In another study, Trx1 overexpression was found to decrease infarct size and improve myocardial function after infarction [69].

The Grx superfamily has also been associated with enhanced cell survival and improved resistance to OS in CVD. In fact, overexpression of Grx1 has been shown to reduce ventricular remodeling and improve cardiac function [70], effects that were later proposed to be associated with the induction of angiogenesis. Nevertheless, Grx may offer protection to the ischemic heart by inhibiting apoptosis as demonstrated by an in vivo study in which Grx up-regulation inhibited EC migration and impaired hind limb revascularization [71]. The cardioprotective effect of Grx1 via a decrease in OS-mediated apoptosis is well documented [72–74]. In human coronary arteries, Grx was expressed (together with Trx) in ECs, fibroblasts, and smooth muscle cells. Specifically, the macrophages infiltrating fibrous plaques of atheroma that are known to produce ROS strongly express Grx and Trx [75]. In the year 2000, a Grx isofrom located in the cytosol was discovered, defined as Grx3 or protein kinase C-interacting cousin of thioredoxin (PICOT). Although there is little data available about this oxidoreductase, it has a promising cardioprotective effect against ischemia/reperfusion-induced cardiac injury [76], as well as in aging and heart failure [77].

Apart from oxidoreductases, Prx proteins are essential for the regulation of OS and H2O2-mediated intracellular signaling. Some studies have shown that different isoforms of Prx protect against atherosclerosis. On the one hand, Prx1 scavenges H2O2 [78] and degrades lipids in macrophages, thereby reducing foam cell formation [79]. On the other hand, Prx2 and Prx4 modify the development of atherosclerotic plaques by altering immune cell infiltration [80, 81]. Moreover, Prx2 protects against neointimal thickening [82] and negatively regulates H2O2 generation and the formation of thrombosis [83, 84], whereas, its deficiency has been shown to promote the progression of the abdominal aortic aneurysm [85]. Indeed, Prx has an important influence on myocardial tissue and the overexpression of Prdx1 in cardiomyocytes was recently proposed to confer protection against cardiac hypertrophy and heart failure in the presence of pressure overload [86]. Prx3 ameliorates inflammation and protects the heart against left ventricular remodeling and failure after myocardial infarction [87], and both Prx3 and Prx6 may have a protective role during ischemia/reperfusion-induced heart injury [88]. Together these data suggest that Prx could be a potential target in therapeutic strategies to combat CVD.

4. Redox proteomics to get inside oxidative stress

The regulation of redox signaling commonly involves the post-translational modification (PTM) of proteins, in which different amino acids, including methionine and tyrosine, and most importantly Cys, are sensitive to oxidation and other modifications [89, 90]. As described above, the oxidation/reduction of thiol proteins has emerged as one of the major mechanisms through which reactive oxidants modulate cell signaling [91]. Accordingly, several proteomics-based techniques have been developed to enrich, identify, and characterize thiol-related redox modifications, referred to as redox proteomics [92]. The main aims of redox proteomics include (1) the identification of proteins that are modified or that interact with regulatory oxidoreductases, (2) the identification of residues susceptible to modification, including
specific ROS-induced redox modifications, and (3) the quantification of the proportions of the modified protein(s) [93]. Crucially, RS\(^{-}\) is very reactive, and thus in order to study reversible Cys oxidations, it is essential to block this reactivity to capture the \textit{in vivo} thiol-redox status. For this reason, one of the initial steps in thiol proteomics is to block free thiols in a sample with an alkylating reagent, such as iodoacetamide or derivatives of maleimide [94]. This also enables the reversibility of thiol modifications to be used to selectively label and/or purify redox-modified proteins. After alkylation, proteins can then be reduced using a thiol reducing agent, such as dithiothreitol or tris (2-carboxyethyl) phosphine, and, newly-formed free thiols can also be studied. Differential thiol isotopic labeling allows samples of different groups to be combined, reducing run-to-run experimental variation and permitting relative quantification between different samples (\textit{Figure 3}) [95]. Although redox proteomics is still in its infancy, assessment of possible biomarkers through mass spectrometry (MS)-driven strategies may be promising for the study of OS in different disease states. In contrast to direct measurement of ROS levels, which is a complex task given the short half-life and high reactivity of these species, redox proteomics allows the detection of the resulting oxidative damage to proteins. Thus, it provides mechanistic insight into cellular toxicity mechanisms. For that reason, some studies employing this approach have been performed in CVDs.

One of the first attempts to apply redox proteome analysis to heart proteins was performed using two different proteomic methods: gel-based (DIGE) and gel-free proteomics (ICAT) [96]. Heart tissue was analyzed with the purpose to identify and quantify redox-sensitive proteins, resulting in the elucidation of 50 proteins as potential targets of H2O2 oxidation through the ICAT method and 26 such proteins with the DIGE method, 13 of which were detected with both methods. Several years later, a different technique, named GELSILOX, was used to investigate the mechanisms of damage produced in the heart by ischemia/reperfusion injury and the effects of ischemic preconditioning in mitochondria purified from cardiomyocytes [97]. Several proteins were identified that had previously been associated with redox changes but had not been distinguished in the context of ischemia/reperfusion damage. Although these were mainly proof-of-concept studies of the methodology used, they provide useful information.
about the redox proteome of the cardiovascular system, including specific sites of oxidation. More recently, the plasma redox proteome was analyzed using FASILOX, a novel multiplexed proteomic strategy of isotope labeling, followed by liquid chromatography (LC)-tandem MS [98]. A plasma signature was proposed for the stratification of young individuals and to detect those with a higher probability of suffering a future cardiovascular event. The same approach was used to analyze aortic valve tissue obtained from patients with calcific aortic valve disease [99], which revealed different protein profiles in calcified valve tissue in patients with and without atherosclerosis. Differences in the redox status of the aortic valve tissue were also found despite their high degree of calcification and affection. Additionally, two specific sites of cysteine oxidation in albumin that had not been described previously were also found. The results of these studies highlight the enormous potential of redox proteomics, an approach that is set to become a key tool to obtain new insights into CVD-related protein modifications.

5. Future perspectives: a focus on redox biology to improve patient management

Overall, excessive OS due to exaggerated ROS production or insufficient antioxidant defense has been closely associated with CVD. Improving our understanding of these pathways may permit the modulation of redox biology, which can be instrumental in reducing the systemic impact of these alterations. From a clinical point of view, the combination of standard clinical evaluation with results from high throughput approaches is essential to take a step forward in precision medicine, currently a highly desired goal in medicine. By definition, precision medicine focuses on selecting the appropriate treatment for each patient based on individual phenotypes. This includes a reliable and accurate risk stratification that would allow better screening of patients at high risk and avoiding the use of unnecessary treatments with regard to potential side effects. Since the disruption in OS balance leads to a vicious cycle, it is crucial to identify high-risk patients to avoid the damage caused by an uncontrolled redox milieu.

CVDs are multifactorial diseases that are usually accompanied by other comorbidities, and OS incites a systemic effect that can damage different organs and systems. From a pharmacological point of view, this implies that modification of the redox state may also affect distinct organs in different patients, which may have beneficial and detrimental effects. This kind of treatment can theoretically improve the function of different systems and may be considered an integral treatment, although this unfortunately leads to less control over potential side effects. The only way to overcome this issue is to intensify research focusing on OS and its relationship with disease. Although molecules related to OS are well known but their possible roles as cardioprotective molecules are still not fully understood. More information about this facet of their activity will be essential to design successful new pharmacological approaches. However, we must be aware that there is a long way to go in this regard. Indeed, it should not be forgotten that alterations that alter multiple metabolic pathways may be detrimental when applied at inappropriate times or doses.

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**Conflict of interest**

The authors declare no conflict of interest.
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