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Review article

Discovery of new therapeutic redox targets for cardioprotection against ischemia/reperfusion injury and heart failure

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Running title: ROS in vascular disease – targets for redox drugs

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Abbreviations: CVD, cardiovascular diseases; GBD, Global Burden of Disease; HFrEF, heart failure with reduced ejection fraction; IHD, ischemic heart disease; I/R, ischemia/reperfusion; NCD, non-communicable diseases; RAAS, renin-angiotensin-aldosterone system; ROS, reactive oxygen species; RONS, reactive oxygen and nitrogen species; SOD, superoxide dismutase.

CONTENTS

Abstract

Global epidemiological studies reported a shift from maternal/infectious communicable diseases to chronic non-communicable diseases and a major part is attributable to atherosclerosis and metabolic disorders. Accordingly, ischemic heart disease was identified as a leading risk factor for global mortality and morbidity with a prevalence of 128 million people. Almost 9 million premature deaths can be attributed to ischemic heart disease and subsequent acute myocardial infarction and heart failure, also representing a substantial socioeconomic burden. As evidenced by typical oxidative stress markers such as lipid peroxidation products or oxidized DNA/RNA bases, the formation of reactive oxygen species by various sources (NADPH oxidases, xanthine oxidase and mitochondrial resperatory chain) plays a central role for the severity of ischemia/reperfusion damage. The underlying mechanisms comprise direct oxidative damage but also adverse redox-regulation of kinase and calcium signaling, inflammation and cardiac remodeling among others. However, reactive oxygen species have not only detrimental effects but are also involved in essential cellular signaling and may even act protective as seen by ischemic pre- and post-conditioning or eustress. These processes and the role of reactive oxygen species are discussed in the present review. We also present and discuss potential targets for redox-based therapies that are either already established in the clinics (e.g. guanylyl cyclase activators and stimulators) or at least successfully tested in preclinical models of myocardial infarction and heart failure (mitochondria-targeted antioxidants).

Keywords: ischemia/reperfusion injury; heart failure; endothelial dysfunction; mitochondrial dysfunction; oxidative stress; redox drugs.

1. Introduction

According to data of the Global Burden of Disease (GBD) Study, the World Health Organization and Global Health Observatory, there was a substantial shift from maternal communicable diseases (mainly infections and malnutrition) to chronic non-communicable diseases (NCDs) such as atherosclerosis and metabolic diseases during the last decades [1]. Chronic NCDs will be responsible for 77% of the global burden of disease by 2030 as projected by the World Health Organization [2]. Currently more than half of these chronic NCDs are of cardiovascular or metabolic origin [3]. Ischemic heart disease (IHD), comprising coronary artery disease and acute coronary syndrome, was identified by the GBD Study investigators as a leading risk factor for global deaths and disability adjusted life years [4]. In 2017, the global prevalence of IHD was 128 million people leading to almost 9 million premature deaths [4], mainly via typical IHD sequelae such as acute myocardial infarction (AMI) and heart failure, the latter with a prognosis of 50% survival within 5 years after diagnosis, and with only limited treatment options [5-7]. Also, the socioeconomic burden for the health care systems but also loss of working time caused by IHD is substantial. In the USA, the average medical costs for heart failure care amount to approximately 25,000 USD per patient annually, where the hospitalization costs contribute the major part (almost 16,000 USD per year) [8].

In patients presenting with AMI, the myocardium is subjected to acute lethal ischemia and reperfusion (I/R) injury, the consequences of which are acute myocardial injury and cardiomyocyte death. This manifests as myocardial infarction (MI) and impaired left ventricular systolic function, which are both strong predictors of clinical outcomes such as heart failure in patients with AMI. Therefore, new treatments are needed to protect the heart against the detrimental effects of acute myocardial I/R injury, in order to improve clinical outocomes in patients with AMI and heart failure. In this article we review the role of oxidative stress and redox biochemistry as targets for cardioprotection.

2. The oxidative stress concept in cardiovascular disease

2.1 Reactive oxygen and nitrogen species related pathomechanism of endothelial dysfunction and cardiovascular damage

Without any doubt, mitochondrial damage and dysfunction contributes to the phenotypic changes observed in acute myocardial I/R injury and heart failure [9]. Alterations of the signaling pathways involving extracellular vesicles are also part of these phenotypic changes [10]. Both pathomechanisms also provide diagnostic opportunities and therapeutic targets for IHD. Endothelial dysfunction as measured by flow-mediated dilation (FMD) or acetylcholine-dependent plethysmography in peripheral arteries represents an early correlate of atherosclerosis and has predictive value for future cardiovascular events and accordingly has broad diagnostic implications [11]. Patients with IHD (comprising coronary artery disease and acute coronary syndrome) [12-17], heart failure [18, 19] and myocardial infarction [20, 21] display impaired endothelial function. Of note, endothelial dysfunction is directly correlated with oxidative stress and vascular inflammation [14, 22], representing a strong diagnostic tool to investigate cardiovascular pathophysiology, morbidity and mortality [23, 24].

The central concept of redox regulation of the vascular tone by the highly protective vasodilator nitric oxide and its harmful antagonist superoxide anion is of great (patho)physiological importance [25, 26] and was first reported by Gryglewski, Palmer and Moncada [27]. A detrimental role of superoxide is supported by the existence of superoxide dismutases (mitochondrial Mn-SOD and cytosolic/extracellular Cu,Zn-SOD), described in the 1960s, with the sole biological function to detoxify superoxide anion radicals [28] and impaired vascular function or even lethality in SOD knockout mice [22, 29, 30]. Superoxide and related or reactive oxygen species (ROS) products (e.g. H_2O_2, hydroxyl radicals and organic peroxides) are mainly formed from primary sources such as NADPH oxidases and (dysfunctional) mitochondrial respiratory chain as well as secondary sources such as xanthine oxidase and

uncoupled nitric oxide synthases (**Figure 1**) [31-35]. Primary sources produce ROS without post-translational modification, whereas secondary sources require initial (redox) modification as known for xanthine dehydrogenase to oxidase conversion and uncoupling of nitric oxide synthases [33, 36]. P450 or heme enzymes can also generate superoxide and other ROS as a side reaction.

Almost any cardiovascular disease is associated with an imbalance between the formation of reactive oxygen and nitrogen species (RONS, including superoxide, hydrogen peroxide and hydroxyl radicals, and •NO, as well as products such as peroxynitrite and hypochlorous acid) and detoxification by low molecular weight antioxidants ROS-degrading enzymes [34, 35]. This imbalance will ultimately lead an altered redox state affecting multiple enzymatic pathways that are under redox-control [37]. Oxidative stress manifests by accumulation of oxidative modifications of biomolecules such as lipid peroxidation products, oxidized DNA/RNA or proteins as well as glutathione/peroxiredoxin redox state (= redox biomarkers), when the damage repair capacity of the cell and organelles is exceeded [38]. In addition, abnormal alterations of gene/protein expression, enzymatic activity and cellular pathways (e.g. apoptosis) may be indicative of oxidative stress [39, 40]. However, some of the RONS (especially nitric oxide and H_2O_2) have a role as cellular messengers and contribute to redox signaling, e.g. via S-nitros(yl)ation, reversible thiol oxidations and ferrous/ferric enzyme equilibria [41-43]. RONS also contribute to a preconditioning-like protective process by upregulating antioxidant enzymes [44], which may also explain the failure of clinical trials using oral administration of unspecific antioxidants such as vitamins C and E (see discussion below) [45].

Direct evidence for a role of oxidative stress in cardiovascular disease (CVD) is based on large clinical trials or meta-analysis demonstrating an association of redox biomarkers with cardiovascular events or mortality [22]. According to a systematic review and meta-analysis in patients with CVD (1,916 subjects), higher 8-hydroxy-2-deoxyguanosine levels were

associated with a cardiovascular higher risk [46]. Another meta-analysis in patients with atherosclerotic CVD (14,509 subjects) showed that higher levels of circulating oxidized low-density lipoprotein (oxLDL) were associated with a higher cardiovascular risk [47]. A study in 10,622 men showed an independent association of all-cause and CVD mortality with redox biomarkers in the serum, derivatives of reactive oxygen metabolites (D-ROM indicative of ROS levels) and total thiol levels (TTL representative of redox state) [48]. A study in 636 individuals showed that higher levels of the antioxidant enzyme glutathione peroxidase-1 were positively correlated with cardiovascular event–free survival [49]. In addition, there are numerous small-cohort clinical studies showing that endothelial function (mostly measured by FMD or acetylcholine-dependent plethysmography) in patients with cardiovascular diseases or risk factors is mutually associated with redox biomarkers such as superoxide dismutase activity as well as circulating glutathione (reduced form), oxLDL/ADMA and malondialdehyde/8-oxo-deoxyguanosine levels [11, 22]. There is also good evidence for an important role of oxidative stress in the pathophysiology of IHD in general [50] as well as ischemia/reperfusion damage [51, 52] and heart failure in particular [53-56].

2.2 Central role of oxidative stress and impaired redox signaling for the pathomechanisms of acute myocardial ischemia/reperfusion injury and heart failure

ROS released acutely in large amounts have been implicated in the cell death associated with myocardial infarction, I/R injury and heart failure [57, 58] and all major processes triggered by ROS are summarized in **Figure 2**. I/R damage occurs when the blood supply to a tissue is compromised for minutes to hours (ischemia) and then restored (reperfusion) [59]. ROS are produced mainly at the beginning of reperfusion, whereas relatively low amounts are generated during ischemia. Ischemia is characterized by a lack of O_2 and substrates which induce metabolic disturbances, cell membrane permeability changes, alterations in ion channel

function, and depletion of ATP. The metabolic derangement that occurs during ischemia may trigger a cascade of events including ROS formation from residual molecular oxygen, loss of nucleotide homeostasis and disruption of Ca^{2+} homeostasis [60, 61]. Therefore, RONS are produced during ischemia, but they are especially produced during reperfusion since one of the first damaging events upon reperfusion is a burst of ROS production from mitochondria but also xanthine oxidase and NADPH oxidases are involved [62, 63]. However, mitochondrial permeability transition pore (mPTP) opening may even be regarded as an earlier damaging event as ROS formation was reported to be secondary to the opening of the pore [64].

The timing of RONS formation in I/R was investigated in detail. Three prominent radical signals were detected during I/R in isolated rabbit hearts using electron paramagnetic resonance spectroscopy [70]: 1) a semiquinone signal (isotropic g = 2.004); 2) a $ROO^{\bullet}$ signal (anisotropic g parallel = 2.033 and g perpendicular = 2.005); 3) a nitrogen centered radical signal (triplet g = 2.000 and aN = 24 G). Normally perfused hearts show mainly the semiquinone signal and only traces of $ROO^{\bullet}$ and nitrogen centered radical signals. The latter rise after onset of ischemia and reach a maximum at 45 min, while the semiquinone signal disappears. Upon reperfusion all three signals increased with an early maximum at 10-20 s after reoxygenation. As superoxide dismutase, deferoxamine or mannitol prevented the accumulation of the $ROO^{\bullet}$ signal, one can conclude that superoxide and hydroxyl radicals are involved in its formation. Moreover, real-time fluorescence measurements of ROS and $[Ca^{2+}]$ in isolated rat hearts during I/R revealed that mPTP opening is crucial for delayed ROS burst at 2-3 min and the rapid drop of $[Ca^{2+}]_i$ at 90 s after reperfusion [64]. ROS were monitored by a mitochondria-targeted H_2O_2-sensitive fluorescent probe (MitoPY1) and $[Ca^{2+}]_i$ was monitored with Indo-1, and both processes were blocked by ischemic preconditioning and the mPTP inhibitor cyclosporin A. On the basis of ROS formation kinetic measurements using the superoxide-sensitive dye hydroethidium along with a manganese-porphyrin (MnTBAP) it was also demonstrated that

ROS play a central role for the cardioprotection conferred by ischemic preconditioning [71], although with somewhat different kinetics than described afore.

Since myocardial ischemia is one of the most frequent causes of heart failure, the initial studies were focused on the role of ROS in I/R injury. The first few minutes of reperfusion are critical, because what happens then initiates long-term tissue damage and dysfunction [72]. The initial burst of ROS production can cause directly oxidative damage to mitochondria disrupting ATP production and in combination with dysregulation of calcium levels can lead to mPTP opening leading to necrotic and apoptotic cell death [73, 74]. These ROS can be released from cardiomyocyte mitochondria (mainly complex I and III), xanthine oxidase, and the (NAD(P)H) oxidase (reviewed in [52]). ROS may activate specific redox sensitive signaling molecules. In the heart, redox-modified proteins include proteins involved in various signaling pathways and/or transcriptional activities. Important candidates for downstream effectors are the redox-sensitive molecular targets p38MAPK and JNK (members of the stress-activated kinase family) and the cell survival kinase Akt [75]. The above-mentioned kinases have been linked to survival and apoptotic pathways activated in cardiomyocytes in response to injury stimuli [76]. In particular, both angiotensin II and H_2O_2 activate Akt to participate in hypertrophy or to prevent apoptosis, respectively [77]. In cardiomyocytes, p38MAPK and JNK are also activated in response to ROS and may lead to hypertrophy or apoptosis [78, 79].

Redox-modified proteins include also proteins involved in calcium handling and contractile function with Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), a central regulator of proteins involved in excitation–contraction coupling, to be a key mediator in this respect. Other redox modified proteins include the ryanodine receptor, the sarco/ endoplasmatic reticulum calcium ATPase (SERCA) and phospholamban (for review see [53, 82]. The redox modification of the above-mentioned proteins induces changes in cytoplasmic Ca^{2+} handling which may result in perturbations of mitochondrial Ca^{2+} and Na^{+}, which in turn limit NADH

and NADPH levels needed for antioxidant activity thereby producing a pro-oxidative shift in the redox state of the mitochondrial matrix and an increased mitochondrial ROS release [53, 55]. ROS-mediated mitochondrial damage will also release damage-associated molecular pattern molecules (DAMPs), which can initiate activation of the innate immune system causing inflammation that contributes to I/R injury and can continue for days after the initial damage [83, 84] contributing to development of heart failure but also conferring cardiac repair [85].

The same ROS sources, mitochondria, xanthine oxidase and NADPH oxidases, may also contribute to different extend to the development of heart failure [65, 66]. Besides the cardiomyocytes, white blood cells and platelets contribute to the aggravated ROS levels in heart failure [67]. High levels of ROS can cause cardiomyocyte apoptosis through a variety of mechanisms such as induction of DNA damage, activation of multiple apoptotic signaling pathways, and stabilization of hypoxia inducible factors [68]. Interestingly, the chronic release of ROS has been linked to the development of left ventricular hypertrophy and heart failure progression. More specifically, increased oxidative stress may contribute to contractile dysfunction, endothelial dysfunction, myocyte apoptosis and necrosis, remodeling of the extracellular matrix, and the progressive downward spiral of heart failure [66, 69]. The chronic release of ROS appears to derive from the nonphagocytic NAD(P)H oxidase and mitochondria [65]. Importantly, angiotensin II-induced hypertrophy and subsequent heart failure is largely determined by mitochondrial ROS formation [80], although reducing mitochondrial ROS formation by genetic overexpression of mitochondria-targeted catalase may be protective in old but not in young mice [81].

Although all enzymatic systems involved in the production or degradation of ROS are present in cardiomyocytes and endothelial cells, fibroblasts play also a crucial role. The

important role of fibroblasts is supported by the fact that the activation of matrix metalloproteinases (MMPs) by ROS suggests that perhaps ROS control not only the behavior of cardiac myocytes, but also that of the cardiac fibroblasts, which are the main contributors to interstitial collagen turnover. Therefore, ROS contribute to fibrosis with phenotypic transformation of fibroblasts to myofibroblasts, collagen deposition, and MMPs activation implicated in the remodeling of the failing myocardium, and progression to end-stage heart failure [65].

Experimental studies support a role for increased oxidative stress in myocardial infarction and in failing myocardium [66]. Clinical studies support the evidence that circulating levels of biomarkers of oxidative stress have been positively correlated not only with the presence of heart failure, but also with functional class according to the New York Heart Association (NYHA) classifications of heart failure [86]. If oxidative stress is not attenuated at the beginning of reperfusion, ROS may impair cardiac function through interaction with anti-apoptotic signaling cascades, calcium handling signaling proteins, activation of the innate immune system and activation of fibroblasts leading to heart failure progression. Therefore, targeting oxidative stress by pharmacological agents is of paramount importance for mediating cardioprotection.

3. Evidence for a role of oxidative stress in acute myocardial ischemia/reperfusion injury and heart failure

Animal studies have provided the molecular proof for the involvement of ROS in CVD, and are summarized in [22]. Deletion of ROS-producing enzymes (e.g. different NADPH oxidase isoforms) improved and overexpression of these enzymes aggravated CVD progression and severity. Likewise, deletion of antioxidant, ROS degrading enzymes (e.g. superoxide dismutases, glutathione peroxidase-1 or heme oxygenase-1) aggravated and overexpression of these enzymes improved CVD phenotypes. Similar observations were made for I/R damage

(e.g. myocardial infarction) and heart failure as summarized in **Table 1**. The table provides an overview of the major studies in this research field, and are mostly based on the personal opinion of the authors and the strength of the data. The search criteria in PubMed besides the animal species were "heart failure" and/or "ischemia/reperfusion" as well as "NADPH oxidase" or "xanthine oxidase" or "mitochondria". Drug-induced heart failure or cardiomyopathy (e.g. by doxorubicin [87]) as well as myocarditis-induced heart failure [88] were excluded as the focus of the present review is ischemic heart failure.

Clinical studies supporting the role of oxidative stress as a pathogenic mediator of IHD have been largely limited to plasma biomarker studies showing elevations in levels of oxidative stress biomarkers, and their role as independent predictors of clinical outcomes (please see **Table 2** for summary of major studies). Unfortunately, many antioxidant therapies have failed to improve clinical outcomes in patients with IHD or AMI (see section 5.1). A systematic review on oxidative stress markers in animal models of acute I/R damage concluded that there is good evidence for an association of oxidative stress markers in preclinical studies although the reported clinical studies observed no evidence of oxidative damage during early reperfusion during kidney transplants or aortic valve replacement employing by-pass surgery [89].

4. Therapeutic interventions in acute myocardial ischemia/reperfusion injury and heart failure that may improve redox balance

4.1. Established cardiovascular drugs

Endothelial dysfunction, atherosclerosis and the majority of CVD show an association with chronic renin-angiotensin-aldosterone system (RAAS) activation providing a primary target for angiotensin-converting enzyme (ACE) inhibitor and AT_1-receptor blockade therapy [90]. Statin therapy represents a mainstay in the treatment of CVD [91]. Importantly, these drugs have potent antioxidant, anti-inflammatory, anti-thrombotic and vasoprotective

properties, besides their primary blood pressure and lipid lowering properties [92]. Clinical trials have shown that statin therapy decreases markers of oxidative stress such as F2-isoprostane (8-isoPF2α) and 3-nitrotyrosine as well as markers of endothelial cell activation (e.g. vascular cell adhesion molecule-1 and E-selectin), a first step in the development of endothelial dysfunction and atherosclerosis, in hypercholesterolemic subjects with or without pre-established coronary artery diseases [93-95]. AT_1-receptor blockade therapy also reduced similar markers of oxidative stress and inflammation in patients with hypercholesterolemia [96]. Of note, statins may also confer protective cardiovascular effects by induction of the Nrf2–heme oxygenase-1 system as well as improved function of endothelial progenitor cells [97]. Accordingly, these drugs show also beneficial effects in patients with heart failure [98]. Beta-blocker therapy, besides lowering catecholamines that contribute to the pathophysiology of heart failure, was also reported to decrease oxidative stress markers such as 4-hydroxynonenal in the failing heart and thereby improve prognosis [99]. The powerful antioxidant effect of classical cardiovascular drugs, beta-blockers and RAAS inhibitors, was demonstrated by normal levels of lipid peroxidation markers malondialdehyde and isoprostanes in heart failure patients under standard therapy recommended for these patients [100]. The third generation beta-blocker nebivolol was shown to act as a potent inhibitor of NADPH oxidase activity [101, 102].

The only organic nitrate in clinical use that is devoid of side effects such as oxidative stress, nitrate tolerance and endothelial dysfunction is pentaerithrityl tetranitrate (PETN) [103, 104]. The molecular explanation for the beneficial effects of PETN, not shared by other nitrates, is the induction of heme oxygenase-1 [105, 106] and hundreds of other genes in an Nrf2-dependent fashion [107].

Sodium glucose transporter 2 inhibitors (e.g. empagliflozin) are highly effective in reducing the risk of hospitalisation for heart failure in patients with or without type 2 diabetes mellitus (87,162 participants) [108]. Dapagliflozin therapy lowered the risk of heart failure

progression or cardiovascular death (4,744 participants) [109] and empagliflozin therapy lowered the risk of hospitalization for heart failure or cardiovascular death (3,730 participants) [110] in HFrEF patients (heart failure with reduced ejection fraction), regardless of the presence or absence of diabetes (see also meta-analysis of both studies [111]). The novel antidiabetic drug classes sodium glucose transporter 2 inhibitors and incretin-based therapeutics (e.g. glucagon-like peptide-1 analogs) display cardioprotective effects in models of myocardial infarction [112-115], which is of great importance as diabetic patients have a significiantly higher cardiovascular risk. These novel drugs also suppress low-grade inflammation in humans [116-118] and animal models of atherosclerosis [119-122]. Mechanistically, empaglifozin treatment reduces myocardial infarct size in non-diabetic mice through STAT-3 mediated protection on microvascular endothelial cells and reduction of oxidative stress [123].

4.2. Life style modifications

Physical exercise is a powerful non-pharmacological intervention that is largely based on antioxidant mechanisms induced by hormesis/preconditioning-like effects of intermittent ROS formation [124-127], as demonstrated by the loss of exercise protective effects upon supplementation of antioxidants [128]. Although large meta-analyses did not reveal a decreased risk of cardiovascular or all-cause mortality by physical exercise in HFrEF patients, there was evidence for improved exercise capacity and therefore quality of life (4,481 and respectively 5,783 analyzed subjects with heart failure) [129, 130]. Reasons for the so far rather limited benefits of physical exercise on hard endpoints such as mortality are most likely due to missing standards for supervised structured training (e.g. intensity, resistance versus aerobic regimen) [131].

Dietary interventions also represent a promising non-pharmacological strategy to improve the prognosis of patients with heart failure. The PREDIMED study (7,447 participants, 288 major CVD events) showed an association of antioxidant-rich Mediterranean diet

(contributed mainly to olive oil consumption) with better cardiovascular health status (blood pressure, lipid profiles, lipoprotein particles) and lower prevalence of metabolic syndrome [132]. A systematic review and meta-analysis (520 subjects) showed an association of polyphenol-rich treatments with lower cardiovascular risk factors in haemodialysis patients [133]. Another randomised controlled trial and an updated meta-analysis (281 subjects) showed that combination therapy with curcuminoid-piperine polyphenols is associated with antioxidant and anti-inflammatory effects (higher SOD activity, lower malondialdehyde and C-reactive protein) in subjects with metabolic syndrome [134]. Similarly, systematic reviews and meta-analyses demonstrated an association of resveratrol supplementation with better endothelial function (by FMD), lower body weight, triglycerides, blood glucose among patients with metabolic syndrome and related cardiovascular disorders [135, 136].

Caloric restriction, particularly intermittent fasting, belongs to this group of non-pharmacological interventions and induces highly protective pathways, including antioxidant mechanisms [137, 138]. However, to date, systematic reviews and meta-analyses have not demonstrated a measurable benefit of intermittent fasting or caloric restriction in general on cardiovascular or all-cause prognosis [139, 140]. At least Ramadan fasting was reported to have no negative effects on the indicence of heart failure symptoms in patients with HFrEF [141].

5. Redox-based therapeutic strategies in acute myocardial ischemia/reperfusion injury and heart failure

As shown in **Figure 3** for the concept of oxidative stress in CVD in general, classical cardiovascular drugs decrease the risk factor-mediated activation of RAAS and inflammatory pathways, which largely defines their antioxidant profile [44, 92]. In addition, cholesterol lowering or direct antioxidant and anti-inflammatory effects by other pleiotropic properties may come into play. Additional redox therapeutic strategies including targeting specific ROS

sources (e.g. inhibitors of NADPH oxidases and xanthine oxidase [146], mitochondrial dysfunction/ROS formation [147, 148]) will be addressed in more detail below. Repair drugs or mimetics of vasoactive products that target oxidatively modified enzymatic regulators of the vascular tone such as endothelial nitric oxide synthase, prostacyclin synthase and soluble guanylyl cyclase have partially been translated into clinical therapy. Examples are the successful activators/stimulators of cGMP-producing soluble guanylyl cyclase [149], phosphodiesterase inhibitors that prevent degradation of cGMP [150] and nitric oxide replacement drugs such as organic nitrates [151] as well as prostacyclin replacement drugs such as iloprost [152]. Endothelin-1 antagonization by $ET_{A/B}$-receptor blockade confers improved prognosis in patients with pulmonary hypertension [153], which may be explained at least in part by decreased oxidative stress and inflammation, as reported by animal studies [154, 155].

On the other hand, despite encouraging preclinical data for pharmacological tetrahydrobiopterin supplementation/genetic inductions and eNOS enhancers, the strategy of preventing oxidative inhibition or uncoupling of endothelial nitric oxide synthase remains largely unexplored in patients with CVD [156]. Similarly, modulators of mitochondrial function and ROS formation remain largely unexplored in patients with CVD, apart from some drugs that are used for the treatment of metabolic disease or diabetes such as glibenclamide [157]. Another example is the experimental clinical study on the inhibitor of mPTP cyclosporine A and beneficial effects in patients with myocardial infarction [158], although for this drug, anti-inflammatory actions may also come into play via immunosuppressive effects. Activators of Nrf2, which regulates antioxidant enzymes such as heme oxygenase-1 and peroxiredoxins, were evaluated in over 90 clinical trials for multiple indications, represent a leading therapeutic principle based induction of endogenous antioxidant pathways [159] and will be discussed in detail below.

Antioxidant therapy in animal models of heart failure was initially reported in 1995 and the concept of decreasing oxidative stress was also proposed for the treatment of heart failure

patients [142]. In experimental models, administration of antioxidant vitamins E and C were protective against heart failure. Vitamin E was shown to reduce I/R damage by suppression of oxidative stress and inflammation [143]. Hypertrophy and heart failure by banding of the ascending aorta in guinea pigs was largely prevented by vitamin E therapy, which was also associated with a more reduced redox state, less lipid peroxidation and ultrastructural abnormalities as well as cardiac function [144]. Infusion of vitamin C into patients with heart failure decreased the number of circulating apoptotic microparticles [145].

5.1. Failure of unspecific antioxidants

Whereas animal data clearly support a role of oxidative stress in CVD and markers of oxidative stress are a hallmark of all CVD [22], there are only few large-scale clinical trials supporting beneficial effects of antioxidant treatment in patients with CVD, e.g. demonstrating improved prognosis by therapy with xanthine oxidase inhibitors [160], α-lipoic acid [161] or tight control of vitamin C plasma levels (EPIC-Norfolk trial) [162]. Also a large number of experimental clinical studies have demonstrated benefit with acute vitamin C infusion on endothelial function in patients with cardiovascular risk factors (reviewed in [11]). In contrast, most large clinical trials and meta-analyses (e.g. HOPE, HOPE-TOO [163]) failed to show any health benefit for the treatment of CVD or for all-cause mortality with non-selective oral antioxidant drugs (reviewed in [45, 163, 164]). There are several possible explanations for this discrepancy between experimental and large clinical studies such as insufficient accumulation of the antioxidant vitamins at sites of oxidative stress upon oral therapy or patients being already treated with established (cardiovascular) drugs with potent pleiotropic antioxidant effects [44]. The latter potential explanation is supported by the observation that NO/cGMP signaling did not improve by vitamin C infusion in coronary artery disease patients treated with ramipril or losartan [165]. The most likely explanation is that ROS, especially hydrogen peroxide as derived from NOX4 isoform [166-168], also confer beneficial signaling in essential cellular

functions [169] and provide the 'eustress' required for maintaining antioxidant defence by preconditioning-like mechanisms [170-172]. Regulation of cell differentiation [173, 174], proliferation [175, 176] and migration [173, 177] are among the beneficial cellular redox processes. Therefore, antioxidant approaches should be based on fine-tuned, species-specific, space- and time-resolved pharmacological targeting [170, 178] and, most importantly, require the full picture of their mode of action [179].

5.2. Ischemic conditioning

The heart can be protected against the lethal effects of acute IRI by subjecting it to brief non-lethal episodes of ischemia and reperfusion, either prior to index ischemia (termed ischemic preconditioning) [180], or at onset of reperfusion (termed ischemic postconditioning) [181]. To circumvent the need to apply the cardioprotective stimulus directly to the heart, it has been shown that the cycles of brief ischemia and reperfusion can be applied to an organ or tissue (such as the limb) away from the heart (termed remote ischemic conditioning) [182], a strategy which is easier to apply and test in the clinical setting. The mechanisms underlying ischemic conditioning-induced cardioprotection have been intensively investigated, and the current paradigm suggests that the cardioprotective stimulus triggers the release of signaling ROS from mitochondria, which then mediate protection by activating cytoprotective protein kinases such as Akt and Erk1/2, and reducing detrimental ROS produced at the onset of reperfusion [183, 184]. Thiol-dependent regulatory mechanisms (e.g. thioredoxin [Trx]/glutaredoxin [Grx]-based) have also been discussed in detail for redox control of ischaemic preconditioning [185] and myocardial remodelling [186].

5.3. Redox drugs in clinical use against ischemia/reperfusion injury and heart failure

According to a systematic review on antioxidant therapy of ST-segment elevation myocardial infarction (STEMI) patients undergoing primary percutaneous coronary

intervention (PCI), combination therapy with edaravone, allopurinol, atorvastatin and nicorandil decreased oxidative stress and myocardial damage as well as ameliorated cardiac function and clinical prognosis [187]. Therapy with deferoxamine and N-acetylcysteine reduced oxidative stress markers but did not improve cardiac function or prognosis. Some experimental studies (a total of 73 subjects) also show that combination therapy with the thiol-based antioxidant N-acetylcysteine and the nitric oxide donor nitroglycerin reduces oxidative stress parameters (e.g. malondialdehyde) and improves cardiac perfusion as well as left ventricular function in patients with acute myocardial infarction [188, 189]. A study of 112 STEMI patients undergoing PCI and administered low-dose, background intravenous nitroglycerin, found that N-acetylcysteine significantly reduced infarct size [190]. A systematic review on the effects of N-acetylcysteine in animal models or experimental human studies of heart failure concluded that there was good evidence for a protective effect of thiol-based antioxidants in preclinical studies but clinical studies are inconclusive and further research is needed [191].

A meta-analysis (3,630 subjects, 525 treated) on the effects of combined therapy with angiotensin-converting enzyme inhibitors and xanthine oxidase inhibitors in post-acute myocardial infarction patients reported more event-free survival in the treatment group [192]. A meta-analysis including 40 studies revealed an improvement of vascular function and markers of oxidative stress by allopurinol or oxypurinol in patients with CVD including heart failure [193]. A cohort study (288 subjects) showed that long-term xanthine oxidase inhibitor (febuxostat) prescription prevented worsening of left ventricular hypertrophy, left ventricular diastolic function and new-onset heart failure with preserved ejection fraction (HFpEF) in patients at risk of heart failure [194]. A population-based study in patients with gout (99,744 subjects) showed no reduced risk of myocardial infarction, stroke, new-onset heart failure, coronary revascularization or all-cause mortality by febuxostat as compared to allopurinol therapy [195]. However, another meta-analysis (10,684 subjects) found no effect of xanthine

oxidase inhibitor therapy on cardiovascular death in hypertensive patients [192]. Finally, a controlled cohort study in patients with chronic heart failure (66 subjects) showed no improvement of arterial stiffness by allopurinol treatment [196] indicating that more clinical data are required. More clinical trials are in progress in patients with chronic heart failure, also comparing the effects of novel xanthine oxidase inhibitors such as febuxostat and topiroxostat with allopurinol [197]. More ongoing studies on effects of xanthine oxidase inhibition in patients with heart failure (e.g. NCT00181155 Phase II, NCT00997542 Phase IV) are presented in [146]. Mechanistically, febuxostat improved oxidative stress parameters, adverse protein kinase signaling (phospho-Erk and phospho-mTOR), mitochondrial as well as cardiac function, apoptosis parameters and arrhythmia in animal models of cardiac hypertrophy, myocardial infarction and heart failure [198-201].

The NO replacement therapy by organic nitrates is a mainstay in cardiovascular therapy of stable angina, congestive heart failure and acute coronary syndromes, despite limitations due to the development of nitrate tolerance and endothelial dysfunction under chronic therapy that are mainly related to the induction of oxidative stress by most organic nitrates [104]. Hydralazine, one of the first antihypertensive drugs in clinical use [202], showed a substantial improvement of heart failure mortality in combination therapy with the nitric oxide donor isosorbide dinitrate (A-HeFT Study) [203, 204]. Hydralazine displays strong antioxidant effects by peroxynitrite scavenging that may help to reduce side effects of organic nitrate therapy such as oxidative stress and nitrate tolerance [205, 206]. Activators and stimulators of the soluble guanylyl cyclase (e.g. Cinaciguat™ (BAY 58-2667) and Riociguat™ (BAY 63-2521)) define a novel class of repair drugs of an oxidatively damaged soluble guanylyl cyclase enzyme [146, 149]. They are approved and marketed for clinical use in the treatment of different forms of pulmonary hypertension and heart failure [207, 208]. More ongoing clinical trials and published cohort studies can be found in [146]. Of note, these drugs may be also effective for other indications such as arterial hypertension, renal fibrosis or failure, liver cirrhosis, erectile

dysfunction, atherosclerosis, restenosis, thrombosis and inflammation (reviewed in [149]). All of these therapeutic concepts of established redox-related drugs are summarized in **Figure 4**.

5.4. Emerging concepts of redox therapy in ischemia/reperfusion injury and heart failure

A wide variety of different pharmacological approaches are under investigation as means to modulate cellular redox state and thereby limit I/R injury and prevent heart failure, and some illustrative examples will be discussed in this section. As mentioned previously, in addition to their cytotoxic effect, RONS have important intracellular and extracellular signaling roles in the heart and vasculature. Consequently, it is increasingly clear that non-targeted redox therapy, while effective at protecting against I/R injury in relatively simple animal models, may be ineffective in the more complex clinical setting. One emerging approach is therefore to try to target the antioxidant to the subcellular source of damaging ROS, while leaving signaling ROS pathways intact. In particular, targeting antioxidant molecules to mitochondria is an appealing approach, given that mitochondrial ROS are believed to be central to the mechanism of mPTP opening, cell death and infarct formation [209].

One example is the mitochondria-targeted coenzyme Q10 (mitoQ) compound in which the direct ROS scavenger coenzyme Q is conjugated to the positively charged triphenylphosphonium, which targets mitoQ to mitochondria [45, 148, 210]. Coenzyme Q10 (CoQ10) is a potent intracellular antioxidant commonly used to treat cardiomyopathy, despite the evidence for benefit in cardiovascular diseases being mixed. A number of clinical trials have been conducted to investigate the effects of coenzyme Q10 supplementation on biomarkers of inflammation and oxidative stress in among coronary artery disease. A 2019 meta-analysis of 13 of trials of CoQ10 supplementation in patients with coronary artery disease found that, despite evidence that CoQ10 increased levels of SOD and catalase, and decreased markers of oxidative stress, there was no effect on GPx levels or inflammatory markers C-reactive protein (CRP), TNF-α, IL-6 [211]. mitoQ decreased ROS levels in cells subject to

hypoxia/reoxygenation and in isolated hearts, it decreased mPTP opening and improved functional recovery following I/R [212, 213]. MitoQ can also improve arterial endothelial function when administered to aged mice [214]. The antioxidant 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO, a spin trap) has also been conjugated to triphenylphosphonium to make mitoTEMPO. When administered to rats, mitoTEMPO can prevent the increase in H_2O_2 levels and diaphragm muscle weakness associated with heart failure [215].

Other mitochondrial targeted compounds have been investigated, including 3,5-seco-4-nor-cholestan-5-one oxime-3-ol (TRO40303, a mPTP inhibitor) [216], which binds specifically to the mitochondrial translocator protein 18 kDa (TSPO), and bendavia (also called elamipretide, MTP-131 or SS-31) [217, 218], a water-soluble tetrapeptide that associates with cardiolipin and confers antioxidant effects. However, despite promising results in animal studies, no benefit of these compounds has been seen in clinical trials in STEMI patients to date [219, 220].

Sirtuins are a family of enzymes that post-transcriptionally regulate proteins by acetylation of lysine residues, in an NAD^+- dependent reaction that is linked to the energy and redox status of the cell via the NAD^+/NADH ratio. Sirtuins regulate various critical biological processes including energy production, oxidative stress, angiogenesis, Ca^{2+} handling, cell death and autophagy. Therefore, pharmacologic activation of sirtuins, and SIRT1 and SIRT3 in particular, can potentially ameliorate the development or progression of heart failure (reviewed in [221]). The natural polyphenol, resveratrol, is of particular interest as it is believed to mediate the benefits of red wine in the cardiovascular system by activating SIRT1. There has been a great deal of controversy about its precise mechanism of action, but it appears to be capable of directly activating SIRT1 via an allosteric mechanism [222]. On the other hand, it was recently shown that resveratrol paradoxically induces oxidation of proteins such as PKG1α, especially during periods of oxidative stress, and that this is the mechanism by which it lowers blood pressure [223]. This emphasizes the important point that antioxidants such as polyphenols can

act as both antioxidants or pro-oxidants under different circumstances [224], e.g. again mimicking preconditioning or hormesis effects [225-227].

Following I/R, increased oxidative and nitrosative stress activates the nuclear enzyme poly(ADP-ribose) polymerase (PARP), which catalyzes the cleavage of NAD^+ into nicotinamide and ADP-ribose [228]. NAD^+ depletion can interfere with glycolysis, the Krebs cycle, mitochondrial electron transport and lead eventually to ATP depletion. In fact, it has been proposed that PARP-mediated ATP depletion is the ultimate cause of cell death following mPTP opening [229]. As such, PARP overactivation contributes to the pathogenesis of cardiac and endothelial dysfunction associated with myocardial infarction and chronic heart failure (reviewed in [230]). Mice lacking PARP exhibit less myocardial injury following I/R [231]. Correspondingly, inhibitors of PARP such as 3-aminobenzamide and LL-2286 reduce infarct size following I/R, as well as preventing postinfarction myocardial remodeling and heart failure in rats [232, 233]. A wide range of PARP inhibitors have been developed, although many of the best-known inhibitors lack specifity [234]. On the other hand, it might be anticipated that the benefit of antioxidants might be partially mediated via inhibition of PARP activation.

Recent evidence suggests that in the setting of heart failure, increased cytoplasmic Na^+ combined with impaired Ca^{2+} release from the sarcoplasmic reticulum alters Na^+ and Ca^{2+} gradients across the mitochondrial inner membrane, leading to altered energy supply and demand and driving mitochondrial oxidation. Consequently, inhibition of mitochondrial Na^+/Ca^{2+} exchange with 7-chloro-5-(2-chlorophenyl)-1,5-dihydro-4,1-benzothiazepin-2(3H)-one (CGP-37157) restores mitochondrial Ca^{2+} handling and prevents sudden death in a guinea pig model of heart failure [235]. As well as ion channels being able to contribute to oxidative stress, conversely, oxidative stress can modify ion channel activity. For example, oxidative inhibition of the Na^+–K^+ pump via S-glutathionylation occurs during myocardial infarction and may contribute to intracellular Na^+ overload [236]. Consequently, therapies that maintain Na^+-K^+ pump activity are cardioprotective [236].

A seminal publication in 2005 showed that inhalation of H_2S gas induces a state of "suspended animation" in mice, by causing reversible hypometabolism [237]. H_2S is well-recognized as a second messenger implicated in protection from oxidative stress by direct scavenging of RONS and indirect antioxidant effects [238]. Multiple targets for H_2S-releasing drugs have been identified and summarised for pre- and postconditioning and remote conditioning in myocardial infarction, reperfusion injury and cardioprotection, such as mPTP, K_{ATP}, PKC, eNOS/cGMP signaling, the Erk/GSK3β pathway and JAK2/STAT-3/iNOS signaling [239]. The H_2S donor sodium hydrosulfide reduced infarct size via a cGMP/protein kinase G/phospholamban pathway, which was independent of nitric oxide synthase activity [240]. Additionally, many studies have shown that H_2S effectively ameliorates I/R injury by activating cardioprotective signaling pathways and by attenuating ROS, indicating that H_2S may represent an effective intervention to mitigate the irreversible myocardial injury associated with I/R [238].

Acute treatment with nitric oxide replacement drugs such as nitroglycerin not only causes direct vasodilation of ischemic vascular areas but also limits I/R damage by inhibition of mPTP opening via cyclophilin D S-nitrosation [241]. Multiple tetrahydrobiopterin replacement therapy, e.g. by folate, improved endothelial function and prevented endothelial nitric oxide synthase uncoupling in patients undergoing elective Coronary Artery Bypass Graft (CABG) [242]. Furthermore, cardiac response in a model of pressure-overload induced hypertrophy and heart failure was improved by tetrahydrobiopterin supplementation [243]. In a canine tachypacing model of heart failure, tetrahydrobiopterin depletion and uncoupling of inducible nitric oxide synthase was associated with electrophysiological abnormalities, whereas tetrahydrobiopterin therapy normalized atrial fibrillation, fractional shortening and cardiac oxidative stress [244]. Additionally, direct targeting of endothelial nitric oxide synthase expression and function by so-called endothelial nitric oxide synthase enhancers such as AVE9488 improves left ventricular remodeling in myocardial infarction [245] or AVE3085

prevents diastolic heart failure [246] or AVE9488 reduces I/R damage [247]. S-glutathionylation at cysteine residues Cys689 and Cys908 represents an important redox switch in endothelial nitric oxide synthase leading to uncoupling of the electron flow within the enzyme and superoxide formation [248, 249]. This redox-regulatory mechanism gained even more biological relevance with results indicating that endothelial nitric oxide synthase S-glutathionylation can be biologically reversed by glutaredoxin-1 (Grx-1) [250] and potentially by thiol-based drugs such as N-acetylcysteine and alpha-lipoic acid. As uncoupling of endothelial nitric oxide synthase is a hallmark of I/R damage and heart failure, reversal of S-glutathionylation of endothelial nitric oxide synthase represents an emerging therapeutic target.

The thiol-based drug alpha-lipoic acid has been reported to have beneficial effects in cardiovascular therapy [251]. Of note, α-lipoic acid is a potent activator of mitochondrial aldehyde dehydrogenase (ALDH-2) and thereby improves detoxification of toxic aldehydes such as 4-hydroxynonenal [252], which contributes to cardiac dysfunction [253]. In addition, α-lipoic acid improves diabetic polyneuropathy [161] by preventing accumulation of advanced glycation endproducts (AGEs) and adverse prooxidant and inflammatory signaling via their receptor (RAGE) [254]. Finally, α-lipoic acid ameliorates endothelial function (measured by venous occlusion plethysmography) in patients with diabetes, potentially by preventing oxidative break-down of the eNOS cofactor tetrahydrobiopterin as α-lipoic acid also normalized the plasma levels of the lipid peroxidation product malondialdehyde [255]. Besides the direct antioxidant effect by scavenging RONS, these thiols may also interact with proteins and enhance enzymatic activity by redox regulation – in principle, any oxidatively inactivated enzyme with a sulfhydryl group involved in its activity can be targeted by N-acetylcysteine or α-lipoic acid (see example of endothelial nitric oxide synthase below). Also ryanodine receptors, ion channels and pumps such as SERCA and various potassium channels, kinases such as Ras/MEKK/c-Jun/Akt/PKC, apoptosis pathway constituents such as p53/caspase, and

central regulators of inflammation such as NFkB represent pharmacological targets to modulate protein S-glutathionylation in CVD [256, 257].

The inhibitor of myeloperoxidase, AZD4831, is currently being investigated in ongoing clinical trials in heart failure patients with preserved ejection fraction (HFpEF) (NCT03756285 and NCT03611153, Phase II), and in acute coronary syndrome patients [146]. Despite the fact that monoamine oxidase genetic deletion or pharmacological inhibition was reported to prevent I/R damage [258], there are currently no ongoing cardiovascular clinical trials to further exploit this redox therapeutic target. Monoamine oxidase inhibitors are currently being developed for neurodegenerative and psychiatric diseases [146]. One of the most promising redox therapeutic approaches is based on inhibitors of NOX isoforms. There are a large number of animal studies reporting the beneficial effects of genetic deletion (**Table 1**) and pharmacological inhibition of NOX enzymes [259-262] in I/R damage and heart failure. Although there are currently no clinical trials on NOX inhibition in heart failure patients, these drugs are tested for multiple other indications [146]. Targeting NOX isoforms may be challenging given that broad (unspecific) inhibition of NADPH oxidases may be detrimental by removing the "protective" ROS as discussed for I/R injury [263]. Accordingly, NOX2/4 double knockout mice showed decreased ROS levels but increased infarct size, which may be due to lack of ROS-mediated activation of hypoxia-inducible factor-1alpha and peroxisome proliferator-activated receptor-alpha providing preconditioning-like protection [264]. Also genetic deficiency of NOX2 subunits and other NOX isoforms in patients with chronic granulomatous disease (CGD) envisages the inherent complications such as higher susceptibility to chronic inflammatory diseases associated with genetic or pharmacological NADPH oxidase inhibition [265].

There is a large body of preclinical evidence that Nrf2 activation is highly protective in models of I/R damage and heart failure [266, 267]. Although Nrf2 activators such as sulforaphane, dimethylfumarate and bardoxolone are currently tested in multiple clinical trials for a broad range of indications including pulmonary hypertension and chronic kidney disease,

as yet there is no clinical trial in patients with CVD [146, 159]. Despite animal data on increased susceptibility of $Nrf2^{-/-}$ mice to heart failure after myocardial infarction [266], the BEACON clinical trial, conducted in patients with chronic kidney disease and type 2 diabetes, had to be terminated early because of increased incidence of heart failure, myocardial infarction, stroke and other cardiovascular complications in the bardoxolone treatment group (reviewed in [268]). The latter drawback might also be responsible for the rather hesitant approach to cardiovascular clinical trials on Nrf2 activators.

All of these therapeutic concepts of emerging redox-related drugs are summarized in **Figure 4**. The beneficial effects of genetic and pharmacological inhibition of ROS-producing enzymes or genetic overexpression of ROS-degrading enzymes on I/R damage and heart failure is also summarized in **Table 1**.

6. Conclusions and clinical implications

Heart failure and other complications of AMI are the leading causes of death and disability in Europe and worldwide. The most effective treatment for limiting myocardial infarct size and preventing heart failure following AMI, is timely myocardial reperfusion using primary percutaneous coronary interventions. However, despite timely treatment, mortality and morbidity following AMI remain significant. Therefore, novel strategies are being introduced in patients or are currently tested in animal models comprising the limitation of infarct size and progression of heart failure by prevention of oxidative damage and adverse redox signaling during I/R episodes (summarized in **Figure 4**). Although there is no doubt that ROS formation and oxidative damage play a major role for I/R injury and onset of heart failure, there are yet now only few “real” redox therapies besides the well-known pleiotropic antioxidant effects of most established cardiovascular drugs. The most prominent example is the preservation of the activity of oxidatively impaired soluble guanylyl cyclase enzyme by activators and stimulators

that are approved and marketed for clinical use in the treatment of different forms of pulmonary hypertension and heart failure. Also xanthine oxidase inhibition and isosorbide dinitrate/hydralazine combination therapy have proven highly beneficial in large scale clinical trials. Besides these 3 successful clinical examples, there are numerous experimental approaches that are mostly based on inhibition of other ROS sources using specific inhibitors, prevention of mitochondrial damage by targeting antioxidants to mitochondria or improvement of NO/cGMP signaling. Finally, non-pharmacological therapeutic approaches such as ischemic conditioning, intermittent fasting or physical exercise are based on antioxidant mechanisms and have proven efficient in animals models or small cohort trials on I/R damage or heart failure. As I/R damage or heart failure represent multi-factorial cardiovascular disorders, a combination therapy comprising a fine-tuned mixture of the above mentioned treatment categories may be most promising. Any redox therapeutic approach should not interfere with the protective effects of ROS in processes such as eustress, hormesis and ischemic preconditioning as well as their involvement in essential redox signaling processes regulating cell differentiation, proliferation and migration.

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References

[1] Lozano, R.; Naghavi, M.; Foreman, K.; Lim, S.; Shibuya, K.; Aboyans, V.; Abraham, J.; Adair, T.; Aggarwal, R.; Ahn, S. Y.; Alvarado, M.; Anderson, H. R.; Anderson, L. M.; Andrews, K. G.; Atkinson, C.; Baddour, L. M.; Barker-Collo, S.; Bartels, D. H.; Bell, M. L.; Benjamin, E. J.; Bennett, D.; Bhalla, K.; Bikbov, B.; Bin Abdulhak, A.; Birbeck, G.; Blyth, F.; Bolliger, I.; Boufous, S.; Bucello, C.; Burch, M.; Burney, P.; Carapetis, J.; Chen, H.; Chou, D.; Chugh, S. S.; Coffeng, L. E.; Colan, S. D.; Colquhoun, S.; Colson, K. E.; Condon, J.; Connor, M. D.; Cooper, L. T.; Corriere, M.; Cortinovis, M.; de Vaccaro, K. C.; Couser, W.; Cowie, B. C.; Criqui, M. H.; Cross, M.; Dabhadkar, K. C.; Dahodwala, N.; De Leo, D.; Degenhardt, L.; Delossantos, A.; Denenberg, J.; Des Jarlais, D. C.; Dharmaratne, S. D.; Dorsey, E. R.; Driscoll, T.; Duber, H.; Ebel, B.; Erwin, P. J.; Espindola, P.; Ezzati, M.; Feigin, V.; Flaxman, A. D.; Forouzanfar, M. H.; Fowkes, F. G.; Franklin, R.; Fransen, M.; Freeman, M. K.; Gabriel, S. E.; Gakidou, E.; Gaspari, F.; Gillum, R. F.; Gonzalez-Medina, D.; Halasa, Y. A.; Haring, D.; Harrison, J. E.; Havmoeller, R.; Hay, R. J.; Hoen, B.; Hotez, P. J.; Hoy, D.; Jacobsen, K. H.; James, S. L.; Jasrasaria, R.; Jayaraman, S.; Johns, N.; Karthikeyan, G.; Kassebaum, N.; Keren, A.; Khoo, J. P.; Knowlton, L. M.; Kobusingye, O.; Koranteng, A.; Krishnamurthi, R.; Lipnick, M.; Lipshultz, S. E.; Ohno, S. L.; Mabweijano, J.; MacIntyre, M. F.; Mallinger, L.; March, L.; Marks, G. B.; Marks, R.; Matsumori, A.; Matzopoulos, R.; Mayosi, B. M.; McAnulty, J. H.; McDermott, M. M.; McGrath, J.; Mensah, G. A.; Merriman, T. R.; Michaud, C.; Miller, M.; Miller, T. R.; Mock, C.; Mocumbi, A. O.; Mokdad, A. A.; Moran, A.; Mulholland, K.; Nair, M. N.; Naldi, L.; Narayan, K. M.; Nasseri, K.; Norman, P.; O'Donnell, M.; Omer, S. B.; Ortblad, K.; Osborne, R.; Ozgediz, D.; Pahari, B.; Pandian, J. D.; Rivero, A. P.; Padilla, R. P.; Perez-Ruiz, F.; Perico, N.; Phillips, D.; Pierce, K.; Pope, C. A., 3rd; Porrini, E.; Pourmalek, F.; Raju, M.; Ranganathan, D.; Rehm, J. T.; Rein, D. B.; Remuzzi, G.; Rivara, F. P.; Roberts, T.; De Leon, F. R.; Rosenfeld, L. C.; Rushton, L.; Sacco, R. L.; Salomon, J. A.; Sampson, U.; Sanman, E.; Schwebel, D. C.; Segui-Gomez, M.; Shepard, D. S.; Singh, D.; Singleton, J.; Sliwa, K.; Smith, E.; Steer, A.; Taylor, J. A.; Thomas, B.; Tleyjeh, I. M.; Towbin, J. A.; Truelsen, T.; Undurraga, E. A.; Venketasubramanian, N.; Vijayakumar, L.; Vos, T.; Wagner, G. R.; Wang, M.; Wang, W.; Watt, K.; Weinstock, M. A.; Weintraub, R.; Wilkinson, J. D.; Woolf, A. D.; Wulf, S.; Yeh, P. H.; Yip, P.; Zabetian, A.; Zheng, Z. J.; Lopez, A. D.; Murray, C. J.; AlMazroa, M. A.; Memish, Z. A. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380:**2095-2128; 2012.

[2] WHO. Projections of mortality and causes of death, 2016 to 2060. *https://www.who.int/healthinfo/global_burden_disease/projections/en/*.

[3] WHO. Global health observatory data on causes of non-communicable disease in 2016. *https://www.who.int/gho/ncd/mortality_morbidity/en/*.

[4] Dai, H.; Much, A. A.; Maor, E.; Asher, E.; Younis, A.; Xu, Y.; Lu, Y.; Liu, X.; Shu, J.; Bragazzi, N. L. Global, regional, and national burden of ischemic heart disease and its attributable risk factors, 1990-2017: results from the global Burden of Disease Study 2017. *Eur Heart J Qual Care Clin Outcomes*; 2020.

[5] Gyongyosi, M.; Haller, P. M.; Blake, D. J.; Martin Rendon, E. Meta-Analysis of Cell Therapy Studies in Heart Failure and Acute Myocardial Infarction. *Circ Res* **123:**301-308; 2018.

[6] Elgendy, I. Y.; Mahtta, D.; Pepine, C. J. Medical Therapy for Heart Failure Caused by Ischemic Heart Disease. *Circ Res* **124:**1520-1535; 2019.

[7] Hausenloy, D. J.; Yellon, D. M. Reperfusion injury salvage kinase signalling: taking a RISK for cardioprotection. *Heart Fail Rev* **12:**217-234; 2007.

[8] Urbich, M.; Globe, G.; Pantiri, K.; Heisen, M.; Bennison, C.; Wirtz, H. S.; Di Tanna, G. L. A Systematic Review of Medical Costs Associated with Heart Failure in the USA (2014-2020). *Pharmacoeconomics* **38:**1219-1236; 2020.

[9] Murphy, E.; Ardehali, H.; Balaban, R. S.; DiLisa, F.; Dorn, G. W., 2nd; Kitsis, R. N.; Otsu, K.; Ping, P.; Rizzuto, R.; Sack, M. N.; Wallace, D.; Youle, R. J.; American Heart Association Council on Basic Cardiovascular Sciences, C. o. C. C.; Council on Functional, G.; Translational, B. Mitochondrial Function, Biology, and Role in Disease: A Scientific Statement From the American Heart Association. *Circ Res* **118:**1960-1991; 2016.

[10] Sluijter, J. P. G.; Davidson, S. M.; Boulanger, C. M.; Buzas, E. I.; de Kleijn, D. P. V.; Engel, F. B.; Giricz, Z.; Hausenloy, D. J.; Kishore, R.; Lecour, S.; Leor, J.; Madonna, R.; Perrino, C.; Prunier, F.; Sahoo, S.; Schiffelers, R. M.; Schulz, R.; Van Laake, L. W.; Ytrehus, K.; Ferdinandy, P. Extracellular vesicles in diagnostics and therapy of the ischaemic heart: Position Paper from the Working Group on Cellular Biology of the Heart of the European Society of Cardiology. *Cardiovasc Res* **114:**19-34; 2018.

[11] Daiber, A.; Steven, S.; Weber, A.; Shuvaev, V. V.; Muzykantov, V. R.; Laher, I.; Li, H.; Lamas, S.; Munzel, T. Targeting vascular (endothelial) dysfunction. *Br J Pharmacol* **174:**1591-1619; 2017.

[12] Schachinger, V.; Britten, M. B.; Zeiher, A. M. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* **101:**1899-1906; 2000.

[13] Celermajer, D. S. Endothelial dysfunction: does it matter? Is it reversible? *J Am Coll Cardiol* **30:**325-333; 1997.

[14] Heitzer, T.; Schlinzig, T.; Krohn, K.; Meinertz, T.; Munzel, T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* **104:**2673-2678; 2001.

[15] Gokce, N.; Keaney, J. F., Jr.; Hunter, L. M.; Watkins, M. T.; Menzoian, J. O.; Vita, J. A. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation* **105:**1567-1572; 2002.

[16] Gori, T.; Muxel, S.; Damaske, A.; Radmacher, M. C.; Fasola, F.; Schaefer, S.; Schulz, A.; Jabs, A.; Parker, J. D.; Munzel, T. Endothelial function assessment: flow-mediated dilation and constriction provide different and complementary information on the presence of coronary artery disease. *Eur Heart J* **33:**363-371; 2012.

[17] Fichtlscherer, S.; Breuer, S.; Zeiher, A. M. Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes: further evidence for the existence of the "vulnerable" patient. *Circulation* **110:**1926-1932; 2004.

[18] Heitzer, T.; Baldus, S.; von Kodolitsch, Y.; Rudolph, V.; Meinertz, T. Systemic endothelial dysfunction as an early predictor of adverse outcome in heart failure. *Arterioscler Thromb Vasc Biol* **25:**1174-1179; 2005.

[19] Ellis, G. R.; Anderson, R. A.; Chirkov, Y. Y.; Morris-Thurgood, J.; Jackson, S. K.; Lewis, M. J.; Horowitz, J. D.; Frenneaux, M. P. Acute effects of vitamin C on platelet responsiveness to nitric oxide donors and endothelial function in patients with chronic heart failure. *J Cardiovasc Pharmacol* **37:**564-570; 2001.

[20] Gupta, N.; Giri, S.; Rathi, V.; Ranga, G. S. Flow Mediated Dilatation, Carotid Intima Media Thickness, Ankle Brachial Pressure Index and Pulse Pressure in Young Male Post Myocardial Infarction Patients in India. *J Clin Diagn Res* **10:**OC35-OC39; 2016.

[21] Simsek, E. C.; Sari, C.; Kucukokur, M.; Ekmekci, C.; Colak, A.; Ozdogan, O. Endothelial dysfunction in patients with myocardial ischemia or infarction and nonobstructive coronary arteries. *J Clin Ultrasound*; 2020.

[22] Daiber, A.; Chlopicki, S. Revisiting pharmacology of oxidative stress and endothelial dysfunction in cardiovascular disease: Evidence for redox-based therapies. *Free Radic Biol Med* **157:**15-37; 2020.

[23] Munzel, T.; Daiber, A. Environmental Stressors and Their Impact on Health and Disease with Focus on Oxidative Stress. *Antioxid Redox Signal* **28:**735-740; 2018.

[24] Steven, S.; Frenis, K.; Oelze, M.; Kalinovic, S.; Kuntic, M.; Bayo Jimenez, M. T.; Vujacic-Mirski, K.; Helmstadter, J.; Kroller-Schon, S.; Munzel, T.; Daiber, A. Vascular Inflammation and Oxidative Stress: Major Triggers for Cardiovascular Disease. *Oxid Med Cell Longev* **2019:**7092151; 2019.

[25] Daiber, A.; Oelze, M.; Steven, S.; Kroller-Schon, S.; Munzel, T. Taking up the cudgels for the traditional reactive oxygen and nitrogen species detection assays and their use in the cardiovascular system. *Redox Biol* **12:**35-49; 2017.

[26] Daiber, A.; Steven, S.; Vujacic-Mirski, K.; Kalinovic, S.; Oelze, M.; Di Lisa, F.; Munzel, T. Regulation of Vascular Function and Inflammation via Cross Talk of Reactive Oxygen and Nitrogen Species from Mitochondria or NADPH Oxidase-Implications for Diabetes Progression. *Int J Mol Sci* **21**; 2020.

[27] Gryglewski, R. J.; Palmer, R. M.; Moncada, S. Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* **320:**454-456; 1986.

[28] McCord, J. M.; Keele, B. B., Jr.; Fridovich, I. An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. *Proc Natl Acad Sci U S A* **68:**1024-1027; 1971.

[29] Fukai, T.; Folz, R. J.; Landmesser, U.; Harrison, D. G. Extracellular superoxide dismutase and cardiovascular disease. *Cardiovasc Res* **55:**239-249; 2002.

[30] Jung, O.; Marklund, S. L.; Geiger, H.; Pedrazzini, T.; Busse, R.; Brandes, R. P. Extracellular superoxide dismutase is a major determinant of nitric oxide bioavailability: in vivo and ex vivo evidence from ecSOD-deficient mice. *Circ Res* **93:**622-629; 2003.

[31] Munzel, T.; Daiber, A.; Ullrich, V.; Mulsch, A. Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expression of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. *Arterioscler Thromb Vasc Biol* **25:**1551-1557; 2005.

[32] Daiber, A.; Oelze, M.; Daub, S.; Steven, S.; Schuff, A.; Kroller-Schon, S.; Hausding, M.; Wenzel, P.; Schulz, E.; Gori, T.; Munzel, T. Vascular Redox Signaling, Redox Switches in Endothelial Nitric Oxide Synthase and Endothelial Dysfunction. In: Laher, I., ed. *Systems Biology of Free Radicals and Antioxidants*. Berlin Heidelberg: Springer-Verlag; 2014: 1177-1211.

[33] Schulz, E.; Wenzel, P.; Munzel, T.; Daiber, A. Mitochondrial redox signaling: Interaction of mitochondrial reactive oxygen species with other sources of oxidative stress. *Antioxid Redox Signal* **20:**308-324; 2014.

[34] Griendling, K. K.; FitzGerald, G. A. Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS. *Circulation* **108:**1912-1916; 2003.

[35] Griendling, K. K.; FitzGerald, G. A. Oxidative stress and cardiovascular injury: Part II: animal and human studies. *Circulation* **108:**2034-2040; 2003.

[36] Daiber, A.; Di Lisa, F.; Oelze, M.; Kroller-Schon, S.; Steven, S.; Schulz, E.; Munzel, T. Crosstalk of mitochondria with NADPH oxidase via reactive oxygen and nitrogen species signalling and its role for vascular function. *Br J Pharmacol* **174:**1670-1689; 2017.

[37] Sies, H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol* **4:**180-183; 2015.
[38] Frijhoff, J.; Winyard, P. G.; Zarkovic, N.; Davies, S. S.; Stocker, R.; Cheng, D.; Knight, A. R.; Taylor, E. L.; Oettrich, J.; Ruskovska, T.; Gasparovic, A. C.; Cuadrado, A.; Weber, D.; Poulsen, H. E.; Grune, T.; Schmidt, H. H.; Ghezzi, P. Clinical Relevance of Biomarkers of Oxidative Stress. *Antioxid Redox Signal* **23:**1144-1170; 2015.
[39] Mikhed, Y.; Gorlach, A.; Knaus, U. G.; Daiber, A. Redox regulation of genome stability by effects on gene expression, epigenetic pathways and DNA damage/repair. *Redox Biol* **5:**275-289; 2015.
[40] Circu, M. L.; Aw, T. Y. Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med* **48:**749-762; 2010.
[41] Rhee, S. G. Redox signaling: hydrogen peroxide as intracellular messenger. *Exp Mol Med* **31:**53-59; 1999.
[42] Stamler, J. S. Redox signaling: nitrosylation and related target interactions of nitric oxide. *Cell* **78:**931-936; 1994.
[43] Ullrich, V.; Kissner, R. Redox signaling: bioinorganic chemistry at its best. *J Inorg Biochem* **100:**2079-2086; 2006.
[44] Chen, A. F.; Chen, D. D.; Daiber, A.; Faraci, F. M.; Li, H.; Rembold, C. M.; Laher, I. Free radical biology of the cardiovascular system. *Clin Sci (Lond)* **123:**73-91; 2012.
[45] Schmidt, H. H.; Stocker, R.; Vollbracht, C.; Paulsen, G.; Riley, D.; Daiber, A.; Cuadrado, A. Antioxidants in Translational Medicine. *Antioxid Redox Signal* **23:**1130-1143; 2015.
[46] Di Minno, A.; Turnu, L.; Porro, B.; Squellerio, I.; Cavalca, V.; Tremoli, E.; Di Minno, M. N. 8-Hydroxy-2-Deoxyguanosine Levels and Cardiovascular Disease: A Systematic Review and Meta-Analysis of the Literature. *Antioxid Redox Signal* **24:**548-555; 2016.
[47] Gao, S.; Zhao, D.; Wang, M.; Zhao, F.; Han, X.; Qi, Y.; Liu, J. Association Between Circulating Oxidized LDL and Atherosclerotic Cardiovascular Disease: A Meta-analysis of Observational Studies. *Can J Cardiol* **33:**1624-1632; 2017.
[48] Schottker, B.; Brenner, H.; Jansen, E. H.; Gardiner, J.; Peasey, A.; Kubinova, R.; Pajak, A.; Topor-Madry, R.; Tamosiunas, A.; Saum, K. U.; Holleczek, B.; Pikhart, H.; Bobak, M. Evidence for the free radical/oxidative stress theory of ageing from the CHANCES consortium: a meta-analysis of individual participant data. *BMC Med* **13:**300; 2015.
[49] Blankenberg, S.; Rupprecht, H. J.; Bickel, C.; Torzewski, M.; Hafner, G.; Tiret, L.; Smieja, M.; Cambien, F.; Meyer, J.; Lackner, K. J. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. *N Engl J Med* **349:**1605-1613; 2003.
[50] Dubois-Deruy, E.; Peugnet, V.; Turkieh, A.; Pinet, F. Oxidative Stress in Cardiovascular Diseases. *Antioxidants (Basel)* **9**; 2020.
[51] Frohlich, G. M.; Meier, P.; White, S. K.; Yellon, D. M.; Hausenloy, D. J. Myocardial reperfusion injury: looking beyond primary PCI. *Eur Heart J* **34:**1714-1722; 2013.
[52] Cadenas, S. ROS and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection. *Free Radic Biol Med* **117:**76-89; 2018.
[53] Munzel, T.; Gori, T.; Keaney, J. F., Jr.; Maack, C.; Daiber, A. Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications. *Eur Heart J* **36:**2555-2564; 2015.
[54] Landmesser, U.; Spiekermann, S.; Dikalov, S.; Tatge, H.; Wilke, R.; Kohler, C.; Harrison, D. G.; Hornig, B.; Drexler, H. Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. *Circulation* **106:**3073-3078; 2002.
[55] Maack, C.; Bohm, M. Targeting mitochondrial oxidative stress in heart failure throttling the afterburner. *J Am Coll Cardiol* **58:**83-86; 2011.

[56] Braunwald, E. Biomarkers in heart failure. *N Engl J Med* **358:**2148-2159; 2008.
[57] Giordano, F. J. Oxygen, oxidative stress, hypoxia, and heart failure. *J Clin Invest* **115:**500-508; 2005.
[58] Chouchani, E. T.; Pell, V. R.; James, A. M.; Work, L. M.; Saeb-Parsy, K.; Frezza, C.; Krieg, T.; Murphy, M. P. A Unifying Mechanism for Mitochondrial Superoxide Production during Ischemia-Reperfusion Injury. *Cell Metab* **23:**254-263; 2016.
[59] Hausenloy, D. J.; Yellon, D. M. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *J Clin Invest* **123:**92-100; 2013.
[60] Yu, H.; Kalogeris, T.; Korthuis, R. J. Reactive species-induced microvascular dysfunction in ischemia/reperfusion. *Free Radic Biol Med* **135:**182-197; 2019.
[61] Andreadou, I.; Iliodromitis, E. K.; Mikros, E.; Constantinou, M.; Agalias, A.; Magiatis, P.; Skaltsounis, A. L.; Kamber, E.; Tsantili-Kakoulidou, A.; Kremastinos, D. T. The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits. *J Nutr* **136:**2213-2219; 2006.
[62] Granger, D. N.; Kvietys, P. R. Reperfusion injury and reactive oxygen species: The evolution of a concept. *Redox Biol* **6:**524-551; 2015.
[63] Duda, M.; Konior, A.; Klemenska, E.; Beresewicz, A. Preconditioning protects endothelium by preventing ET-1-induced activation of NADPH oxidase and xanthine oxidase in post-ischemic heart. *J Mol Cell Cardiol* **42:**400-410; 2007.
[64] Andrienko, T.; Pasdois, P.; Rossbach, A.; Halestrap, A. P. Real-Time Fluorescence Measurements of ROS and [Ca2+] in Ischemic / Reperfused Rat Hearts: Detectable Increases Occur only after Mitochondrial Pore Opening and Are Attenuated by Ischemic Preconditioning. *PLoS One* **11:**e0167300; 2016.
[65] Sorescu, D.; Griendling, K. K. Reactive oxygen species, mitochondria, and NAD(P)H oxidases in the development and progression of heart failure. *Congest Heart Fail* **8:**132-140; 2002.
[66] Heymes, C.; Bendall, J. K.; Ratajczak, P.; Cave, A. C.; Samuel, J. L.; Hasenfuss, G.; Shah, A. M. Increased myocardial NADPH oxidase activity in human heart failure. *J Am Coll Cardiol* **41:**2164-2171; 2003.
[67] Ijsselmuiden, A. J.; Musters, R. J.; de Ruiter, G.; van Heerebeek, L.; Alderse-Baas, F.; van Schilfgaarde, M.; Leyte, A.; Tangelder, G. J.; Laarman, G. J.; Paulus, W. J. Circulating white blood cells and platelets amplify oxidative stress in heart failure. *Nat Clin Pract Cardiovasc Med* **5:**811-820; 2008.
[68] Loor, G.; Kondapalli, J.; Iwase, H.; Chandel, N. S.; Waypa, G. B.; Guzy, R. D.; Vanden Hoek, T. L.; Schumacker, P. T. Mitochondrial oxidant stress triggers cell death in simulated ischemia-reperfusion. *Biochim Biophys Acta* **1813:**1382-1394; 2011.
[69] Cesselli, D.; Jakoniuk, I.; Barlucchi, L.; Beltrami, A. P.; Hintze, T. H.; Nadal-Ginard, B.; Kajstura, J.; Leri, A.; Anversa, P. Oxidative stress-mediated cardiac cell death is a major determinant of ventricular dysfunction and failure in dog dilated cardiomyopathy. *Circ Res* **89:**279-286; 2001.
[70] Zweier, J. L.; Kuppusamy, P.; Williams, R.; Rayburn, B. K.; Smith, D.; Weisfeldt, M. L.; Flaherty, J. T. Measurement and characterization of postischemic free radical generation in the isolated perfused heart. *J Biol Chem* **264:**18890-18895; 1989.
[71] Kevin, L. G.; Camara, A. K.; Riess, M. L.; Novalija, E.; Stowe, D. F. Ischemic preconditioning alters real-time measure of O2 radicals in intact hearts with ischemia and reperfusion. *Am J Physiol Heart Circ Physiol* **284:**H566-574; 2003.
[72] Eltzschig, H. K.; Eckle, T. Ischemia and reperfusion--from mechanism to translation. *Nat Med* **17:**1391-1401; 2011.
[73] Murphy, E.; Steenbergen, C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. *Physiol Rev* **88:**581-609; 2008.

[74] Di Lisa, F.; Bernardi, P. Modulation of Mitochondrial Permeability Transition in Ischemia-Reperfusion Injury of the Heart. Advantages and Limitations. *Curr Med Chem* **22:**2480-2487; 2015.
[75] Griendling, K. K.; Sorescu, D.; Ushio-Fukai, M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ Res* **86:**494-501; 2000.
[76] Feuerstein, G. Z.; Young, P. R. Apoptosis in cardiac diseases: stress- and mitogen-activated signaling pathways. *Cardiovasc Res* **45:**560-569; 2000.
[77] Ushio-Fukai, M.; Alexander, R. W.; Akers, M.; Yin, Q.; Fujio, Y.; Walsh, K.; Griendling, K. K. Reactive oxygen species mediate the activation of Akt/protein kinase B by angiotensin II in vascular smooth muscle cells. *J Biol Chem* **274:**22699-22704; 1999.
[78] Clerk, A.; Michael, A.; Sugden, P. H. Stimulation of multiple mitogen-activated protein kinase sub-families by oxidative stress and phosphorylation of the small heat shock protein, HSP25/27, in neonatal ventricular myocytes. *Biochem J* **333 (Pt 3):**581-589; 1998.
[79] Laderoute, K. R.; Webster, K. A. Hypoxia/reoxygenation stimulates Jun kinase activity through redox signaling in cardiac myocytes. *Circ Res* **80:**336-344; 1997.
[80] Dai, D. F.; Johnson, S. C.; Villarin, J. J.; Chin, M. T.; Nieves-Cintron, M.; Chen, T.; Marcinek, D. J.; Dorn, G. W., 2nd; Kang, Y. J.; Prolla, T. A.; Santana, L. F.; Rabinovitch, P. S. Mitochondrial oxidative stress mediates angiotensin II-induced cardiac hypertrophy and Galphaq overexpression-induced heart failure. *Circ Res* **108:**837-846; 2011.
[81] Basisty, N.; Dai, D. F.; Gagnidze, A.; Gitari, L.; Fredrickson, J.; Maina, Y.; Beyer, R. P.; Emond, M. J.; Hsieh, E. J.; MacCoss, M. J.; Martin, G. M.; Rabinovitch, P. S. Mitochondrial-targeted catalase is good for the old mouse proteome, but not for the young: 'reverse' antagonistic pleiotropy? *Aging Cell* **15:**634-645; 2016.
[82] Pagliaro, P.; Penna, C. Redox signalling and cardioprotection: translatability and mechanism. *Br J Pharmacol* **172:**1974-1995; 2015.
[83] Arslan, F.; de Kleijn, D. P.; Pasterkamp, G. Innate immune signaling in cardiac ischemia. *Nat Rev Cardiol* **8:**292-300; 2011.
[84] Hernandez-Resendiz, S.; Chinda, K.; Ong, S. B.; Cabrera-Fuentes, H.; Zazueta, C.; Hausenloy, D. J. The Role of Redox Dysregulation in the Inflammatory Response to Acute Myocardial Ischaemia-reperfusion Injury - Adding Fuel to the Fire. *Curr Med Chem* **25:**1275-1293; 2018.
[85] Frangogiannis, N. G. Regulation of the inflammatory response in cardiac repair. *Circ Res* **110:**159-173; 2012.
[86] Avgeropoulou, C.; Andreadou, I.; Markantonis-Kyroudis, S.; Demopoulou, M.; Missovoulos, P.; Androulakis, A.; Kallikazaros, I. The Ca2+-sensitizer levosimendan improves oxidative damage, BNP and pro-inflammatory cytokine levels in patients with advanced decompensated heart failure in comparison to dobutamine. *Eur J Heart Fail* **7:**882-887; 2005.
[87] van Dalen, E. C.; van der Pal, H. J.; Kremer, L. C. Different dosage schedules for reducing cardiotoxicity in people with cancer receiving anthracycline chemotherapy. *Cochrane Database Syst Rev* **3:**CD005008; 2016.
[88] Sukumaran, V.; Veeraveedu, P. T.; Gurusamy, N.; Yamaguchi, K.; Lakshmanan, A. P.; Ma, M.; Suzuki, K.; Kodama, M.; Watanabe, K. Cardioprotective effects of telmisartan against heart failure in rats induced by experimental autoimmune myocarditis through the modulation of angiotensin-converting enzyme-2/angiotensin 1-7/mas receptor axis. *Int J Biol Sci* **7:**1077-1092; 2011.
[89] de Vries, D. K.; Kortekaas, K. A.; Tsikas, D.; Wijermars, L. G.; van Noorden, C. J.; Suchy, M. T.; Cobbaert, C. M.; Klautz, R. J.; Schaapherder, A. F.; Lindeman, J. H.

Oxidative damage in clinical ischemia/reperfusion injury: a reappraisal. *Antioxid Redox Signal* **19:**535-545; 2013.

[90] Goodfriend, T. L.; Elliott, M. E.; Catt, K. J. Angiotensin receptors and their antagonists. *N Engl J Med* **334:**1649-1654; 1996.

[91] Patel, T. N.; Shishehbor, M. H.; Bhatt, D. L. A review of high-dose statin therapy: targeting cholesterol and inflammation in atherosclerosis. *Eur Heart J* **28:**664-672; 2007.

[92] Steven, S.; Munzel, T.; Daiber, A. Exploiting the Pleiotropic Antioxidant Effects of Established Drugs in Cardiovascular Disease. *Int J Mol Sci* **16:**18185-18223; 2015.

[93] De Caterina, R.; Cipollone, F.; Filardo, F. P.; Zimarino, M.; Bernini, W.; Lazzerini, G.; Bucciarelli, T.; Falco, A.; Marchesani, P.; Muraro, R.; Mezzetti, A.; Ciabattoni, G. Low-density lipoprotein level reduction by the 3-hydroxy-3-methylglutaryl coenzyme-A inhibitor simvastatin is accompanied by a related reduction of F2-isoprostane formation in hypercholesterolemic subjects: no further effect of vitamin E. *Circulation* **106:**2543-2549; 2002.

[94] Desideri, G.; Croce, G.; Tucci, M.; Passacquale, G.; Broccoletti, S.; Valeri, L.; Santucci, A.; Ferri, C. Effects of bezafibrate and simvastatin on endothelial activation and lipid peroxidation in hypercholesterolemia: evidence of different vascular protection by different lipid-lowering treatments. *J Clin Endocrinol Metab* **88:**5341-5347; 2003.

[95] Shishehbor, M. H.; Aviles, R. J.; Brennan, M. L.; Fu, X.; Goormastic, M.; Pearce, G. L.; Gokce, N.; Keaney, J. F., Jr.; Penn, M. S.; Sprecher, D. L.; Vita, J. A.; Hazen, S. L. Association of nitrotyrosine levels with cardiovascular disease and modulation by statin therapy. *JAMA* **289:**1675-1680; 2003.

[96] Wassmann, S.; Hilgers, S.; Laufs, U.; Bohm, M.; Nickenig, G. Angiotensin II type 1 receptor antagonism improves hypercholesterolemia-associated endothelial dysfunction. *Arterioscler Thromb Vasc Biol* **22:**1208-1212; 2002.

[97] Hibbert, B.; Simard, T.; Ramirez, F. D.; Pourdjabbar, A.; Raizman, J. E.; Maze, R.; Wilson, K. R.; Hawken, S.; O'Brien, E. R. The effect of statins on circulating endothelial progenitor cells in humans: a systematic review. *J Cardiovasc Pharmacol* **62:**491-496; 2013.

[98] Munzel, T.; Camici, G. G.; Maack, C.; Bonetti, N. R.; Fuster, V.; Kovacic, J. C. Impact of Oxidative Stress on the Heart and Vasculature: Part 2 of a 3-Part Series. *J Am Coll Cardiol* **70:**212-229; 2017.

[99] Nakamura, K.; Murakami, M.; Miura, D.; Yunoki, K.; Enko, K.; Tanaka, M.; Saito, Y.; Nishii, N.; Miyoshi, T.; Yoshida, M.; Oe, H.; Toh, N.; Nagase, S.; Kohno, K.; Morita, H.; Matsubara, H.; Kusano, K. F.; Ohe, T.; Ito, H. Beta-Blockers and Oxidative Stress in Patients with Heart Failure. *Pharmaceuticals (Basel)* **4:**1088-1100; 2011.

[100] Tingberg, E.; Ohlin, A. K.; Gottsater, A.; Ohlin, H. Lipid peroxidation is not increased in heart failure patients on modern pharmacological therapy. *Int J Cardiol* **112:**275-281; 2006.

[101] Mollnau, H.; Schulz, E.; Daiber, A.; Baldus, S.; Oelze, M.; August, M.; Wendt, M.; Walter, U.; Geiger, C.; Agrawal, R.; Kleschyov, A. L.; Meinertz, T.; Munzel, T. Nebivolol prevents vascular NOS III uncoupling in experimental hyperlipidemia and inhibits NADPH oxidase activity in inflammatory cells. *Arterioscler Thromb Vasc Biol* **23:**615-621; 2003.

[102] Oelze, M.; Daiber, A.; Brandes, R. P.; Hortmann, M.; Wenzel, P.; Hink, U.; Schulz, E.; Mollnau, H.; von Sandersleben, A.; Kleschyov, A. L.; Mulsch, A.; Li, H.; Forstermann, U.; Munzel, T. Nebivolol inhibits superoxide formation by NADPH oxidase and endothelial dysfunction in angiotensin II-treated rats. *Hypertension* **48:**677-684; 2006.

[103] Munzel, T.; Meinertz, T.; Tebbe, U.; Schneider, H. T.; Stalleicken, D.; Wargenau, M.; Gori, T.; Klingmann, I.; Investigators, C. S. Efficacy of the long-acting nitro vasodilator

pentaerithrityl tetranitrate in patients with chronic stable angina pectoris receiving anti-anginal background therapy with beta-blockers: a 12-week, randomized, double-blind, placebo-controlled trial. *Eur Heart J* **35:**895-903; 2014.

[104] Munzel, T.; Daiber, A.; Gori, T. Nitrate therapy: new aspects concerning molecular action and tolerance. *Circulation* **123:**2132-2144; 2011.

[105] Oberle, S.; Abate, A.; Grosser, N.; Vreman, H. J.; Dennery, P. A.; Schneider, H. T.; Stalleicken, D.; Schroder, H. Heme oxygenase-1 induction may explain the antioxidant profile of pentaerythrityl trinitrate. *Biochem Biophys Res Commun* **290:**1539-1544; 2002.

[106] Wenzel, P.; Oelze, M.; Coldewey, M.; Hortmann, M.; Seeling, A.; Hink, U.; Mollnau, H.; Stalleicken, D.; Weiner, H.; Lehmann, J.; Li, H.; Forstermann, U.; Munzel, T.; Daiber, A. Heme oxygenase-1: a novel key player in the development of tolerance in response to organic nitrates. *Arterioscler Thromb Vasc Biol* **27:**1729-1735; 2007.

[107] Schuhmacher, S.; Oelze, M.; Bollmann, F.; Kleinert, H.; Otto, C.; Heeren, T.; Steven, S.; Hausding, M.; Knorr, M.; Pautz, A.; Reifenberg, K.; Schulz, E.; Gori, T.; Wenzel, P.; Munzel, T.; Daiber, A. Vascular dysfunction in experimental diabetes is improved by pentaerithrityl tetranitrate but not isosorbide-5-mononitrate therapy. *Diabetes* **60:**2608-2616; 2011.

[108] Kramer, C. K.; Ye, C.; Campbell, S.; Retnakaran, R. Comparison of New Glucose-Lowering Drugs on Risk of Heart Failure in Type 2 Diabetes: A Network Meta-Analysis. *JACC Heart Fail* **6:**823-830; 2018.

[109] McMurray, J. J. V.; Solomon, S. D.; Inzucchi, S. E.; Kober, L.; Kosiborod, M. N.; Martinez, F. A.; Ponikowski, P.; Sabatine, M. S.; Anand, I. S.; Belohlavek, J.; Bohm, M.; Chiang, C. E.; Chopra, V. K.; de Boer, R. A.; Desai, A. S.; Diez, M.; Drozdz, J.; Dukat, A.; Ge, J.; Howlett, J. G.; Katova, T.; Kitakaze, M.; Ljungman, C. E. A.; Merkely, B.; Nicolau, J. C.; O'Meara, E.; Petrie, M. C.; Vinh, P. N.; Schou, M.; Tereshchenko, S.; Verma, S.; Held, C.; DeMets, D. L.; Docherty, K. F.; Jhund, P. S.; Bengtsson, O.; Sjostrand, M.; Langkilde, A. M.; Committees, D.-H. T.; Investigators. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. *N Engl J Med* **381:**1995-2008; 2019.

[110] Packer, M.; Anker, S. D.; Butler, J.; Filippatos, G.; Pocock, S. J.; Carson, P.; Januzzi, J.; Verma, S.; Tsutsui, H.; Brueckmann, M.; Jamal, W.; Kimura, K.; Schnee, J.; Zeller, C.; Cotton, D.; Bocchi, E.; Bohm, M.; Choi, D. J.; Chopra, V.; Chuquiure, E.; Giannetti, N.; Janssens, S.; Zhang, J.; Gonzalez Juanatey, J. R.; Kaul, S.; Brunner-La Rocca, H. P.; Merkely, B.; Nicholls, S. J.; Perrone, S.; Pina, I.; Ponikowski, P.; Sattar, N.; Senni, M.; Seronde, M. F.; Spinar, J.; Squire, I.; Taddei, S.; Wanner, C.; Zannad, F.; Investigators, E. M.-R. T. Cardiovascular and Renal Outcomes with Empagliflozin in Heart Failure. *N Engl J Med* **383:**1413-1424; 2020.

[111] Zannad, F.; Ferreira, J. P.; Pocock, S. J.; Anker, S. D.; Butler, J.; Filippatos, G.; Brueckmann, M.; Ofstad, A. P.; Pfarr, E.; Jamal, W.; Packer, M. SGLT2 inhibitors in patients with heart failure with reduced ejection fraction: a meta-analysis of the EMPEROR-Reduced and DAPA-HF trials. *Lancet* **396:**819-829; 2020.

[112] Timmers, L.; Henriques, J. P.; de Kleijn, D. P.; Devries, J. H.; Kemperman, H.; Steendijk, P.; Verlaan, C. W.; Kerver, M.; Piek, J. J.; Doevendans, P. A.; Pasterkamp, G.; Hoefer, I. E. Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. *J Am Coll Cardiol* **53:**501-510; 2009.

[113] Hocher, B.; Sharkovska, Y.; Mark, M.; Klein, T.; Pfab, T. The novel DPP-4 inhibitors linagliptin and BI 14361 reduce infarct size after myocardial ischemia/reperfusion in rats. *Int J Cardiol* **167:**87-93; 2013.

[114] Andreadou, I.; Efentakis, P.; Balafas, E.; Togliatto, G.; Davos, C. H.; Varela, A.; Dimitriou, C. A.; Nikolaou, P. E.; Maratou, E.; Lambadiari, V.; Ikonomidis, I.;

Kostomitsopoulos, N.; Brizzi, M. F.; Dimitriadis, G.; Iliodromitis, E. K. Empagliflozin Limits Myocardial Infarction in Vivo and Cell Death in Vitro: Role of STAT3, Mitochondria, and Redox Aspects. *Front Physiol* **8:**1077; 2017.

[115] Oshima, H.; Miki, T.; Kuno, A.; Mizuno, M.; Sato, T.; Tanno, M.; Yano, T.; Nakata, K.; Kimura, Y.; Abe, K.; Ohwada, W.; Miura, T. Empagliflozin, an SGLT2 inhibitor, reduced the mortality rate after acute myocardial infarction with modification of cardiac metabolomes and anti-oxidants in diabetic rats. *J Pharmacol Exp Ther*; 2018.

[116] Bonnet, F.; Scheen, A. J. Effects of SGLT2 inhibitors on systemic and tissue low-grade inflammation: The potential contribution to diabetes complications and cardiovascular disease. *Diabetes Metab* **44:**457-464; 2018.

[117] Atkin, S. L.; Katsiki, N.; Banach, M.; Mikhailidis, D. P.; Pirro, M.; Sahebkar, A. Effect of dipeptidyl peptidase-4 inhibitors on circulating tumor necrosis factor-alpha concentrations: A systematic review and meta-analysis of controlled trials. *J Diabetes Complications* **31:**1458-1464; 2017.

[118] Lee, Y. S.; Jun, H. S. Anti-Inflammatory Effects of GLP-1-Based Therapies beyond Glucose Control. *Mediators Inflamm* **2016:**3094642; 2016.

[119] Matsubara, J.; Sugiyama, S.; Sugamura, K.; Nakamura, T.; Fujiwara, Y.; Akiyama, E.; Kurokawa, H.; Nozaki, T.; Ohba, K.; Konishi, M.; Maeda, H.; Izumiya, Y.; Kaikita, K.; Sumida, H.; Jinnouchi, H.; Matsui, K.; Kim-Mitsuyama, S.; Takeya, M.; Ogawa, H. A dipeptidyl peptidase-4 inhibitor, des-fluoro-sitagliptin, improves endothelial function and reduces atherosclerotic lesion formation in apolipoprotein E-deficient mice. *J Am Coll Cardiol* **59:**265-276; 2012.

[120] Shah, Z.; Kampfrath, T.; Deiuliis, J. A.; Zhong, J.; Pineda, C.; Ying, Z.; Xu, X.; Lu, B.; Moffatt-Bruce, S.; Durairaj, R.; Sun, Q.; Mihai, G.; Maiseyeu, A.; Rajagopalan, S. Long-term dipeptidyl-peptidase 4 inhibition reduces atherosclerosis and inflammation via effects on monocyte recruitment and chemotaxis. *Circulation* **124:**2338-2349; 2011.

[121] Nakatsu, Y.; Kokubo, H.; Bumdelger, B.; Yoshizumi, M.; Yamamotoya, T.; Matsunaga, Y.; Ueda, K.; Inoue, Y.; Inoue, M. K.; Fujishiro, M.; Kushiyama, A.; Ono, H.; Sakoda, H.; Asano, T. The SGLT2 Inhibitor Luseogliflozin Rapidly Normalizes Aortic mRNA Levels of Inflammation-Related but Not Lipid-Metabolism-Related Genes and Suppresses Atherosclerosis in Diabetic ApoE KO Mice. *Int J Mol Sci* **18**; 2017.

[122] Han, J. H.; Oh, T. J.; Lee, G.; Maeng, H. J.; Lee, D. H.; Kim, K. M.; Choi, S. H.; Jang, H. C.; Lee, H. S.; Park, K. S.; Kim, Y. B.; Lim, S. The beneficial effects of empagliflozin, an SGLT2 inhibitor, on atherosclerosis in ApoE (-/-) mice fed a western diet. *Diabetologia* **60:**364-376; 2017.

[123] Nikolaou, P. E.; Efentakis, P.; Qourah, F. A.; Femmino, S.; Makridakis, M.; Kanaki, Z.; Varela, A.; Tsoumani, M.; Davos, C. H.; Dimitriou, C. A.; Tasouli, A.; Dimitriadis, G.; Kostomitsopoulos, N.; Zuurbier, C. J.; Vlahou, A.; Klinakis, A.; Brizzi, M. F.; Iliodromitis, E. K.; Andreadou, I. Chronic Empaglifozin treatment reduces myocardial infarct size in non-diabetic mice through STAT-3 mediated protection on microvascular endothelial cells and reduction of oxidative stress. *Antioxid Redox Signal*; 2020.

[124] Chicco, A. J.; McCarty, H.; Reed, A. H.; Story, R. R.; Westerlind, K. C.; Turner, R. T.; Hayward, R. Resistance exercise training attenuates alcohol-induced cardiac oxidative stress. *Eur J Cardiovasc Prev Rehabil* **13:**74-79; 2006.

[125] da Silva, M. F.; Natali, A. J.; da Silva, E.; Gomes, G. J.; Teodoro, B. G.; Cunha, D. N.; Drummond, L. R.; Drummond, F. R.; Moura, A. G.; Belfort, F. G.; de Oliveira, A.; Maldonado, I. R.; Alberici, L. C. Attenuation of Ca2+ homeostasis, oxidative stress, and mitochondrial dysfunctions in diabetic rat heart: insulin therapy or aerobic exercise? *J Appl Physiol (1985)* **119:**148-156; 2015.

[126] Radak, Z.; Chung, H. Y.; Goto, S. Exercise and hormesis: oxidative stress-related adaptation for successful aging. *Biogerontology* **6:**71-75; 2005.

[127] Golbidi, S.; Laher, I. Molecular mechanisms in exercise-induced cardioprotection. *Cardiol Res Pract* **2011:**972807; 2011.
[128] Ristow, M.; Zarse, K.; Oberbach, A.; Kloting, N.; Birringer, M.; Kiehntopf, M.; Stumvoll, M.; Kahn, C. R.; Bluher, M. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A* **106:**8665-8670; 2009.
[129] Bjarnason-Wehrens, B.; Nebel, R.; Jensen, K.; Hackbusch, M.; Grilli, M.; Gielen, S.; Schwaab, B.; Rauch, B.; German Society of Cardiovascular, P.; Rehabilitation. Exercise-based cardiac rehabilitation in patients with reduced left ventricular ejection fraction: The Cardiac Rehabilitation Outcome Study in Heart Failure (CROS-HF): A systematic review and meta-analysis. *Eur J Prev Cardiol* **27:**929-952; 2020.
[130] Long, L.; Mordi, I. R.; Bridges, C.; Sagar, V. A.; Davies, E. J.; Coats, A. J.; Dalal, H.; Rees, K.; Singh, S. J.; Taylor, R. S. Exercise-based cardiac rehabilitation for adults with heart failure. *Cochrane Database Syst Rev* **1:**CD003331; 2019.
[131] Edelmann, F.; Pieske, B. [Exercise training in heart failure]. *Herz* **38:**578-586; 2013.
[132] Ros, E.; Martinez-Gonzalez, M. A.; Estruch, R.; Salas-Salvado, J.; Fito, M.; Martinez, J. A.; Corella, D. Mediterranean diet and cardiovascular health: Teachings of the PREDIMED study. *Adv Nutr* **5:**330S-336S; 2014.
[133] Marx, W.; Kelly, J.; Marshall, S.; Nakos, S.; Campbell, K.; Itsiopoulos, C. The Effect of Polyphenol-Rich Interventions on Cardiovascular Risk Factors in Haemodialysis: A Systematic Review and Meta-Analysis. *Nutrients* **9**; 2017.
[134] Panahi, Y.; Hosseini, M. S.; Khalili, N.; Naimi, E.; Majeed, M.; Sahebkar, A. Antioxidant and anti-inflammatory effects of curcuminoid-piperine combination in subjects with metabolic syndrome: A randomized controlled trial and an updated meta-analysis. *Clin Nutr* **34:**1101-1108; 2015.
[135] Akbari, M.; Tamtaji, O. R.; Lankarani, K. B.; Tabrizi, R.; Dadgostar, E.; Kolahdooz, F.; Jamilian, M.; Mirzaei, H.; Asemi, Z. The Effects of Resveratrol Supplementation on Endothelial Function and Blood Pressures Among Patients with Metabolic Syndrome and Related Disorders: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *High Blood Press Cardiovasc Prev* **26:**305-319; 2019.
[136] Asgary, S.; Karimi, R.; Momtaz, S.; Naseri, R.; Farzaei, M. H. Effect of resveratrol on metabolic syndrome components: A systematic review and meta-analysis. *Rev Endocr Metab Disord* **20:**173-186; 2019.
[137] Golbidi, S.; Daiber, A.; Korac, B.; Li, H.; Essop, M. F.; Laher, I. Health Benefits of Fasting and Caloric Restriction. *Curr Diab Rep* **17:**123; 2017.
[138] Mattson, M. P.; Wan, R. Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. *J Nutr Biochem* **16:**129-137; 2005.
[139] Horne, B. D.; Muhlestein, J. B.; Anderson, J. L. Health effects of intermittent fasting: hormesis or harm? A systematic review. *Am J Clin Nutr* **102:**464-470; 2015.
[140] Clar, C.; Al-Khudairy, L.; Loveman, E.; Kelly, S. A.; Hartley, L.; Flowers, N.; Germano, R.; Frost, G.; Rees, K. Low glycaemic index diets for the prevention of cardiovascular disease. *Cochrane Database Syst Rev* **7:**CD004467; 2017.
[141] Abazid, R. M.; Khalaf, H. H.; Sakr, H. I.; Altorbak, N. A.; Alenzi, H. S.; Awad, Z. M.; Smettei, O. A.; Elsanan, M. A.; Widyan, A. M.; Azazy, A. S.; Chamsi-Pasha, H. W. Effects of Ramadan fasting on the symptoms of chronic heart failure. *Saudi Med J* **39:**395-400; 2018.
[142] Singh, N.; Dhalla, A. K.; Seneviratne, C.; Singal, P. K. Oxidative stress and heart failure. *Mol Cell Biochem* **147:**77-81; 1995.
[143] Wallert, M.; Ziegler, M.; Wang, X.; Maluenda, A.; Xu, X.; Yap, M. L.; Witt, R.; Giles, C.; Kluge, S.; Hortmann, M.; Zhang, J.; Meikle, P.; Lorkowski, S.; Peter, K. alpha-Tocopherol preserves cardiac function by reducing oxidative stress and inflammation in ischemia/reperfusion injury. *Redox Biol* **26:**101292; 2019.

[144] Dhalla, A. K.; Hill, M. F.; Singal, P. K. Role of oxidative stress in transition of hypertrophy to heart failure. *J Am Coll Cardiol* **28:**506-514; 1996.
[145] Rossig, L.; Hoffmann, J.; Hugel, B.; Mallat, Z.; Haase, A.; Freyssinet, J. M.; Tedgui, A.; Aicher, A.; Zeiher, A. M.; Dimmeler, S. Vitamin C inhibits endothelial cell apoptosis in congestive heart failure. *Circulation* **104:**2182-2187; 2001.
[146] Casas, A. I.; Nogales, C.; Mucke, H. A. M.; Petraina, A.; Cuadrado, A.; Rojo, A. I.; Ghezzi, P.; Jaquet, V.; Augsburger, F.; Dufrasne, F.; Soubhye, J.; Deshwal, S.; Di Sante, M.; Kaludercic, N.; Di Lisa, F.; Schmidt, H. On the Clinical Pharmacology of Reactive Oxygen Species. *Pharmacol Rev* **72:**801-828; 2020.
[147] Murphy, M. P.; Smith, R. A. Targeting antioxidants to mitochondria by conjugation to lipophilic cations. *Annu Rev Pharmacol Toxicol* **47:**629-656; 2007.
[148] Smith, R. A.; Hartley, R. C.; Murphy, M. P. Mitochondria-targeted small molecule therapeutics and probes. *Antioxid Redox Signal* **15:**3021-3038; 2011.
[149] Evgenov, O. V.; Pacher, P.; Schmidt, P. M.; Hasko, G.; Schmidt, H. H.; Stasch, J. P. NO-independent stimulators and activators of soluble guanylate cyclase: discovery and therapeutic potential. *Nat Rev Drug Discov* **5:**755-768; 2006.
[150] Blanco-Rivero, J.; Xavier, F. E. Therapeutic Potential of Phosphodiesterase Inhibitors for Endothelial Dysfunction- Related Diseases. *Curr Pharm Des* **26:**3633-3651; 2020.
[151] Munzel, T.; Daiber, A.; Gori, T. More answers to the still unresolved question of nitrate tolerance. *Eur Heart J* **34:**2666-2673; 2013.
[152] Gryglewski, R. J. Prostacyclin among prostanoids. *Pharmacol Rep* **60:**3-11; 2008.
[153] Pulido, T.; Adzerikho, I.; Channick, R. N.; Delcroix, M.; Galie, N.; Ghofrani, H. A.; Jansa, P.; Jing, Z. C.; Le Brun, F. O.; Mehta, S.; Mittelholzer, C. M.; Perchenet, L.; Sastry, B. K.; Sitbon, O.; Souza, R.; Torbicki, A.; Zeng, X.; Rubin, L. J.; Simonneau, G.; Investigators, S. Macitentan and morbidity and mortality in pulmonary arterial hypertension. *N Engl J Med* **369:**809-818; 2013.
[154] Oelze, M.; Knorr, M.; Kroller-Schon, S.; Kossmann, S.; Gottschlich, A.; Rummler, R.; Schuff, A.; Daub, S.; Doppler, C.; Kleinert, H.; Gori, T.; Daiber, A.; Munzel, T. Chronic therapy with isosorbide-5-mononitrate causes endothelial dysfunction, oxidative stress, and a marked increase in vascular endothelin-1 expression. *Eur Heart J* **34:**3206-3216; 2013.
[155] Steven, S.; Oelze, M.; Brandt, M.; Ullmann, E.; Kroller-Schon, S.; Heeren, T.; Tran, L. P.; Daub, S.; Dib, M.; Stalleicken, D.; Wenzel, P.; Munzel, T.; Daiber, A. Pentaerythritol Tetranitrate In Vivo Treatment Improves Oxidative Stress and Vascular Dysfunction by Suppression of Endothelin-1 Signaling in Monocrotaline-Induced Pulmonary Hypertension. *Oxid Med Cell Longev* **2017:**4353462; 2017.
[156] Daiber, A.; Xia, N.; Steven, S.; Oelze, M.; Hanf, A.; Kroller-Schon, S.; Munzel, T.; Li, H. New Therapeutic Implications of Endothelial Nitric Oxide Synthase (eNOS) Function/Dysfunction in Cardiovascular Disease. *Int J Mol Sci* **20**; 2019.
[157] Gangji, A. S.; Cukierman, T.; Gerstein, H. C.; Goldsmith, C. H.; Clase, C. M. A systematic review and meta-analysis of hypoglycemia and cardiovascular events: a comparison of glyburide with other secretagogues and with insulin. *Diabetes Care* **30:**389-394; 2007.
[158] Piot, C.; Croisille, P.; Staat, P.; Thibault, H.; Rioufol, G.; Mewton, N.; Elbelghiti, R.; Cung, T. T.; Bonnefoy, E.; Angoulvant, D.; Macia, C.; Raczka, F.; Sportouch, C.; Gahide, G.; Finet, G.; Andre-Fouet, X.; Revel, D.; Kirkorian, G.; Monassier, J. P.; Derumeaux, G.; Ovize, M. Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *N Engl J Med* **359:**473-481; 2008.
[159] Cuadrado, A.; Manda, G.; Hassan, A.; Alcaraz, M. J.; Barbas, C.; Daiber, A.; Ghezzi, P.; Leon, R.; Lopez, M. G.; Oliva, B.; Pajares, M.; Rojo, A. I.; Robledinos-Anton, N.; Valverde, A. M.; Guney, E.; Schmidt, H. Transcription Factor NRF2 as a Therapeutic

Target for Chronic Diseases: A Systems Medicine Approach. *Pharmacol Rev* **70:**348-383; 2018.

[160] Bredemeier, M.; Lopes, L. M.; Eisenreich, M. A.; Hickmann, S.; Bongiorno, G. K.; d'Avila, R.; Morsch, A. L. B.; da Silva Stein, F.; Campos, G. G. D. Xanthine oxidase inhibitors for prevention of cardiovascular events: a systematic review and meta-analysis of randomized controlled trials. *BMC Cardiovasc Disord* **18:**24; 2018.

[161] Ziegler, D.; Nowak, H.; Kempler, P.; Vargha, P.; Low, P. A. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis. *Diabet Med* **21:**114-121; 2004.

[162] Khaw, K. T.; Bingham, S.; Welch, A.; Luben, R.; Wareham, N.; Oakes, S.; Day, N. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. European Prospective Investigation into Cancer and Nutrition. *Lancet* **357:**657-663; 2001.

[163] Bjelakovic, G.; Nikolova, D.; Gluud, L. L.; Simonetti, R. G.; Gluud, C. Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention: Systematic Review and Meta-analysis. *Jama* **297:**842-857; 2007.

[164] Munzel, T.; Gori, T.; Bruno, R. M.; Taddei, S. Is oxidative stress a therapeutic target in cardiovascular disease? *Eur Heart J* **31:**2741-2748; 2010.

[165] Hornig, B.; Landmesser, U.; Kohler, C.; Ahlersmann, D.; Spiekermann, S.; Christoph, A.; Tatge, H.; Drexler, H. Comparative effect of ace inhibition and angiotensin II type 1 receptor antagonism on bioavailability of nitric oxide in patients with coronary artery disease: role of superoxide dismutase. *Circulation* **103:**799-805; 2001.

[166] Schurmann, C.; Rezende, F.; Kruse, C.; Yasar, Y.; Lowe, O.; Fork, C.; van de Sluis, B.; Bremer, R.; Weissmann, N.; Shah, A. M.; Jo, H.; Brandes, R. P.; Schroder, K. The NADPH oxidase Nox4 has anti-atherosclerotic functions. *Eur Heart J* **36:**3447-3456; 2015.

[167] Schroder, K.; Zhang, M.; Benkhoff, S.; Mieth, A.; Pliquett, R.; Kosowski, J.; Kruse, C.; Luedike, P.; Michaelis, U. R.; Weissmann, N.; Dimmeler, S.; Shah, A. M.; Brandes, R. P. Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase. *Circ Res* **110:**1217-1225; 2012.

[168] Sanchez-Gomez, F. J.; Calvo, E.; Breton-Romero, R.; Fierro-Fernandez, M.; Anilkumar, N.; Shah, A. M.; Schroder, K.; Brandes, R. P.; Vazquez, J.; Lamas, S. NOX4-dependent Hydrogen peroxide promotes shear stress-induced SHP2 sulfenylation and eNOS activation. *Free Radic Biol Med* **89:**419-430; 2015.

[169] Zhang, M.; Brewer, A. C.; Schroder, K.; Santos, C. X.; Grieve, D. J.; Wang, M.; Anilkumar, N.; Yu, B.; Dong, X.; Walker, S. J.; Brandes, R. P.; Shah, A. M. NADPH oxidase-4 mediates protection against chronic load-induced stress in mouse hearts by enhancing angiogenesis. *Proc Natl Acad Sci U S A* **107:**18121-18126; 2010.

[170] Egea, J.; Fabregat, I.; Frapart, Y. M.; Ghezzi, P.; Gorlach, A.; Kietzmann, T.; Kubaichuk, K.; Knaus, U. G.; Lopez, M. G.; Olaso-Gonzalez, G.; Petry, A.; Schulz, R.; Vina, J.; Winyard, P.; Abbas, K.; Ademowo, O. S.; Afonso, C. B.; Andreadou, I.; Antelmann, H.; Antunes, F.; Aslan, M.; Bachschmid, M. M.; Barbosa, R. M.; Belousov, V.; Berndt, C.; Bernlohr, D.; Bertran, E.; Bindoli, A.; Bottari, S. P.; Brito, P. M.; Carrara, G.; Casas, A. I.; Chatzi, A.; Chondrogianni, N.; Conrad, M.; Cooke, M. S.; Costa, J. G.; Cuadrado, A.; My-Chan Dang, P.; De Smet, B.; Debelec-Butuner, B.; Dias, I. H. K.; Dunn, J. D.; Edson, A. J.; El Assar, M.; El-Benna, J.; Ferdinandy, P.; Fernandes, A. S.; Fladmark, K. E.; Forstermann, U.; Giniatullin, R.; Giricz, Z.; Gorbe, A.; Griffiths, H.; Hampl, V.; Hanf, A.; Herget, J.; Hernansanz-Agustin, P.; Hillion, M.; Huang, J.; Ilikay, S.; Jansen-Durr, P.; Jaquet, V.; Joles, J. A.; Kalyanaraman, B.; Kaminskyy, D.; Karbaschi, M.; Kleanthous, M.; Klotz, L. O.; Korac, B.; Korkmaz, K. S.; Koziel, R.; Kracun, D.; Krause, K. H.; Kren, V.; Krieg, T.; Laranjinha, J.; Lazou, A.; Li, H.;

Martinez-Ruiz, A.; Matsui, R.; McBean, G. J.; Meredith, S. P.; Messens, J.; Miguel, V.; Mikhed, Y.; Milisav, I.; Milkovic, L.; Miranda-Vizuete, A.; Mojovic, M.; Monsalve, M.; Mouthuy, P. A.; Mulvey, J.; Munzel, T.; Muzykantov, V.; Nguyen, I. T. N.; Oelze, M.; Oliveira, N. G.; Palmeira, C. M.; Papaevgeniou, N.; Pavicevic, A.; Pedre, B.; Peyrot, F.; Phylactides, M.; Pircalabioru, G. G.; Pitt, A. R.; Poulsen, H. E.; Prieto, I.; Rigobello, M. P.; Robledinos-Anton, N.; Rodriguez-Manas, L.; Rolo, A. P.; Rousset, F.; Ruskovska, T.; Saraiva, N.; Sasson, S.; Schroder, K.; Semen, K.; Seredenina, T.; Shakirzyanova, A.; Smith, G. L.; Soldati, T.; Sousa, B. C.; Spickett, C. M.; Stancic, A.; Stasia, M. J.; Steinbrenner, H.; Stepanic, V.; Steven, S.; Tokatlidis, K.; Tuncay, E.; Turan, B.; Ursini, F.; Vacek, J.; Vajnerova, O.; Valentova, K.; Van Breusegem, F.; Varisli, L.; Veal, E. A.; Yalcin, A. S.; Yelisyeyeva, O.; Zarkovic, N.; Zatloukalova, M.; Zielonka, J.; Touyz, R. M.; Papapetropoulos, A.; Grune, T.; Lamas, S.; Schmidt, H.; Di Lisa, F.; Daiber, A. European contribution to the study of ROS: A summary of the findings and prospects for the future from the COST action BM1203 (EU-ROS). *Redox Biol* **13:**94-162; 2017.

[171] Sies, H.; Berndt, C.; Jones, D. P. Oxidative Stress. *Annu Rev Biochem* **86:**715-748; 2017.

[172] Levonen, A. L.; Hill, B. G.; Kansanen, E.; Zhang, J.; Darley-Usmar, V. M. Redox regulation of antioxidants, autophagy, and the response to stress: implications for electrophile therapeutics. *Free Radic Biol Med* **71:**196-207; 2014.

[173] Ushio-Fukai, M.; Urao, N. Novel role of NADPH oxidase in angiogenesis and stem/progenitor cell function. *Antioxid Redox Signal* **11:**2517-2533; 2009.

[174] Ludin, A.; Gur-Cohen, S.; Golan, K.; Kaufmann, K. B.; Itkin, T.; Medaglia, C.; Lu, X. J.; Ledergor, G.; Kollet, O.; Lapidot, T. Reactive oxygen species regulate hematopoietic stem cell self-renewal, migration and development, as well as their bone marrow microenvironment. *Antioxid Redox Signal* **21:**1605-1619; 2014.

[175] Mofarrahi, M.; Brandes, R. P.; Gorlach, A.; Hanze, J.; Terada, L. S.; Quinn, M. T.; Mayaki, D.; Petrof, B.; Hussain, S. N. Regulation of proliferation of skeletal muscle precursor cells by NADPH oxidase. *Antioxid Redox Signal* **10:**559-574; 2008.

[176] Petry, A.; Djordjevic, T.; Weitnauer, M.; Kietzmann, T.; Hess, J.; Gorlach, A. NOX2 and NOX4 mediate proliferative response in endothelial cells. *Antioxid Redox Signal* **8:**1473-1484; 2006.

[177] Hurd, T. R.; Degennaro, M.; Lehmann, R. Redox regulation of cell migration and adhesion. *Trends Cell Biol*; 2011.

[178] Casas, A. I.; Dao, V. T.; Daiber, A.; Maghzal, G. J.; Di Lisa, F.; Kaludercic, N.; Leach, S.; Cuadrado, A.; Jaquet, V.; Seredenina, T.; Krause, K. H.; Lopez, M. G.; Stocker, R.; Ghezzi, P.; Schmidt, H. H. Reactive Oxygen-Related Diseases: Therapeutic Targets and Emerging Clinical Indications. *Antioxid Redox Signal* **23:**1171-1185; 2015.

[179] Forman, H. J.; Davies, K. J.; Ursini, F. How do nutritional antioxidants really work: nucleophilic tone and para-hormesis versus free radical scavenging in vivo. *Free Radic Biol Med* **66:**24-35; 2014.

[180] Murry, C. E.; Jennings, R. B.; Reimer, K. A. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* **74:**1124-1136; 1986.

[181] Zhao, Z. Q.; Corvera, J. S.; Halkos, M. E.; Kerendi, F.; Wang, N. P.; Guyton, R. A.; Vinten-Johansen, J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* **285:**H579-588; 2003.

[182] Przyklenk, K.; Bauer, B.; Ovize, M.; Kloner, R. A.; Whittaker, P. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. *Circulation* **87:**893-899; 1993.

[183] Hausenloy, D. J. Cardioprotection techniques: preconditioning, postconditioning and remote conditioning (basic science). *Curr Pharm Des* **19:**4544-4563; 2013.
[184] Hausenloy, D. J.; Yellon, D. M. Ischaemic conditioning and reperfusion injury. *Nat Rev Cardiol* **13:**193-209; 2016.
[185] Das, D. K. Thioredoxin regulation of ischemic preconditioning. *Antioxid Redox Signal* **6:**405-412; 2004.
[186] Kuster, G. M.; Siwik, D. A.; Pimentel, D. R.; Colucci, W. S. Role of reversible, thioredoxin-sensitive oxidative protein modifications in cardiac myocytes. *Antioxid Redox Signal* **8:**2153-2159; 2006.
[187] Ekelof, S.; Jensen, S. E.; Rosenberg, J.; Gogenur, I. Reduced oxidative stress in STEMI patients treated by primary percutaneous coronary intervention and with antioxidant therapy: a systematic review. *Cardiovasc Drugs Ther* **28:**173-181; 2014.
[188] Arstall, M. A.; Yang, J.; Stafford, I.; Betts, W. H.; Horowitz, J. D. N-acetylcysteine in combination with nitroglycerin and streptokinase for the treatment of evolving acute myocardial infarction. Safety and biochemical effects. *Circulation* **92:**2855-2862; 1995.
[189] Horowitz, J. D.; Henry, C. A.; Syrjanen, M. L.; Louis, W. J.; Fish, R. D.; Smith, T. W.; Antman, E. M. Combined use of nitroglycerin and N-acetylcysteine in the management of unstable angina pectoris. *Circulation* **77:**787-794; 1988.
[190] Pasupathy, S.; Tavella, R.; Grover, S.; Raman, B.; Procter, N. E. K.; Du, Y. T.; Mahadavan, G.; Stafford, I.; Heresztyn, T.; Holmes, A.; Zeitz, C.; Arstall, M.; Selvanayagam, J.; Horowitz, J. D.; Beltrame, J. F. Early Use of N-acetylcysteine With Nitrate Therapy in Patients Undergoing Primary Percutaneous Coronary Intervention for ST-Segment-Elevation Myocardial Infarction Reduces Myocardial Infarct Size (the NACIAM Trial [N-acetylcysteine in Acute Myocardial Infarction]). *Circulation* **136:**894-903; 2017.
[191] Bartekova, M.; Barancik, M.; Ferenczyova, K.; Dhalla, N. S. Beneficial Effects of N-acetylcysteine and N-mercaptopropionylglycine on Ischemia Reperfusion Injury in the Heart. *Curr Med Chem* **25:**355-366; 2018.
[192] Borghi, C.; Omboni, S.; Reggiardo, G.; Bacchelli, S.; Esposti, D. D.; Ambrosioni, E.; Project, S. W. Effects of the concomitant administration of xanthine oxidase inhibitors with zofenopril or other ACE-inhibitors in post-myocardial infarction patients: a meta-analysis of individual data of four randomized, double-blind, prospective studies. *BMC Cardiovasc Disord* **18:**112; 2018.
[193] Higgins, P.; Dawson, J.; Lees, K. R.; McArthur, K.; Quinn, T. J.; Walters, M. R. Xanthine oxidase inhibition for the treatment of cardiovascular disease: a systematic review and meta-analysis. *Cardiovasc Ther* **30:**217-226; 2012.
[194] Pan, J. A.; Lin, H.; Wang, C. Q.; Zhang, J. F.; Gu, J. Association between long-term prescription of febuxostat and the progression of heart failure with preserved ejection fraction in patients with hypertension and asymptomatic hyperuricemia. *Heart Vessels* **35:**1446-1453; 2020.
[195] Zhang, M.; Solomon, D. H.; Desai, R. J.; Kang, E. H.; Liu, J.; Neogi, T.; Kim, S. C. Assessment of Cardiovascular Risk in Older Patients With Gout Initiating Febuxostat Versus Allopurinol: Population-Based Cohort Study. *Circulation* **138:**1116-1126; 2018.
[196] Alem, M. M.; Alshehri, A. M.; Cahusac, P. M.; Walters, M. R. Effect of Xanthine Oxidase Inhibition on Arterial Stiffness in Patients With Chronic Heart Failure. *Clin Med Insights Cardiol* **12:**1179546818779584; 2018.
[197] Sakuma, M.; Toyoda, S.; Arikawa, T.; Koyabu, Y.; Kato, T.; Adachi, T.; Suwa, H.; Narita, J. I.; Anraku, K.; Ishimura, K.; Yamauchi, F.; Sato, Y.; Inoue, T.; For Excited, U. A. s. I. The effects of xanthine oxidase inhibitor in patients with chronic heart failure complicated with hyperuricemia: a prospective randomized controlled clinical trial of topiroxostat vs allopurinol-study protocol. *Clin Exp Nephrol* **22:**1379-1386; 2018.

[198] Xu, X.; Hu, X.; Lu, Z.; Zhang, P.; Zhao, L.; Wessale, J. L.; Bache, R. J.; Chen, Y. Xanthine oxidase inhibition with febuxostat attenuates systolic overload-induced left ventricular hypertrophy and dysfunction in mice. *J Card Fail* **14:**746-753; 2008.

[199] Nambu, H.; Takada, S.; Maekawa, S.; Matsumoto, J.; Kakutani, N.; Furihata, T.; Shirakawa, R.; Katayama, T.; Nakajima, T.; Yamanashi, K.; Obata, Y.; Nakano, I.; Tsuda, M.; Saito, A.; Fukushima, A.; Yokota, T.; Nio-Kobayashi, J.; Yasui, H.; Higashikawa, K.; Kuge, Y.; Anzai, T.; Sabe, H.; Kinugawa, S. Inhibition of xanthine oxidase in the acute phase of myocardial infarction prevents skeletal muscle abnormalities and exercise intolerance. *Cardiovasc Res*; 2020.

[200] Lee, T. M.; Lin, S. Z.; Chang, N. C. Effects of urate-lowering agents on arrhythmia vulnerability in post-infarcted rat hearts. *J Pharmacol Sci* **131:**28-36; 2016.

[201] Wang, S.; Li, Y.; Song, X.; Wang, X.; Zhao, C.; Chen, A.; Yang, P. Febuxostat pretreatment attenuates myocardial ischemia/reperfusion injury via mitochondrial apoptosis. *J Transl Med* **13:**209; 2015.

[202] Magee, L. A.; Abalos, E.; von Dadelszen, P.; Sibai, B.; Easterling, T.; Walkinshaw, S.; Group, C. S. How to manage hypertension in pregnancy effectively. *Br J Clin Pharmacol* **72:**394-401; 2011.

[203] Taylor, A. L.; Lindenfeld, J.; Ziesche, S.; Walsh, M. N.; Mitchell, J. E.; Adams, K.; Tam, S. W.; Ofili, E.; Sabolinski, M. L.; Worcel, M.; Cohn, J. N. Outcomes by gender in the African-American Heart Failure Trial. *J Am Coll Cardiol* **48:**2263-2267; 2006.

[204] Taylor, A. L.; Ziesche, S.; Yancy, C.; Carson, P.; D'Agostino, R., Jr.; Ferdinand, K.; Taylor, M.; Adams, K.; Sabolinski, M.; Worcel, M.; Cohn, J. N. Combination of isosorbide dinitrate and hydralazine in blacks with heart failure. *N Engl J Med* **351:**2049-2057; 2004.

[205] Daiber, A.; Mulsch, A.; Hink, U.; Mollnau, H.; Warnholtz, A.; Oelze, M.; Munzel, T. The oxidative stress concept of nitrate tolerance and the antioxidant properties of hydralazine. *Am J Cardiol* **96:**25i-36i; 2005.

[206] Daiber, A.; Oelze, M.; Coldewey, M.; Kaiser, K.; Huth, C.; Schildknecht, S.; Bachschmid, M.; Nazirisadeh, Y.; Ullrich, V.; Mulsch, A.; Munzel, T.; Tsilimingas, N. Hydralazine is a powerful inhibitor of peroxynitrite formation as a possible explanation for its beneficial effects on prognosis in patients with congestive heart failure. *Biochem Biophys Res Commun* **338:**1865-1874; 2005.

[207] Mitrovic, V.; Hernandez, A. F.; Meyer, M.; Gheorghiade, M. Role of guanylate cyclase modulators in decompensated heart failure. *Heart Fail Rev* **14:**309-319; 2009.

[208] Mielniczuk, L. M.; Swiston, J. R.; Mehta, S. Riociguat: A Novel Therapeutic Option for Pulmonary Arterial Hypertension and Chronic Thromboembolic Pulmonary Hypertension. *Can J Cardiol* **30:**1233-1240; 2014.

[209] Di Lisa, F.; Bernardi, P. Mitochondria and ischemia-reperfusion injury of the heart: fixing a hole. *Cardiovasc Res* **70:**191-199; 2006.

[210] Dao, V. T.; Casas, A. I.; Maghzal, G. J.; Seredenina, T.; Kaludercic, N.; Robledinos-Anton, N.; Di Lisa, F.; Stocker, R.; Ghezzi, P.; Jaquet, V.; Cuadrado, A.; Schmidt, H. H. Pharmacology and Clinical Drug Candidates in Redox Medicine. *Antioxid Redox Signal* **23:**1113-1129; 2015.

[211] Jorat, M. V.; Tabrizi, R.; Kolahdooz, F.; Akbari, M.; Salami, M.; Heydari, S. T.; Asemi, Z. The effects of coenzyme Q10 supplementation on biomarkers of inflammation and oxidative stress in among coronary artery disease: a systematic review and meta-analysis of randomized controlled trials. *Inflammopharmacology* **27:**233-248; 2019.

[212] Neuzil, J.; Widen, C.; Gellert, N.; Swettenham, E.; Zobalova, R.; Dong, L. F.; Wang, X. F.; Lidebjer, C.; Dalen, H.; Headrick, J. P.; Witting, P. K. Mitochondria transmit apoptosis signalling in cardiomyocyte-like cells and isolated hearts exposed to experimental ischemia-reperfusion injury. *Redox Rep* **12:**148-162; 2007.

[213] Davidson, S. M.; Yellon, D. M.; Murphy, M. P.; Duchen, M. R. Slow calcium waves and redox changes precede mitochondrial permeability transition pore opening in the intact heart during hypoxia and reoxygenation. *Cardiovasc Res* **93:**445-453; 2012.

[214] Gioscia-Ryan, R. A.; LaRocca, T. J.; Sindler, A. L.; Zigler, M. C.; Murphy, M. P.; Seals, D. R. Mitochondria-targeted antioxidant (MitoQ) ameliorates age-related arterial endothelial dysfunction in mice. *J Physiol* **592:**2549-2561; 2014.

[215] Laitano, O.; Ahn, B.; Patel, N.; Coblentz, P. D.; Smuder, A. J.; Yoo, J. K.; Christou, D. D.; Adhihetty, P. J.; Ferreira, L. F. Pharmacological targeting of mitochondrial reactive oxygen species counteracts diaphragm weakness in chronic heart failure. *J Appl Physiol (1985)* **120:**733-742; 2016.

[216] Schaller, S.; Paradis, S.; Ngoh, G. A.; Assaly, R.; Buisson, B.; Drouot, C.; Ostuni, M. A.; Lacapere, J. J.; Bassissi, F.; Bordet, T.; Berdeaux, A.; Jones, S. P.; Morin, D.; Pruss, R. M. TRO40303, a new cardioprotective compound, inhibits mitochondrial permeability transition. *J Pharmacol Exp Ther* **333:**696-706; 2010.

[217] Dai, W.; Shi, J.; Gupta, R. C.; Sabbah, H. N.; Hale, S. L.; Kloner, R. A. Bendavia, a mitochondria-targeting peptide, improves postinfarction cardiac function, prevents adverse left ventricular remodeling, and restores mitochondria-related gene expression in rats. *J Cardiovasc Pharmacol* **64:**543-553; 2014.

[218] Shi, J.; Dai, W.; Hale, S. L.; Brown, D. A.; Wang, M.; Han, X.; Kloner, R. A. Bendavia restores mitochondrial energy metabolism gene expression and suppresses cardiac fibrosis in the border zone of the infarcted heart. *Life Sci* **141:**170-178; 2015.

[219] Atar, D.; Arheden, H.; Berdeaux, A.; Bonnet, J. L.; Carlsson, M.; Clemmensen, P.; Cuvier, V.; Danchin, N.; Dubois-Rande, J. L.; Engblom, H.; Erlinge, D.; Firat, H.; Halvorsen, S.; Hansen, H. S.; Hauke, W.; Heiberg, E.; Koul, S.; Larsen, A. I.; Le Corvoisier, P.; Nordrehaug, J. E.; Paganelli, F.; Pruss, R. M.; Rousseau, H.; Schaller, S.; Sonou, G.; Tuseth, V.; Veys, J.; Vicaut, E.; Jensen, S. E. Effect of intravenous TRO40303 as an adjunct to primary percutaneous coronary intervention for acute ST-elevation myocardial infarction: MITOCARE study results. *Eur Heart J* **36:**112-119; 2015.

[220] Gibson, C. M.; Giugliano, R. P.; Kloner, R. A.; Bode, C.; Tendera, M.; Janosi, A.; Merkely, B.; Godlewski, J.; Halaby, R.; Korjian, S.; Daaboul, Y.; Chakrabarti, A. K.; Spielman, K.; Neal, B. J.; Weaver, W. D. EMBRACE STEMI study: a Phase 2a trial to evaluate the safety, tolerability, and efficacy of intravenous MTP-131 on reperfusion injury in patients undergoing primary percutaneous coronary intervention. *Eur Heart J* **37:**1296-1303; 2016.

[221] Tanno, M.; Kuno, A.; Horio, Y.; Miura, T. Emerging beneficial roles of sirtuins in heart failure. *Basic Res Cardiol* **107:**273; 2012.

[222] Hubbard, B. P.; Gomes, A. P.; Dai, H.; Li, J.; Case, A. W.; Considine, T.; Riera, T. V.; Lee, J. E.; E, S. Y.; Lamming, D. W.; Pentelute, B. L.; Schuman, E. R.; Stevens, L. A.; Ling, A. J.; Armour, S. M.; Michan, S.; Zhao, H.; Jiang, Y.; Sweitzer, S. M.; Blum, C. A.; Disch, J. S.; Ng, P. Y.; Howitz, K. T.; Rolo, A. P.; Hamuro, Y.; Moss, J.; Perni, R. B.; Ellis, J. L.; Vlasuk, G. P.; Sinclair, D. A. Evidence for a common mechanism of SIRT1 regulation by allosteric activators. *Science* **339:**1216-1219; 2013.

[223] Prysyazhna, O.; Wolhuter, K.; Switzer, C.; Santos, C.; Yang, X.; Lynham, S.; Shah, A. M.; Eaton, P.; Burgoyne, J. R. Blood Pressure-Lowering by the Antioxidant Resveratrol Is Counterintuitively Mediated by Oxidation of cGMP-Dependent Protein Kinase. *Circulation* **140:**126-137; 2019.

[224] Halliwell, B. Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? *Arch Biochem Biophys* **476:**107-112; 2008.

[225] Gruber, J.; Tang, S. Y.; Halliwell, B. Evidence for a trade-off between survival and fitness caused by resveratrol treatment of Caenorhabditis elegans. *Ann N Y Acad Sci* **1100:**530-542; 2007.
[226] Kanner, J. Polyphenols by Generating H2O2, Affect Cell Redox Signaling, Inhibit PTPs and Activate Nrf2 Axis for Adaptation and Cell Surviving: In Vitro, In Vivo and Human Health. *Antioxidants (Basel)* **9**; 2020.
[227] Calabrese, E. J. Preconditioning is hormesis part I: Documentation, dose-response features and mechanistic foundations. *Pharmacol Res* **110:**242-264; 2016.
[228] Zhang, L. Q.; Qi, G. X.; Jiang, D. M.; Tian, W.; Zou, J. L. Increased poly(ADP-ribosyl)ation in peripheral leukocytes and the reperfused myocardium tissue of rats with ischemia/reperfusion injury: prevention by 3-aminobenzamide treatment. *Shock* **37:**492-500; 2012.
[229] Schriewer, J. M.; Peek, C. B.; Bass, J.; Schumacker, P. T. ROS-mediated PARP activity undermines mitochondrial function after permeability transition pore opening during myocardial ischemia-reperfusion. *J Am Heart Assoc* **2:**e000159; 2013.
[230] Ungvari, Z.; Gupte, S. A.; Recchia, F. A.; Batkai, S.; Pacher, P. Role of oxidative-nitrosative stress and downstream pathways in various forms of cardiomyopathy and heart failure. *Curr Vasc Pharmacol* **3:**221-229; 2005.
[231] Zingarelli, B.; Salzman, A. L.; Szabo, C. Genetic disruption of poly (ADP-ribose) synthetase inhibits the expression of P-selectin and intercellular adhesion molecule-1 in myocardial ischemia/reperfusion injury. *Circ Res* **83:**85-94; 1998.
[232] Zingarelli, B.; Cuzzocrea, S.; Zsengeller, Z.; Salzman, A. L.; Szabo, C. Protection against myocardial ischemia and reperfusion injury by 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase. *Cardiovasc Res* **36:**205-215; 1997.
[233] Palfi, A.; Toth, A.; Hanto, K.; Deres, P.; Szabados, E.; Szereday, Z.; Kulcsar, G.; Kalai, T.; Hideg, K.; Gallyas, F., Jr.; Sumegi, B.; Toth, K.; Halmosi, R. PARP inhibition prevents postinfarction myocardial remodeling and heart failure via the protein kinase C/glycogen synthase kinase-3beta pathway. *J Mol Cell Cardiol* **41:**149-159; 2006.
[234] Wahlberg, E.; Karlberg, T.; Kouznetsova, E.; Markova, N.; Macchiarulo, A.; Thorsell, A. G.; Pol, E.; Frostell, A.; Ekblad, T.; Oncu, D.; Kull, B.; Robertson, G. M.; Pellicciari, R.; Schuler, H.; Weigelt, J. Family-wide chemical profiling and structural analysis of PARP and tankyrase inhibitors. *Nat Biotechnol* **30:**283-288; 2012.
[235] Liu, T.; Takimoto, E.; Dimaano, V. L.; DeMazumder, D.; Kettlewell, S.; Smith, G.; Sidor, A.; Abraham, T. P.; O'Rourke, B. Inhibiting mitochondrial Na+/Ca2+ exchange prevents sudden death in a Guinea pig model of heart failure. *Circ Res* **115:**44-54; 2014.
[236] Figtree, G. A.; Keyvan Karimi, G.; Liu, C. C.; Rasmussen, H. H. Oxidative regulation of the Na(+)-K(+) pump in the cardiovascular system. *Free Radic Biol Med* **53:**2263-2268; 2012.
[237] Blackstone, E.; Morrison, M.; Roth, M. B. H2S induces a suspended animation-like state in mice. *Science* **308:**518; 2005.
[238] Andreadou, I.; Schulz, R.; Papapetropoulos, A.; Turan, B.; Ytrehus, K.; Ferdinandy, P.; Daiber, A.; Di Lisa, F. The role of mitochondrial reactive oxygen species, NO and H2 S in ischaemia/reperfusion injury and cardioprotection. *J Cell Mol Med* **24:**6510-6522; 2020.
[239] Andreadou, I.; Iliodromitis, E. K.; Rassaf, T.; Schulz, R.; Papapetropoulos, A.; Ferdinandy, P. The role of gasotransmitters NO, H2S and CO in myocardial ischaemia/reperfusion injury and cardioprotection by preconditioning, postconditioning and remote conditioning. *Br J Pharmacol* **172:**1587-1606; 2015.
[240] Bibli, S. I.; Andreadou, I.; Chatzianastasiou, A.; Tzimas, C.; Sanoudou, D.; Kranias, E.; Brouckaert, P.; Coletta, C.; Szabo, C.; Kremastinos, D. T.; Iliodromitis, E. K.;

Papapetropoulos, A. Cardioprotection by H2S engages a cGMP-dependent protein kinase G/phospholamban pathway. *Cardiovasc Res* **106:**432-442; 2015.

[241] Bibli, S. I.; Papapetropoulos, A.; Iliodromitis, E. K.; Daiber, A.; Randriamboavonjy, V.; Steven, S.; Brouckaert, P.; Chatzianastasiou, A.; Kypreos, K. E.; Hausenloy, D. J.; Fleming, I.; Andreadou, I. Nitroglycerine limits infarct size through S-nitrosation of cyclophilin D: a novel mechanism for an old drug. *Cardiovasc Res* **115:**625-636; 2019.

[242] Antoniades, C.; Shirodaria, C.; Warrick, N.; Cai, S.; de Bono, J.; Lee, J.; Leeson, P.; Neubauer, S.; Ratnatunga, C.; Pillai, R.; Refsum, H.; Channon, K. M. 5-methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and endothelial nitric oxide synthase coupling. *Circulation* **114:**1193-1201; 2006.

[243] Moens, A. L.; Ketner, E. A.; Takimoto, E.; Schmidt, T. S.; O'Neill, C. A.; Wolin, M. S.; Alp, N. J.; Channon, K. M.; Kass, D. A. Bi-modal dose-dependent cardiac response to tetrahydrobiopterin in pressure-overload induced hypertrophy and heart failure. *J Mol Cell Cardiol* **51:**564-569; 2011.

[244] Nishijima, Y.; Sridhar, A.; Bonilla, I.; Velayutham, M.; Khan, M.; Terentyeva, R.; Li, C.; Kuppusamy, P.; Elton, T. S.; Terentyev, D.; Gyorke, S.; Zweier, J. L.; Cardounel, A. J.; Carnes, C. A. Tetrahydrobiopterin depletion and NOS2 uncoupling contribute to heart failure-induced alterations in atrial electrophysiology. *Cardiovasc Res* **91:**71-79; 2011.

[245] Fraccarollo, D.; Widder, J. D.; Galuppo, P.; Thum, T.; Tsikas, D.; Hoffmann, M.; Ruetten, H.; Ertl, G.; Bauersachs, J. Improvement in left ventricular remodeling by the endothelial nitric oxide synthase enhancer AVE9488 after experimental myocardial infarction. *Circulation* **118:**818-827; 2008.

[246] Westermann, D.; Riad, A.; Richter, U.; Jager, S.; Savvatis, K.; Schuchardt, M.; Bergmann, N.; Tolle, M.; Nagorsen, D.; Gotthardt, M.; Schultheiss, H. P.; Tschope, C. Enhancement of the endothelial NO synthase attenuates experimental diastolic heart failure. *Basic Res Cardiol* **104:**499-509; 2009.

[247] Frantz, S.; Adamek, A.; Fraccarollo, D.; Tillmanns, J.; Widder, J. D.; Dienesch, C.; Schafer, A.; Podolskaya, A.; Held, M.; Ruetten, H.; Ertl, G.; Bauersachs, J. The eNOS enhancer AVE 9488: a novel cardioprotectant against ischemia reperfusion injury. *Basic Res Cardiol* **104:**773-779; 2009.

[248] Chen, C. A.; Wang, T. Y.; Varadharaj, S.; Reyes, L. A.; Hemann, C.; Talukder, M. A.; Chen, Y. R.; Druhan, L. J.; Zweier, J. L. S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. *Nature* **468:**1115-1118; 2010.

[249] Zweier, J. L.; Chen, C. A.; Druhan, L. J. S-glutathionylation reshapes our understanding of endothelial nitric oxide synthase uncoupling and nitric oxide/reactive oxygen species-mediated signaling. *Antioxid Redox Signal* **14:**1769-1775; 2011.

[250] Chen, C. A.; De Pascali, F.; Basye, A.; Hemann, C.; Zweier, J. L. Redox modulation of endothelial nitric oxide synthase by glutaredoxin-1 through reversible oxidative post-translational modification. *Biochemistry* **52:**6712-6723; 2013.

[251] Wollin, S. D.; Jones, P. J. Alpha-lipoic acid and cardiovascular disease. *J Nutr* **133:**3327-3330; 2003.

[252] Gomes, K. M.; Bechara, L. R.; Lima, V. M.; Ribeiro, M. A.; Campos, J. C.; Dourado, P. M.; Kowaltowski, A. J.; Mochly-Rosen, D.; Ferreira, J. C. Aldehydic load and aldehyde dehydrogenase 2 profile during the progression of post-myocardial infarction cardiomyopathy: benefits of Alda-1. *Int J Cardiol* **179:**129-138; 2015.

[253] Wang, J.; Wang, H.; Hao, P.; Xue, L.; Wei, S.; Zhang, Y.; Chen, Y. Inhibition of aldehyde dehydrogenase 2 by oxidative stress is associated with cardiac dysfunction in diabetic rats. *Mol Med* **17:**172-179; 2011.

[254] Bierhaus, A.; Chevion, S.; Chevion, M.; Hofmann, M.; Quehenberger, P.; Illmer, T.; Luther, T.; Berentshtein, E.; Tritschler, H.; Muller, M.; Wahl, P.; Ziegler, R.; Nawroth, P. P. Advanced glycation end product-induced activation of NF-kappaB is suppressed by alpha-lipoic acid in cultured endothelial cells. *Diabetes* **46:**1481-1490; 1997.

[255] Heitzer, T.; Finckh, B.; Albers, S.; Krohn, K.; Kohlschutter, A.; Meinertz, T. Beneficial effects of alpha-lipoic acid and ascorbic acid on endothelium-dependent, nitric oxide-mediated vasodilation in diabetic patients: relation to parameters of oxidative stress. *Free Radic Biol Med* **31:**53-61; 2001.

[256] Mieyal, J. J.; Gallogly, M. M.; Qanungo, S.; Sabens, E. A.; Shelton, M. D. Molecular mechanisms and clinical implications of reversible protein S-glutathionylation. *Antioxid Redox Signal* **10:**1941-1988; 2008.

[257] Berndt, C.; Lillig, C. H.; Holmgren, A. Thiol-based mechanisms of the thioredoxin and glutaredoxin systems: implications for diseases in the cardiovascular system. *Am J Physiol Heart Circ Physiol* **292:**H1227-1236; 2007.

[258] Kaludercic, N.; Mialet-Perez, J.; Paolocci, N.; Parini, A.; Di Lisa, F. Monoamine oxidases as sources of oxidants in the heart. *J Mol Cell Cardiol* **73C:**34-42; 2014.

[259] Zhang, M.; Perino, A.; Ghigo, A.; Hirsch, E.; Shah, A. M. NADPH oxidases in heart failure: poachers or gamekeepers? *Antioxid Redox Signal* **18:**1024-1041; 2013.

[260] Octavia, Y.; Brunner-La Rocca, H. P.; Moens, A. L. NADPH oxidase-dependent oxidative stress in the failing heart: From pathogenic roles to therapeutic approach. *Free Radic Biol Med* **52:**291-297; 2012.

[261] Altenhofer, S.; Kleikers, P. W.; Radermacher, K. A.; Scheurer, P.; Rob Hermans, J. J.; Schiffers, P.; Ho, H.; Wingler, K.; Schmidt, H. H. The NOX toolbox: validating the role of NADPH oxidases in physiology and disease. *Cell Mol Life Sci* **69:**2327-2343; 2012.

[262] Zhang, Y.; Murugesan, P.; Huang, K.; Cai, H. NADPH oxidases and oxidase crosstalk in cardiovascular diseases: novel therapeutic targets. *Nat Rev Cardiol* **17:**170-194; 2020.

[263] Kleikers, P. W.; Wingler, K.; Hermans, J. J.; Diebold, I.; Altenhofer, S.; Radermacher, K. A.; Janssen, B.; Gorlach, A.; Schmidt, H. H. NADPH oxidases as a source of oxidative stress and molecular target in ischemia/reperfusion injury. *J Mol Med (Berl)* **90:**1391-1406; 2012.

[264] Matsushima, S.; Kuroda, J.; Ago, T.; Zhai, P.; Ikeda, Y.; Oka, S.; Fong, G. H.; Tian, R.; Sadoshima, J. Broad suppression of NADPH oxidase activity exacerbates ischemia/reperfusion injury through inadvertent downregulation of hypoxia-inducible factor-1alpha and upregulation of peroxisome proliferator-activated receptor-alpha. *Circ Res* **112:**1135-1149; 2013.

[265] O'Neill, S.; Brault, J.; Stasia, M. J.; Knaus, U. G. Genetic disorders coupled to ROS deficiency. *Redox Biol* **6:**135-156; 2015.

[266] Strom, J.; Chen, Q. M. Loss of Nrf2 promotes rapid progression to heart failure following myocardial infarction. *Toxicol Appl Pharmacol* **327:**52-58; 2017.

[267] Howden, R. Nrf2 and cardiovascular defense. *Oxid Med Cell Longev* **2013:**104308; 2013.

[268] Wojcik, T.; Szczesny, E.; Chlopicki, S. Detrimental effects of chemotherapeutics and other drugs on the endothelium: A call for endothelial toxicity profiling. *Pharmacol Rep* **67:**811-817; 2015.

[269] Kroller-Schon, S.; Steven, S.; Kossmann, S.; Scholz, A.; Daub, S.; Oelze, M.; Xia, N.; Hausding, M.; Mikhed, Y.; Zinssius, E.; Mader, M.; Stamm, P.; Treiber, N.; Scharffetter-Kochanek, K.; Li, H.; Schulz, E.; Wenzel, P.; Munzel, T.; Daiber, A. Molecular mechanisms of the crosstalk between mitochondria and NADPH oxidase through reactive oxygen species-studies in white blood cells and in animal models. *Antioxid Redox Signal* **20:**247-266; 2014.

[270] Daiber, A. Redox signaling (cross-talk) from and to mitochondria involves mitochondrial pores and reactive oxygen species. *Biochim Biophys Acta* **1797:**897-906; 2010.
[271] van Nierop, B. J.; van Assen, H. C.; van Deel, E. D.; Niesen, L. B.; Duncker, D. J.; Strijkers, G. J.; Nicolay, K. Phenotyping of left and right ventricular function in mouse models of compensated hypertrophy and heart failure with cardiac MRI. *PLoS One* **8:**e55424; 2013.
[272] Gong, X.; Yu, Z.; Huang, Z.; Xie, L.; Zhou, N.; Wang, J.; Liang, Y.; Qin, S.; Nie, Z.; Wei, L.; Li, Z.; Wang, S.; Su, Y.; Ge, J. Protective effects of cardiac resynchronization therapy in a canine model with experimental heart failure by improving mitochondrial function: a mitochondrial proteomics study. *J Interv Card Electrophysiol*; 2020.
[273] Zhang, Z.; Ding, L.; Jin, Z.; Gao, G.; Li, H.; Zhang, L.; Zhang, L.; Lu, X.; Hu, L.; Lu, B.; Yu, X.; Hu, T. Nebivolol protects against myocardial infarction injury via stimulation of beta 3-adrenergic receptors and nitric oxide signaling. *PLoS One* **9:**e98179; 2014.
[274] Kim, E. N.; Kim, C. J.; Kim, S. R.; Song, J. A.; Choe, H.; Kim, K. B.; Choi, J. S.; Oh, S. J. High serum CRP influences myocardial miRNA profiles in ischemia-reperfusion injury of rat heart. *PLoS One* **14:**e0216610; 2019.
[275] Munzel, T.; Daiber, A.; Mulsch, A. Explaining the phenomenon of nitrate tolerance. *Circ Res* **97:**618-628; 2005.
[276] LeLeiko, R. M.; Vaccari, C. S.; Sola, S.; Merchant, N.; Nagamia, S. H.; Thoenes, M.; Khan, B. V. Usefulness of elevations in serum choline and free F2)-isoprostane to predict 30-day cardiovascular outcomes in patients with acute coronary syndrome. *Am J Cardiol* **104:**638-643; 2009.
[277] Infanger, D. W.; Cao, X.; Butler, S. D.; Burmeister, M. A.; Zhou, Y.; Stupinski, J. A.; Sharma, R. V.; Davisson, R. L. Silencing nox4 in the paraventricular nucleus improves myocardial infarction-induced cardiac dysfunction by attenuating sympathoexcitation and periinfarct apoptosis. *Circ Res* **106:**1763-1774; 2010.
[278] Looi, Y. H.; Grieve, D. J.; Siva, A.; Walker, S. J.; Anilkumar, N.; Cave, A. C.; Marber, M.; Monaghan, M. J.; Shah, A. M. Involvement of Nox2 NADPH oxidase in adverse cardiac remodeling after myocardial infarction. *Hypertension* **51:**319-325; 2008.
[279] Somasuntharam, I.; Boopathy, A. V.; Khan, R. S.; Martinez, M. D.; Brown, M. E.; Murthy, N.; Davis, M. E. Delivery of Nox2-NADPH oxidase siRNA with polyketal nanoparticles for improving cardiac function following myocardial infarction. *Biomaterials* **34:**7790-7798; 2013.
[280] Frantz, S.; Brandes, R. P.; Hu, K.; Rammelt, K.; Wolf, J.; Scheuermann, H.; Ertl, G.; Bauersachs, J. Left ventricular remodeling after myocardial infarction in mice with targeted deletion of the NADPH oxidase subunit gp91PHOX. *Basic Res Cardiol* **101:**127-132; 2006.
[281] Kloner, R. A.; Hale, S. L.; Dai, W.; Gorman, R. C.; Shuto, T.; Koomalsingh, K. J.; Gorman, J. H., 3rd; Sloan, R. C.; Frasier, C. R.; Watson, C. A.; Bostian, P. A.; Kypson, A. P.; Brown, D. A. Reduction of ischemia/reperfusion injury with bendavia, a mitochondria-targeting cytoprotective Peptide. *J Am Heart Assoc* **1:**e001644; 2012.
[282] Carpi, A.; Menabo, R.; Kaludercic, N.; Pelicci, P.; Di Lisa, F.; Giorgio, M. The cardioprotective effects elicited by p66(Shc) ablation demonstrate the crucial role of mitochondrial ROS formation in ischemia/reperfusion injury. *Biochim Biophys Acta* **1787:**774-780; 2009.
[283] Akizuki, S.; Yoshida, S.; Chambers, D. E.; Eddy, L. J.; Parmley, L. F.; Yellon, D. M.; Downey, J. M. Infarct size limitation by the xanthine oxidase inhibitor, allopurinol, in closed-chest dogs with small infarcts. *Cardiovasc Res* **19:**686-692; 1985.

[284] Werns, S. W.; Shea, M. J.; Mitsos, S. E.; Dysko, R. C.; Fantone, J. C.; Schork, M. A.; Abrams, G. D.; Pitt, B.; Lucchesi, B. R. Reduction of the size of infarction by allopurinol in the ischemic-reperfused canine heart. *Circulation* **73:**518-524; 1986.
[285] Engberding, N.; Spiekermann, S.; Schaefer, A.; Heineke, A.; Wiencke, A.; Muller, M.; Fuchs, M.; Hilfiker-Kleiner, D.; Hornig, B.; Drexler, H.; Landmesser, U. Allopurinol attenuates left ventricular remodeling and dysfunction after experimental myocardial infarction: a new action for an old drug? *Circulation* **110:**2175-2179; 2004.
[286] Iida, S.; Chu, Y.; Francis, J.; Weiss, R. M.; Gunnett, C. A.; Faraci, F. M.; Heistad, D. D. Gene transfer of extracellular superoxide dismutase improves endothelial function in rats with heart failure. *Am J Physiol Heart Circ Physiol* **289:**H525-532; 2005.
[287] Iida, S.; Chu, Y.; Weiss, R. M.; Kang, Y. M.; Faraci, F. M.; Heistad, D. D. Vascular effects of a common gene variant of extracellular superoxide dismutase in heart failure. *Am J Physiol Heart Circ Physiol* **291:**H914-920; 2006.
[288] Liu, X.; Wei, J.; Peng, D. H.; Layne, M. D.; Yet, S. F. Absence of heme oxygenase-1 exacerbates myocardial ischemia/reperfusion injury in diabetic mice. *Diabetes* **54:**778-784; 2005.
[289] Chen, Z.; Chua, C. C.; Gao, J.; Chua, K. W.; Ho, Y. S.; Hamdy, R. C.; Chua, B. H. Prevention of ischemia/reperfusion-induced cardiac apoptosis and injury by melatonin is independent of glutathione peroxdiase 1. *J Pineal Res* **46:**235-241; 2009.
[290] Di Minno, A.; Turnu, L.; Porro, B.; Squellerio, I.; Cavalca, V.; Tremoli, E.; Di Minno, M. N. 8-Hydroxy-2-deoxyguanosine levels and heart failure: A systematic review and meta-analysis of the literature. *Nutr Metab Cardiovasc Dis* **27:**201-208; 2017.
[291] Rivera, M.; Rosello-Lleti, E.; Garcia de Burgos, F.; Bertomeu, V.; Paya, R.; Cortes, R.; Martinez-Dolz, L.; Jordan, A.; Perez-Bosca, J. L.; Salvador, A.; Marin, F.; Sogorb, F.; Valero, R.; Miro, V.; Portoles, M. [8-hydroxy-2'-deoxyguanosine and lipid peroxidation in patients with heart failure]. *Rev Esp Cardiol* **59:**1140-1145; 2006.
[292] Schwedhelm, E.; Bartling, A.; Lenzen, H.; Tsikas, D.; Maas, R.; Brummer, J.; Gutzki, F. M.; Berger, J.; Frolich, J. C.; Boger, R. H. Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: a matched case-control study. *Circulation* **109:**843-848; 2004.
[293] Sties, S. W.; Andreato, L. V.; de Carvalho, T.; Gonzales, A. I.; Angarten, V. G.; Ulbrich, A. Z.; de Mara, L. S.; Netto, A. S.; da Silva, E. L.; Andrade, A. Influence of exercise on oxidative stress in patients with heart failure. *Heart Fail Rev* **23:**225-235; 2018.
[294] Pignatelli, P.; Pastori, D.; Carnevale, R.; Farcomeni, A.; Cangemi, R.; Nocella, C.; Bartimoccia, S.; Vicario, T.; Saliola, M.; Lip, G. Y.; Violi, F. Serum NOX2 and urinary isoprostanes predict vascular events in patients with atrial fibrillation. *Thromb Haemost* **113:**617-624; 2015.
[295] Calvieri, C.; Tanzilli, G.; Bartimoccia, S.; Cangemi, R.; Arrivi, A.; Dominici, M.; Cammisotto, V.; Viceconte, N.; Mangieri, E.; Frati, G.; Violi, F. Interplay between Oxidative Stress and Platelet Activation in Coronary Thrombus of STEMI Patients. *Antioxidants (Basel)* **7**; 2018.
[296] Pastori, D.; Carnevale, R.; Bartimoccia, S.; Nocella, C.; Tanzilli, G.; Cangemi, R.; Vicario, T.; Catena, M.; Violi, F.; Pignatelli, P. Does Mediterranean Diet Reduce Cardiovascular Events and Oxidative Stress in Atrial Fibrillation? *Antioxid Redox Signal* **23:**682-687; 2015.
[297] Vassalle, C.; Bianchi, S.; Battaglia, D.; Landi, P.; Bianchi, F.; Carpeggiani, C. Elevated levels of oxidative stress as a prognostic predictor of major adverse cardiovascular events in patients with coronary artery disease. *J Atheroscler Thromb* **19:**712-717; 2012.
[298] Wang, B.; Pan, J.; Wang, L.; Zhu, H.; Yu, R.; Zou, Y. Associations of plasma 8-isoprostane levels with the presence and extent of coronary stenosis in patients with coronary artery disease. *Atherosclerosis* **184:**425-430; 2006.

[299] Chu, C. S.; Lee, K. T.; Cheng, K. H.; Lee, M. Y.; Kuo, H. F.; Lin, T. H.; Su, H. M.; Voon, W. C.; Sheu, S. H.; Lai, W. T. Postchallenge responses of nitrotyrosine and TNF-alpha during 75-g oral glucose tolerance test are associated with the presence of coronary artery diseases in patients with prediabetes. *Cardiovasc Diabetol* **11:**21; 2012.
[300] Vita, J. A.; Brennan, M. L.; Gokce, N.; Mann, S. A.; Goormastic, M.; Shishehbor, M. H.; Penn, M. S.; Keaney, J. F., Jr.; Hazen, S. L. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. *Circulation* **110:**1134-1139; 2004.
[301] Tang, W. H.; Iqbal, N.; Wu, Y.; Hazen, S. L. Usefulness of cardiac biomarker score for risk stratification in stable patients undergoing elective cardiac evaluation across glycemic status. *Am J Cardiol* **111:**465-470; 2013.
[302] Baldus, S.; Heeschen, C.; Meinertz, T.; Zeiher, A. M.; Eiserich, J. P.; Munzel, T.; Simoons, M. L.; Hamm, C. W.; Investigators, C. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. *Circulation* **108:**1440-1445; 2003.
[303] Nicholls, S. J.; Tang, W. H.; Brennan, D.; Brennan, M. L.; Mann, S.; Nissen, S. E.; Hazen, S. L. Risk prediction with serial myeloperoxidase monitoring in patients with acute chest pain. *Clin Chem* **57:**1762-1770; 2011.
[304] Cheng, M. L.; Chen, C. M.; Gu, P. W.; Ho, H. Y.; Chiu, D. T. Elevated levels of myeloperoxidase, white blood cell count and 3-chlorotyrosine in Taiwanese patients with acute myocardial infarction. *Clin Biochem* **41:**554-560; 2008.

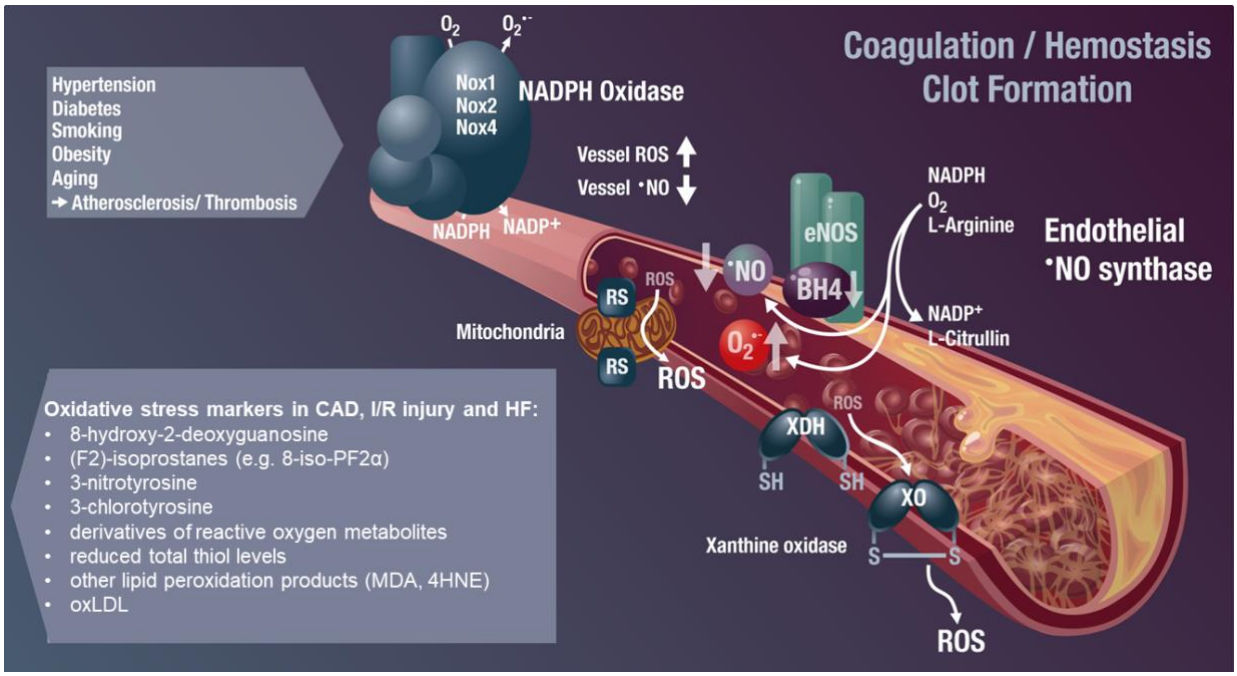

Figure 1. Classical cardiovascular risk factors, primary and secondary ROS sources and markers of oxidative stress. The scheme illustrates the activation of primary vascular ROS sources such as NADPH oxidases (Nox1, Nox2 and Nox4) and mitochondria (via mitochondrial redox switches [RS]), also by activated immune cells. The ROS from primary sources will generate secondary ROS sources such as xanthine oxidase (conversion of the dehydrogenase [XDH] to the oxidase [XO] form) and uncoupled eNOS (oxidative depletion of tetrahydrobiopterin [BH4] and other redox switches), all of which contributes to vascular dysfunction (for review see [269, 270]). Mitochondrial ROS formation and release can be stimulated by thiol-oxidation in different mitochondrial structures (e.g. mitochondrial permeability transition pore constituents such as cyclophilin D, $p66^{shc}$ or monoamine oxidases); xanthine dehydrogenase is converted to the oxidase form by oxidation of critical thiol residues; the protective action of eNOS to produce •NO is switched to adverse superoxide formation by oxidative depletion of BH4, adverse phosphorylation by redox-activated kinases, S-glutathionylation or oxidative disruption of the zinc-sulfur-complex at the dimer binding interface, called the "uncoupling" process. These changes (increased vascular oxidative stress and release of inflammatory signaling molecules) will lead to endothelial cell activation and priming for the adhesion of additional immune cells as well as platelets and switch the vasodilatory, antiaggregatory and antiatherosclerotic phenotype of the endothelium to a vasoconstrictory, proaggregatory and proatherosclerotic one. These processes lead to late-stage cardiovascular complications such as atherosclerosis with plaque formation and thrombosis that are associated with accumulation of the mentioned oxidative stress markers. Modified from [92] with permission.

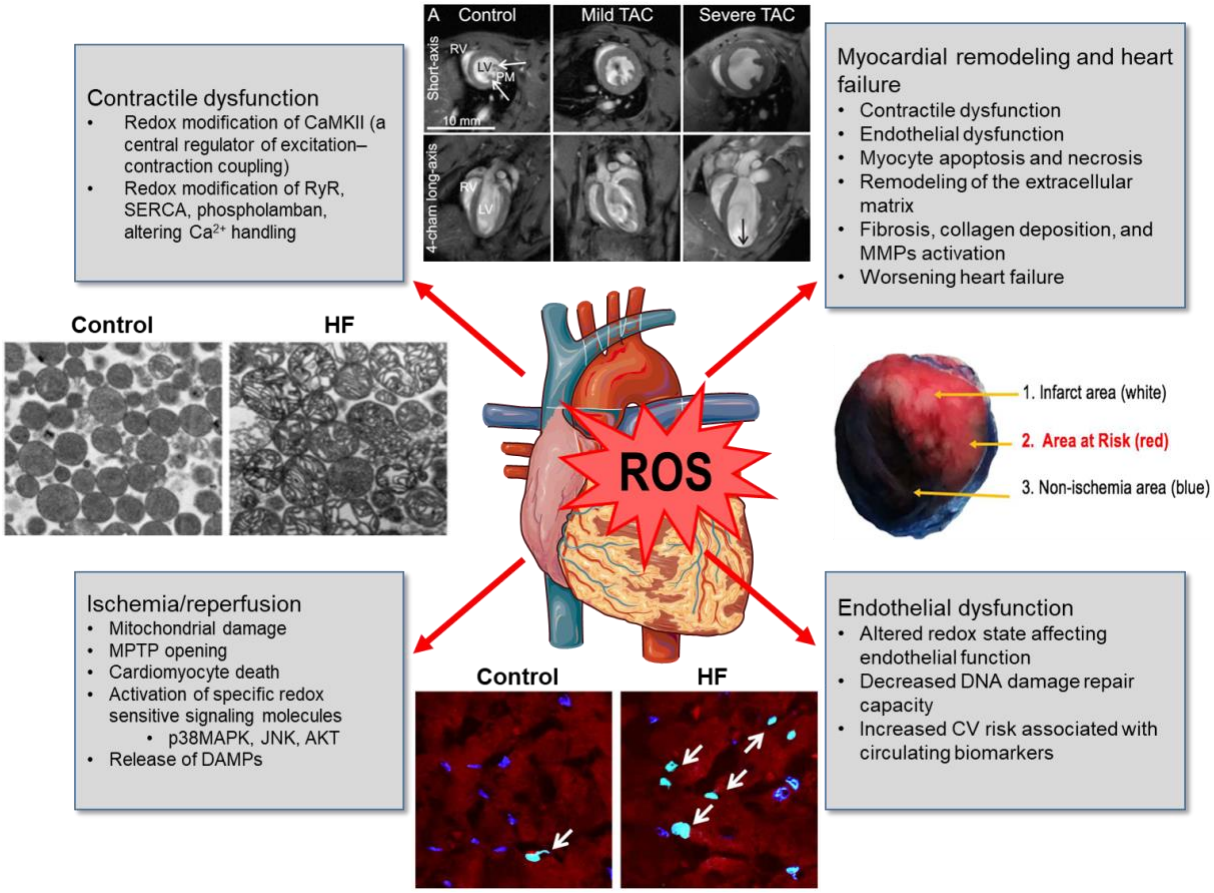

Figure 2. Involvement of ROS in pathophysiological processes of myocardial infarction (MI) and heart failure and classical imaging techniques. I/R induced ROS formation contributes to adverse processes such as contractile dysfunction, remodeling, mitochondrial damage and endothelial dysfunction. The images represent examples of classical imaging techniques to characterize cardiac I/R damage and heart failure. (**Upper image**) End diastolic short-axis and long-axis images from control mice and mice subjected to a mild and severe transverse aortic constriction (TAC) causing heart failure as assessed by magnetic resonance imaging. Left ventricle (LV), right ventricle (RV) and the papillary muscles (PM) are shown. Reproduced from [271] with permission. (**Left image**) Mitochondrial swelling, disappearing of mitochondrial crista, and decrease of electronic density was observed in HF group was detected by transmission electronic microscopy. Reproduced from [272] with permission. (**Lower image**) TUNEL-stained heart sections from sham group and MI group provide a read-out of myocardial apoptosis (arrows indicate apoptotic cells). Reproduced from [273] with permission. (**Right image**) Evans blue and 2,3,5-triphenyltetrazolium chloride (TTC) staining of the infarcted heart. Reproduced from [274] with permission. Central heart cartoon taken from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License.

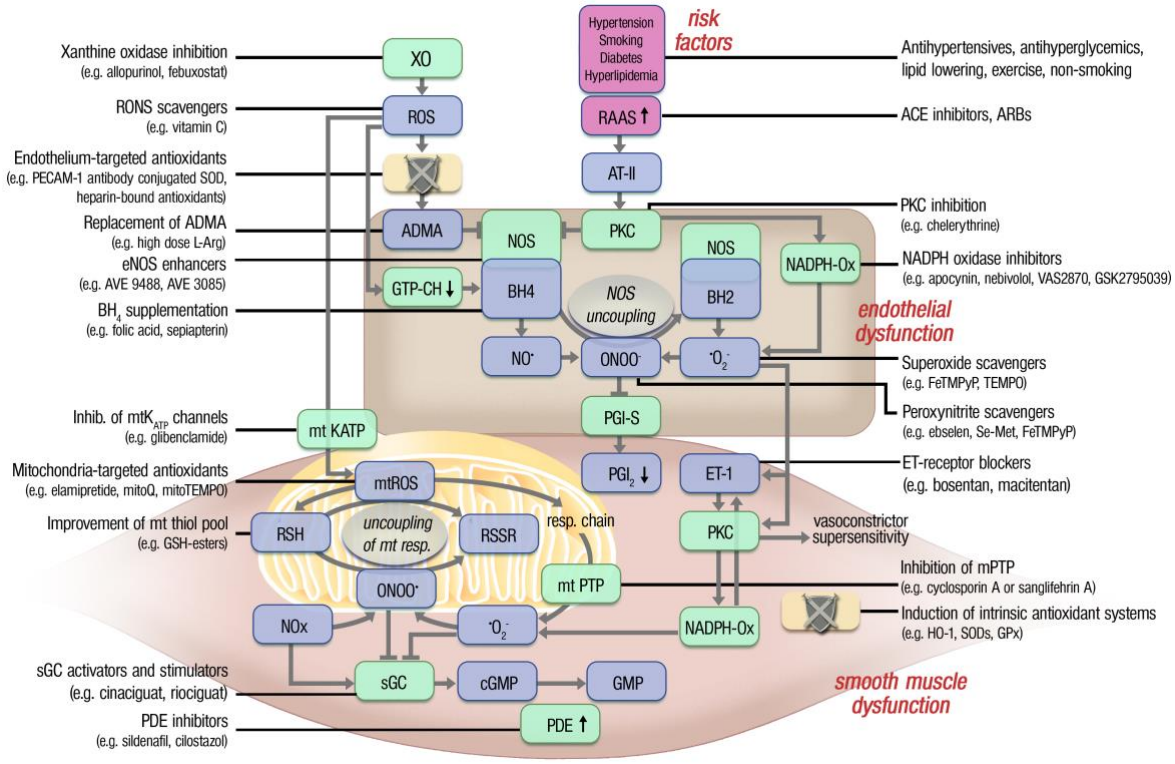

Figure 3. Scheme illustrating the mechanisms underlying vascular (endothelial) dysfunction by oxidative stress [275] and their modulation by clinical and experimental interventions. Known cardiovascular risk factors (e.g. smoking, hypertension, hyperlipidemia, diabetes) activate the renin-angiotensin-aldosterone-system (RAAS) leading to elevated angiotensin-II levels as well as increased endothelial and smooth muscle superoxide ($O_2^{\bullet-}$) formation from NADPH oxidase activation by protein kinase C (PKC) and from the mitochondria. Superoxide reacts with $^{\bullet}NO$, thereby decreases $^{\bullet}NO$ bioavailability in favor of peroxynitrite ($ONOO^-$) formation. Peroxynitrite causes uncoupling of endothelial NOS due to oxidation of tetrahydrobiopterin (BH_4) to BH_2 and nitration/inactivation of prostacyclin synthase (PGI_2S). Direct proteasome-dependent degradation of the BH_4 synthase GTP-cyclohydrolase (GTP-CH) further contributes to eNOS uncoupling. Uncoupled NOS produces superoxide instead of $^{\bullet}NO$ and nitrated PGI_2S produces no prostacyclin (PGI_2) but activated cyclooxygenase-2 (due to increased peroxide tone) generates vasoconstrictive prostaglandin H_2. Inhibition of smooth muscle soluble guanylyl cyclase (sGC) by superoxide and peroxynitrite contributes to vascular dysfunction as well as increased inactivation of cyclic GMP (cGMP) by phosphodiesterases (PDE) and oxidative stress increases the sensitivity to vasoconstrictors such as endothelin-1 (ET-1), which vice versa increases NADPH oxidase activity. Mitochondrial ROS formation is modulated by oxidative activation of ATP-dependent potassium channels (K_{ATP}) leading to altered mitochondrial membrane potential and permeability. Upon uncoupling of mitochondrial respiratory complexes, the mitochondrial permeability transition pore (mPTP) may be oxidatively opened allowing mtROS to escape to the cytosol activating the PKC-NADPH-Ox system. Reported (non-)pharmacological interventions are shown for the different mechanisms along with examples of experimental or clinical drugs. Modified and updated from Chen, Chen, Daiber et al. *Clin. Sci.* **2012** [44] with permission.

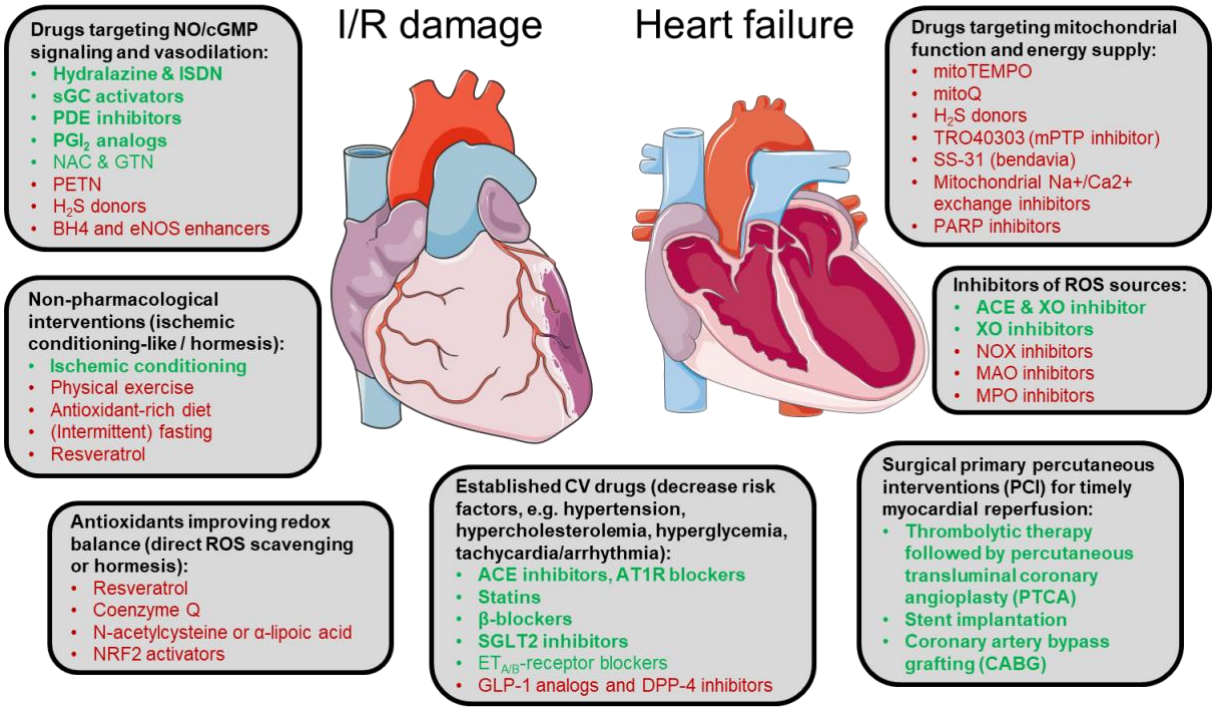

Figure 4. Summary of established and experimental therapies of I/R damage and heart failure including redox-based therapies. Clinically established therapies (green bold letters) represent treatments that were applied to larger human cohorts and proved beneficial in either I/R damage or heart failure. Green standard letters indicate therapies that were tested in larger cohorts but do not represent the recommended standard therapy of I/R damage and heart failure, or were tested in related cardiovascular diseases. Experimental therapies (red letters) represent treatment that are still at the preclinical stage, were only tested in small cohort studies or showed no clear benefit in patients. Abbreviations: NO, nitric oxide; cGMP, cyclic guanosine monophosphate; ISDN, isosorbide dinitrate; sGC, soluble guanylyl cyclase; PDE, phosphodiesterase; PGI_2, prostacyclin; NAC, N-acetylcysteine; GTN, nitroglycerin; PETN, pentaerithrityl tetranitrate; H_2S, hydrogen sulphide; BH4, tetrahydrobiopterin; eNOS, endothelial nitric oxide synthase; NRF2, nuclear factor-E2-related factor 2; CV, cardiovascular; ACE, angiotensin-converting enzyme; AT1R, angiotensin-receptor (type 1); SGLT2, sodium-glucose co-transporter 2; $ET_{A/B}$, type A or B of endothelin receptor; GLP-1, glucagon-like peptide-1; DPP-4, dipeptidyl peptidase-4; mitoTEMPO, mitochondria-targeted 2,2,6,6-tetramethyl-1-piperidinyloxyl; mitoQ, mitochondria-targeted coenzyme Q10; mPTP, mitochondrial permeability transition pore; PARP, poly(ADP-ribose) polymerase; XO, xanthine oxidase; NOX, NADPH oxidase; MAO, monoamine oxidase; MPO, myeloperoxidase. Heart cartoons taken from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License.

Table 1. Important preclinical animal studies on the association of oxidative stress as well as ROS-producing or –degrading enzymatic systems with ischemic heart disease, ischemic major cardiovascular events such as myocardial infarction and heart failure.

Studies on oxidative stress and health effects	Genetic or pharmacological manipulation and outcome	Reference
Induction of myocardial infarction and cardiac failure by permanent ligation and effects of genetic p47phox deletion in mice	p47phox$^{-/-}$ improved cardiac function (LV ejection fraction), LV remodelling and nitric oxide bioavailability, whereas decreased superoxide formation, xanthine oxidase activity, hypertrophy, apoptosis and fibrosis and increased survival were observed.	[276]
Myocardial infarction-induced heart failure by coronary arterial ligation and effects of adenoviral Nox4 silencing in mice	Adenoviral Nox4 silencing or Cu,Zn-SOD overexpression improved cardiac function and decreased ROS levels as well as apoptosis.	[277]
Induction of myocardial infarction, cardiac remodelling and dysfunction by coronary arterial ligation and effects of genetic Nox2 deletion in mice	Nox2$^{-/-}$ showed less LV dilation and dysfunction, hypertrophy, apoptosis and fibrosis. Also 3-NT levels were decreased.	[278]
Delivery of Nox2-siRNA via nanoparticles to the post-ischemic heart of mice undergoing myocardial infarction by coronary arterial ligation	Nox2-siRNA improved cardiac function (fractional shortening) and decreased superoxide formation by phorbol ester-stimulated NADPH oxidase activity.	[279]
Induction of left ventricular hypertrophy and heart failure coronary artery ligation and effects of genetic Nox2 deletion in mice	Nox2$^{-/-}$ **did not** affect left ventricular remodelling and ROS levels.	[280]
Effects of the mitochondria-targeted cytoprotective and antioxidant peptide Bendavia on I/R damage in sheep and rabbits (catheter-based coronary vessel occlusion) as well as guinea pigs (isolated Langedorff model)	Bendavia decreased infarct size in sheep and rabbit upon I/R without influencing the infarct risk zone. Infarct size was also reduced by Bendavia in the guinea pig Langendorff model to a similar extent as by cyclosporine A. Bendavia prevented cell death, oxidative burst and collapse of $\Delta\Psi m$ of isolated cardiomyocytes subjected to I/R.	[281]
I/R damage in isolated mouse hearts (Langedorff model) and effects of genetic p66shc deletion	p66shc-/- reduced cell death (lactate dehydrogenase release) and oxidative stress parameters (MDA and tropomyosin oxidation) by different mechanisms as by	[282]
Induction of myocardial infarction by coronary vessel occlusion and effects of xanthine oxidase inhibition by allopurinol in dogs	Xanthine oxidase inhibition by allopurinol (or metabolite oxypurinol) decreased risk zone by trend and infarct size significantly.	[283]
Induction of myocardial infarction by coronary vessel occlusion and effects of xanthine oxidase inhibition by allopurinol +/- SOD in dogs	Xanthine oxidase inhibition by allopurinol did not affect risk zone but decreased infarct size significantly. SOD had additive protective effects by trend.	[284]
Induction of myocardial infarction and cardiac failure by permanent ligation and effects of xanthine oxidase inhibition by allopurinol post-MI in mice	Xanthine oxidase inhibition by allopurinol did not change infarct size but largely improved cardiac function (fractional shortening and ejection fraction) and fibrosis. Allopurinol decreased xanthine oxidase activity and superoxide formation.	[285]
Heart failure by coronary arterial ligation and effects of adenoviral ecSOD transfection in rats	Adenoviral ecSOS overexpression decreased superoxide and peroxynitrite formation and improved endothelium-dependent relaxation.	[286]; [287]

I/R damage by coronary arterial ligation for 1 hour with 24 hours reperfusion and effects of genetic heme oxygenase-1 deletion or overexpression in diabetic mice	$Hmox1^{-/-}$ increased cardiac oxidative stress 4-fold and caused larger infarcts as well as ventricular thrombi. Cardiac-specific overexpression of HO-1 largely prevented these complications.	[288]
I/R damage in isolated mouse hearts (Langedorff model) and effects of genetic glutathione peroxidase-1 deletion	$Gpx1^{-/-}$ aggravated infarct size, cardiac apoptosis, end-diastolic pressure and cell death (lactate dehydrogenase release).	[289]

Abbreviations used are: LV, left ventricular; ROS, reactive oxygen species; SOD, superoxide dismutase; 3-NT (=3-nitrotyrosine); MDA, malondialdehyde; I/R, ischemia/reperfusion; ΔΨm, mitochondrial membrane potential; MI, myocardial infarction; ecSOD, extracellular superoxide dismutase.

Table 2. Important clinical trials or meta-analysis on the association of oxidative stress markers with ischemic heart disease, ischemic major cardiovascular events such as myocardial infarction and heart failure.

Study category	Study description[a]	Marker and outcome	Included subjects	Reference
Systematic review and meta-analysis	Association of 8-hydroxy-2-deoxyguanosine levels with heart failure	8-OHdG ↑ indicates higher risk	140 Ctr, 446 HF patients	[290]
Clinical trial	Association of 8-hydroxy-2-deoxyguanosine levels and lipid peroxidation products with heart failure	8-OHdG ↑ and lipid peroxidation indicate higher risk	12 Ctr, 78 HF patients	[291]
Clinical trial	Association of free (F2)-isoprostane with 30-day cardiovascular outcomes in patients with acute coronary syndrome	8-isoPF2α ↑ indicates higher risk	108 CVD patients, 26 events	[276]
Matched case-control study	Association of urinary 8-iso-prostaglandin F2alpha with cardiometabolic risk factors in patients with coronary heart disease	8-isoPF2α ↑ indicates higher risk	93 Ctr, 93 CHD patients	[292]
Meta-analysis	Influence of exercise on oxidative stress parameters and improvement of cardiorespiratory capacity in patients with heart failure – high intensity training better than moderate training	iNOS mRNA and protein, 3-NT, MDA ↓, whereas catalase, GPx, SOD ↑ in the exercise group	353 HF patients, 207 undergoing training	[293]
Clinical trial	Serum NOX2 and urinary isoprostane levels predict vascular events in patients with atrial fibrillation	8-iso-PGF2α and serum sNOX2-dp ↑ are predictive of CV events and total mortality	1,002 anticoagulated AF patients; 78 CV deaths, 31 non-CV deaths and 125 CV events[b] occurred	[294]
Clinical trial	Interplay between oxidative stress and platelet activation in coronary thrombus of STEMI patients	sNOX2-dp, oxLDLs, sCD40L, and sP-selectin in ↑ STEMI patients	10 patients with stable angina, 32 STEMI patients	[295]
Prospective cohort study	Mediterranean diet reduces cardiovascular events and oxidative stress in patients with atrial fibrillation	8-isoPF2α, sNOX2-dp and CV events such as HF ↓ in the Mediterranean diet group	709 anticoagulated AF patients	[296]
Clinical trial	Association of oxidative stress markers with major adverse cardiovascular events in patients with coronary artery disease	D-ROM ↑ and OXY-Adsorbent Test indicates higher risk	97 CAD patients	[297]
Clinical trial	Association of 8-isoprostane levels with the presence and extent of coronary stenosis in patients with coronary artery disease	8-isoPF2α ↑ indicates higher risk	241 CAD patients	[298]
Clinical trial	Association of 3-nitrotyrosine with the presence of coronary artery disease in patients with prediabetes	3-NT ↑ indicates higher risk	120 prediabetic patients	[299]

Clinical trial	Association of serum myeloperoxidase levels with endothelial dysfunction or major adverse cardiovascular events in humans	MPO ↑ indicates higher risk	298 subjects; 3,635 subjects	[300]; [301]
Clinical trial	Association of serum myeloperoxidase levels with CV event risk in patients with acute coronary syndromes or chest pain	MPO ↑ indicates higher risk	1,090 ACS patients; 490 with chest pain	[302]; [303]
Clinical trial	Association of serum 3-chlorotyrosine levels with acute myocardial infarction in patients	3-Cl-Tyr ↑ indicates higher risk	53 Ctr, 77 AMI patients	[304]

Abbreviations used are: Ctr, control; D-ROM, derivatives of reactive oxygen metabolites, indicating ROS levels; 8-OHdG, 8-hydroxy-2-deoxyguanosine; HF, heart failure; CVD, cardiovascular disease; 8-isoPGF2α, 8-iso-prostaglandin F2α; CHD, coronary heart disease; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; GPx, glutathione peroxidase; SOD, superoxide dismutase; AF, atrial fibrillation; sNOX2-dp, soluble NADPH oxidase (isoform 2)-derived peptide (= marker of NOX2 activity); oxLDL, oxidized low-density lipoprotein; sCD40L, soluble CD40 ligand; STEMI, ST-segment elevation myocardial infarction; CAD, coronary artery disease; 3-NT, 3-nitrotyrosine; MPO, myeloperoxidase; ACS, acute coronary syndrome; 3-Cl-Tyr, 3-chlorotyrosine; AMI, acute myocardial infarction.

[a] Updated from [22] with permission.

[b] CV events included fatal/nonfatal ischaemic stroke, fatal/nonfatal myocardial infarction (MI), cardiac revascularisation and transient ischaemic attack.