



## Redox-related biomarkers in human cardiovascular disease - classical footprints and beyond

Andreas Daiber<sup>a,b,\*,1</sup>, Omar Hahad<sup>a,b,1</sup>, Ioanna Andreadou<sup>c</sup>, Sebastian Steven<sup>a</sup>, Steffen Daub<sup>a,1</sup>, Thomas Münzel<sup>a,b,\*\*,1</sup>

<sup>a</sup> Department of Cardiology, Molecular Cardiology, University Medical Center, Mainz, Germany

<sup>b</sup> German Center for Cardiovascular Research (DZHK), Partner Site Rhine-Main, Langenbeckstr. 1, 55131, Mainz, Germany

<sup>c</sup> Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

### ARTICLE INFO

#### Keywords:

Redox biomarker  
Oxidative stress  
Cardiovascular disease  
Comorbidities

### ABSTRACT

Global epidemiological studies show that chronic non-communicable diseases such as atherosclerosis and metabolic disorders represent the leading cause of premature mortality and morbidity. Cardiovascular disease such as ischemic heart disease is a major contributor to the global burden of disease and the socioeconomic health costs. Clinical and epidemiological data show an association of typical oxidative stress markers such as lipid peroxidation products, 3-nitrotyrosine or oxidized DNA/RNA bases with all major cardiovascular diseases. This supports the concept that the formation of reactive oxygen and nitrogen species by various sources (NADPH oxidases, xanthine oxidase and mitochondrial respiratory chain) represents a hallmark of the leading cardiovascular comorbidities such as hyperlipidemia, hypertension and diabetes. These reactive oxygen and nitrogen species can lead to oxidative damage but also adverse redox signaling at the level of kinases, calcium handling, inflammation, epigenetic control, circadian clock and proteasomal system. The *in vivo* footprints of these adverse processes (redox biomarkers) are discussed in the present review with focus on their clinical relevance, whereas the details of their mechanisms of formation and technical aspects of their detection are only briefly mentioned. The major categories of redox biomarkers are summarized and explained on the basis of suitable examples. Also the potential prognostic value of redox biomarkers is critically discussed to understand what kind of information they can provide but also what they cannot achieve.

### 1. Introduction

Within the last decades the global burden of disease experienced a shift from maternal, perinatal and nutritional causes as well as communicable (infectious) diseases to non-communicable diseases (e.g. atherosclerosis, hypertension and diabetes), which may be best explained by the demographic shift in population, exacerbation of environmental pollution but also improved global nutrition, medication and sanitation [1,2]. The future projections of the World Health Organization (WHO), based on data of the Global Health Observatory, predict a 77% share of non-communicable diseases in the global burden of disease in 2030 [3,4]. In 2016, 44% of all global deaths caused by

chronic non-communicable diseases were of cardiovascular origin [5], with ischemic heart disease (IHD) as a leading risk factor and a global prevalence of 128 million people as well as 9 million attributable premature deaths mainly caused by myocardial infarction (MI) and heart failure [6]. Importantly, non-communicable diseases represent or generate comorbidities (e.g. hyperlipidemia, hypertension or diabetes) that have great impact on cardiovascular risk and mortality [7]. These lines of evidence highlight the importance of comorbidities for the assessment of overall cardiovascular risk and the need for more and better biomarkers to characterize and provide early diagnosis of cardiovascular risk factors.

The mentioned comorbidities are all associated with an increased burden of oxidative stress (Fig. 1) [8,9], which is characterized by

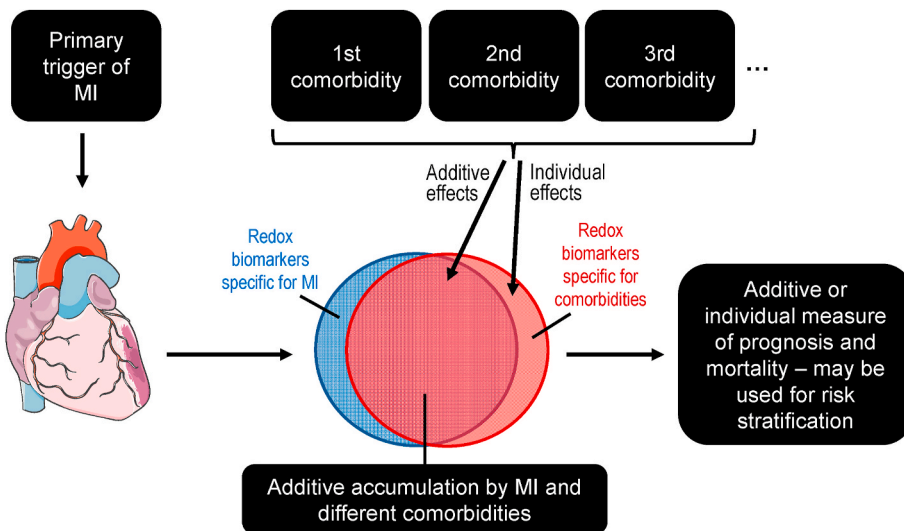
\* Corresponding author. Universitätsmedizin der Johannes Gutenberg, Universität Zentrum für Kardiologie 1, Labor für Molekulare Kardiologie, Geb. 605, Raum 3.262, Langenbeckstr. 1, 55131, Mainz, Germany.

\*\* Corresponding author. Universitätsmedizin der Johannes Gutenberg, Universität Zentrum für Kardiologie 1, Labor für Molekulare Kardiologie, Geb. 605, Raum 3.262, Langenbeckstr. 1, 55131, Mainz, Germany.

E-mail addresses: [daiber@uni-mainz.de](mailto:daiber@uni-mainz.de) (A. Daiber), [tmuenzel@uni-mainz.de](mailto:tmuenzel@uni-mainz.de) (T. Münzel).

<sup>1</sup> These authors contributed equally and should be considered as joint first and senior authors.

Abbreviations			
3-NT	3-nitrotyrosine;	IHD	ischemic heart disease
4-HNE	4-hydroxynonenal	I/R	ischemia/reperfusion
8-iso-PGF2 $\alpha$	8-iso-prostaglandin F2 $\alpha$	LDL	low-density lipoprotein
8-OHdG	8-hydroxy-2-deoxyguanosine;	MDA	malondialdehyde
8-OHG	8-hydroxyguanosine (also 8-oxoGuo)	MI	myocardial infarction
AF	atrial fibrillation	MPO	myeloperoxidase
AGE	advanced glycation endproduct	NOS	nitric oxide synthase
CAD	coronary artery disease	NOX	NADPH oxidase
cGMP	cyclic guanosine monophosphate	OR	odds ratio
CI	confidence interval	RNS	reactive nitrogen species
COPD	chronic obstructive pulmonary disease	ROS	reactive oxygen species
CVD	cardiovascular disease	RR	relative risk
reduced glutathione		SMD	standardized mean difference
GSSG	oxidized glutathione disulfide;	sNox2-dp	soluble nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) 2-derived peptide;
HR	hazard ratio	SOD	superoxide dismutase
HIF-1 $\alpha$	hypoxia-inducible factor-1 $\alpha$	WHO	World Health Organization
		XO	xanthine oxidase



**Fig. 1. Concept of redox biomarkers for comorbidities in myocardial infarction (MI).** Identification of specific redox biomarkers for different comorbidities (e.g. hypertension, hyperlipidemia or diabetes) in major cardiovascular events (e.g. MI) would significantly advance redox diagnostic approaches. Currently most redox biomarkers belong to the category of „additive accumulation” that could at least be used as prognostic tools to identify patients with multiple comorbidities representing the group of highest cardiovascular risk. Heart cartoon from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License.

excessive formation or insufficient break-down of reactive oxygen and nitrogen species (ROS<sup>2</sup> and RNS,<sup>3</sup> definitions as explained in Ref. [10] and the footnotes below) or inadequate repair of the resulting oxidative damage [11,12]. The resulting accumulation of oxidatively modified biomolecules and dysregulation of cellular redox signaling can be used as a diagnostic tool, in the form of defined redox biomarkers, to assess the magnitude of oxidative damage and alterations in redox pathways [13]. The concept of oxidative stress as a trigger of disease and death dates back to the year 1956, when Harman in 1956 postulated the “free radical theory of aging” [14]. However, also more than 60 years later, we rarely find drugs in clinical use that are by definition acting via an antioxidant mechanism, thereby questioning the “free radical theory of aging” [15,16]. In contrast, most cardiovascular mainstay drugs such as AT<sub>1</sub>-receptor blockers, angiotensin-converting enzyme inhibitors, statins, beta-blockers and many more possess potent pleiotropic antioxidant properties [17,18].

Although most large clinical trials dedicated to the therapeutic efficacy of classical antioxidants such as vitamins C and E failed to demonstrate a clear benefit for cardiovascular prognosis (for review see Refs. [19–21]), there is clear evidence that most cardiovascular diseases (CVD) are associated with or even triggered by oxidative stress [22,23]. This fact is also well supported by phenotypic changes in animal models of CVD with genetic deletion or overexpression of enzymes involved in the synthesis or degradation of ROS and RNS [18,19,24,25]. Further support for the oxidative stress concept comes from modern approaches using systems biology to identify therapeutic targets based on redox processes within a specific disease and systematic overviews of redox drug candidates in advanced clinical phase II-III trials [26,27]. Overall the discrepancy between the rather disappointing outcome of antioxidant trials and the established presence of redox biomarkers as well as druggable redox targets in CVD highlights the need of better knowledge on sources of ROS and RNS, their interaction, discrimination between beneficial (physiological) redox signaling and detrimental (pathophysiological) oxidative damage pathways [10]. This knowledge gap can be potentially filled by better characterization and application of redox biomarkers, which may be used for personalized or precision medicine approaches to identify patients with high burden of oxidative stress and to apply optimized redox therapeutic strategies [28], as already suggested for cancer and cardiovascular therapy in particular as well as pharmacotherapy guidance and validation of drug efficacy in general [29,30]. In the subsequent paragraphs we will discuss different approaches to identify and quantify ROS and RNS formation in CVD by their footprints in the form of redox biomarkers with a critical assessment of their specificity and prognostic value.

<sup>2</sup> Throughout this report, the term ROS refers almost exclusively to hydrogen peroxide and superoxide anion radical since both species are involved in redox signaling and are formed by a number of enzymatic sources. Other ROS include the hydroxyl radical or singlet oxygen, which however have most likely no specific roles in redox signaling but rather contribute to unspecific oxidative damage and oxidative stress. A large number of organic peroxides (e.g. lipid peroxides) are also covered by the term ROS but these species are only marginally discussed in the present overview. Other ROS such as hypochlorite are also not in the focus of the present work.

<sup>3</sup> Throughout this report, the term RNS refers almost exclusively to peroxynitrite, peroxyntrous acid and derived free radicals (e.g. hydroxyl radicals and nitrogen dioxide radicals). Peroxynitrite anion has a high specificity for activated thiols and readily reacts with carbon dioxide (the latter supports radical reactions). Peroxynitrous acid also has a high specificity for activated thiols but also reacts with transition metal complexes. RNS also comprises nitric oxide, which upon reaction with oxygen can form nitrogen dioxide radicals or nitrosating species such as N<sub>2</sub>O<sub>3</sub> and has a high affinity for transition metal complexes such as iron(II), all of which contributes to the potent redox signaling properties of nitric oxide. However, in the context of “oxidation of biomolecules” the term RNS does not refer to nitric oxide due to its weak reactivity towards biomolecules in general.

## 2. Redox biomarkers

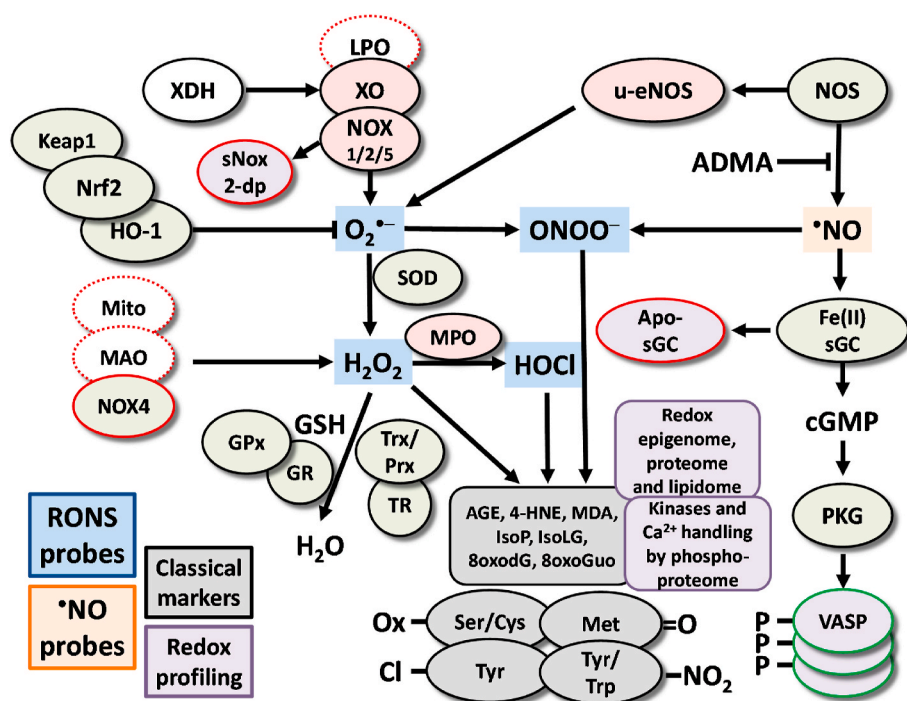
### 2.1. Sources of ROS/RNS and their major role in health and disease

Primary sources of ROS such as NADPH oxidases and the mitochondrial respiratory chain can generate secondary sources of ROS by oxidative conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XO) or NO-producing endothelial nitric oxide synthase (eNOS) to the uncoupled superoxide-generating enzyme [31]. NADPH oxidases are highly specialized ROS producing protein complexes with almost no other biological function [21]. Mitochondria, under normal conditions, produce ROS as a byproduct of the excessive use of oxygen, transport of electrons from reducing factors along the respiratory complexes and escape of these electrons to molecular oxygen (up to 1% of the total flow of electrons by estimation) [32]. In pathological situations, e.g. upon ischemia/reperfusion (I/R) the (redox) activation of p66<sup>Shc</sup> or monoamine oxidases (MAO), activation of mitochondrial ATP-sensitive potassium channels and opening of the mitochondrial permeability transition pore (mPTP) can dramatically increase the formation and release of ROS from mitochondria [25]. Also, a ROS-induced ROS formation via redox-based amplification mechanisms was described [33, 34]. ROS can exert beneficial and detrimental effects, which makes their therapeutic targeting so difficult and so far mostly unsuccessful (reviewed extensively in Ref. [10]). They can have protective effects via preconditioning-like mechanisms, kill pathogens during host defense or even contribute to essential cellular redox signaling (e.g. in cell differentiation, proliferation and migration). Vice versa, after exceeding a certain threshold, ROS have adverse effects including oxidative damage of various biomolecules, activation of inflammatory and cell death (e.g. apoptotic) pathways. Dysregulated redox signaling, e.g. by dysregulation of protein kinase pathways and calcium handling [35,36], epigenetic homeostasis [37–39], lipid metabolism [36] and vascular function [18,40], is counter-balanced by oxidatively activated antioxidant defense systems like NRF2/KEAP1 or hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) that control multiple ROS/RNS-degrading enzymes (e.g. heme oxygenase-1 (HO-1), superoxide dismutases (SOD) or thiol-based reductases/peroxidases) [12,26,41]. An example of this kind of dysregulated redox signaling was established for peripartum cardiomyopathy [42]. Redox biomarkers should help to discriminate these fundamentally different properties of ROS/RNS in health and disease enabling the search for novel redox therapeutic drugs.

### 2.2. What information should redox biomarkers provide and what basic requirements should they fulfill?

As discussed in detail by Frijhoff and colleagues, redox biomarkers should fulfill the following minimal requirements and provide the following minimal level of information [43]: By definition of the WHO, a biomarker represents any substance, structure or functional parameter that can be measured in a biological sample or the whole organism with prognostic value regarding the progression/severity of a disease or the health outcome [44]. In order to add clarity to these criteria, a clinically useful biomarker should be specific for a certain disease (diagnostic), have prognostic value or reflect the disease state or in other words the health risk of an individual. This information will allow monitoring of the therapeutic efficacy of drugs in already diagnosed illness or help to choose the appropriate drug and initiate therapy in so far undiagnosed disease states. For routine use in the clinics, a biomarker should possess a sufficient stability during measurement and storage and should be easily accessible in widely obtainable tissues or preferably in blood. Finally, cost-effectiveness is a major criterion in modern health care systems and reproducibly on a large scale. Criteria for biomarkers in general and redox biomarkers in particular were further refined by Ghezzi and coworkers providing more useful definitions (e.g. for discrimination between passive and active biomarkers) [15,45].

Besides these minimal essential requirements a biomarker should



**Fig. 2. Redox pathways associated with putative biomarkers of oxidative stress [10,43].** The processes that lead to oxidative modifications of proteins, lipids and nucleotides are highly complex. Enzymes, such as XO, NOX and NOS, as well as organelles such as mitochondria, can produce ROS and RNS. ROS/RNS from primary sources can trigger secondary ROS sources (e.g. conversion of XDH to XO or uncoupling of eNOS) [31]. ROS can serve as substrates for other enzymes to generate additional types of ROS, such as the generation of HOCl from  $H_2O_2$  by MPO. Importantly, some ROS sources are solely producing detrimental ROS (filled red color, e.g. NOX1/2/5), some have physiological functions but generate ROS as a side product or in the presence of certain substrates (dashed red line, e.g. LPO, Mito) or even produce mostly beneficial ROS (filled green color with red line, e.g. NOX4). Cellular systems and enzymes, including the SOD, GSH/GPx and Trx/Prx system, counterbalance the production of ROS (filled green color). Oxidative stress affects cGMP signaling through its effects on nitric oxide ( $NO$ ) production, scavenging and on the  $NO$  producing enzyme eNOS as well as the  $NO$  receptor sGC (by oxidative heme depletion to the apo-sGC) and its target kinase PKG. In principle, all of these mentioned ROS producing, detoxifying and oxidatively modified enzymes and systems represent redox biomarkers as their expression or activity will provide information on the redox balance. ROS and RNS can be directly detected using specific probes (e.g. by chemiluminescence, fluorescence based HPLC or EPR-based methods) [46,57]. ROS and RNS also leave footprints in vivo in the form of classical oxidative stress markers such as oxidized amino acids in proteins or oxidized nucleotides in DNA/RNA as well as free or protein-bound reactive aldehydes from glucotoxicity and lipid peroxidation (filled grey color) [43]. In addition, functional readout of signaling pathways (e.g. redox-regulated kinases and calcium-handling [35,36], epigenetic [37–39] and lipidome profiles [36], P-VASP as the endpoint of the  $NO/cGMP$  signaling cascade and novel activity markers such as sNox2-dp or apo-sGC; all filled purple color) can significantly add to our understanding and identification of redox imbalances. For the sake of clarity, we could not show and discuss all existing ROS/RNS sources, species and targets in the present scheme, but focused on the most important ones according to the cited references. **Abbreviations:** AGE, advanced glycation end products; cGMP, cyclic guanosine monophosphate; EPR, electron paramagnetic resonance; GSH, glutathione; GPx, GSH peroxidase; GR, GSH reductase;  $H_2O_2$ , hydrogen peroxide; HO-1, heme oxygenase-1; HOCl, hypochlorous acid; LPO, lipoxygenases; MAO, monoamine oxidase; MPO, myeloperoxidase; NOS, nitric oxide synthase; NOX, NADPH oxidase; ONOO<sup>-</sup>, peroxynitrite; PKG, protein kinase G; Prx, peroxiredoxin; P-VASP, phospho (Ser239)-vasodilator-stimulated phosphoprotein; RNS, reactive nitrogen species; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; sNox2-dp, soluble Nox2-derived peptide; SOD, superoxide dismutase; Trx, thioredoxin; TR, Trx reductase; u-eNOS, uncoupled eNOS; XDH, xanthine dehydrogenase; XO, xanthine oxidase. Reused from Ref. [43] and updated from Refs. [10,57,161] with permission under the terms of the Creative Commons Attribution Noncommercial License. Copyright © [43]; Published



by Mary Ann Liebert, Inc. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

fulfill, it would be also desirable to learn more about the nature of ROS/RNS (helpful for approaches using specific ROS/RNS scavengers) as well as the cellular site and organ system of their formation (helpful to understand the pathophysiological role of ROS/RNS in the respective disease) [10]. However, for the simple conclusion that a certain disease is associated with excessive ROS and RNS formation (oxidative stress), their chemical identity is not required and accordingly sophisticated ROS and RNS detection techniques are not necessary but classical oxidative stress biomarkers (e.g. 3-nitrotyrosine (3-NT)- or 4-hydroxynonenal (4-HNE)-positive proteins or 8-oxo-G-positive RNA) will suffice for qualitative assessment of oxidative stress [46]. This approach may also be enough for simple tests of redox drugs, where it will be sufficient to see whether the oxidative phenotype in diseased animals or challenged cells changes and the tested drug successfully suppresses oxidative stress mediated damage. These tests can be further refined by treatment with specific inhibitors of ROS/RNS sources (e.g. NOX inhibitors, XO inhibitors) or specific scavengers (e.g. SOD mimetics, mitochondria-targeted antioxidants), which will add to further mechanistic insights, even for classical oxidative stress marker-based assays.

### 2.3. Categories of redox biomarkers

Redox biomarkers can be separated into different categories as also defined in the past (Fig. 2) [10,43]. They comprise the classical oxidative stress markers that in most cases represent endproducts of oxidation reactions such as lipid peroxidation, oxidized amino acids (free or protein-bound), oxidized DNA/RNA bases (free or DNA/RNA-bound) and changes in thiol redox state (simplest example is reduced glutathione (GSH)/oxidized glutathione disulfide (GSSG) ratio). Another category of redox biomarkers relies on expression/activity of enzymes that are involved in the formation (e.g. NOX, XO) or breakdown (e.g. SODs, peroxiredoxins) of ROS/RNS. Also the modulation of reporter molecules for signaling cascades (e.g. phosphorylated vasodilator-stimulated phosphoprotein [P-VASP] as the endtarget of NO/cyclic guanosine monophosphate (cGMP) signaling) or concentrations of endogenous inhibitors (e.g. asymmetric dimethyl arginine as inhibitor of eNOS [47]) as well as eNOS uncoupling surrogates (e.g. S-glutathionylation of eNOS) can serve as redox biomarkers [48]. As these rather traditional redox biomarkers, at least if not a plethora of them is determined in the same sample, provide only limited mechanistic insights regarding ROS/RNS identity or pathophysiology and are considered not specific at all for a certain disease or comorbidity, we will here focus on the evaluation of their prognostic value and their additivity in the presence of different comorbidities in human disease studies. Finally, redox profiling represents an emerging technique to obtain a complete picture of the redox changes that characterize a certain disease. Examples are redox lipidomics that characterizes the landscape of oxidized fatty acid products by means of mass spectroscopy, kinase activity networks using phosphoproteomics or epigenetic profiling. These emerging techniques may yield specific redox profiles of certain disease and also provide detailed mechanistic insights.

### 2.4. Direct detection of ROS and RNS formation using fluorescence, chemiluminescence or UV/Vis absorption

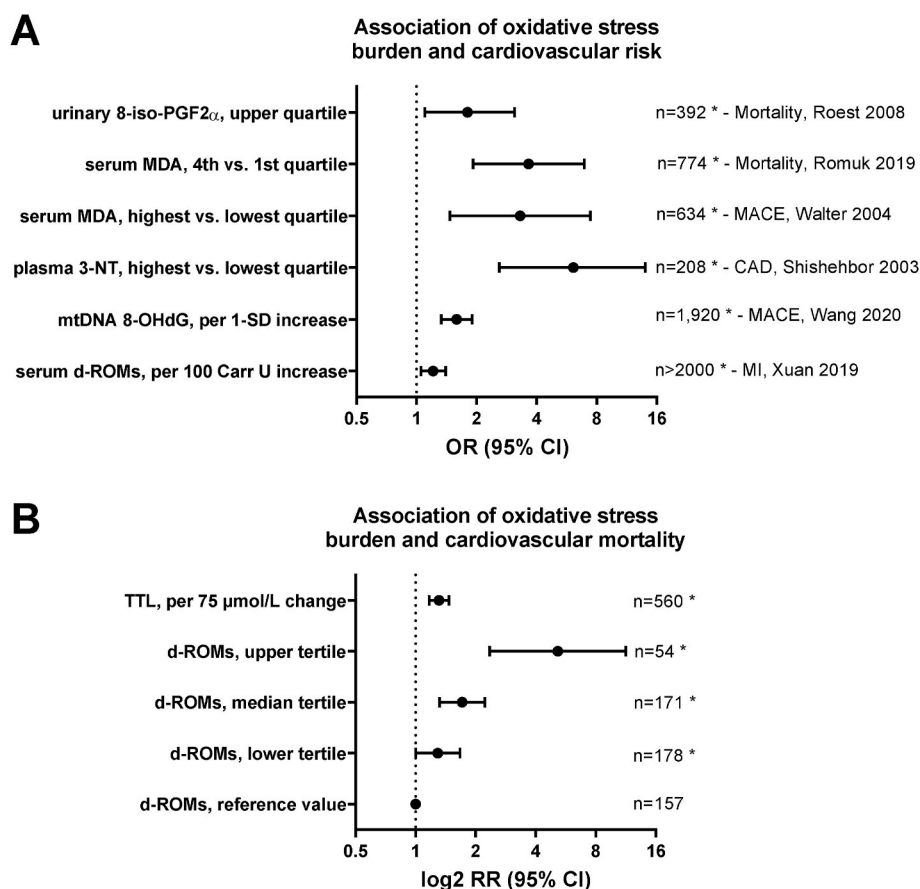
The direct measurement of ROS and RNS formation represents a redox biomarker, although not easily applicable in large cohort studies as the assays need to be applied to freshly harvested tissue or biological fluid samples (at least the incubation of the biological samples with the probes). These direct ROS and RNS assays can provide information on the involved reactive species and are mostly based on fluorescence or

chemiluminescence dyes combined with HPLC- or plate reader-based detection/quantification methods as well as on UV/Vis photometric assays. These techniques will not be discussed further here as they were reviewed in detail in the past. The classical “old” assays such as hydroethidium-dependent fluorescence microtopography in tissue cryosections to assess vascular ROS formation, lucigenin-enhanced chemiluminescence in tissue membrane fractions to quantify NADPH oxidase activity and luminol derivative (L-012)-enhanced chemiluminescence in whole blood to determine oxidative burst of leukocytes were critically discussed extensively in the past [46]. The measurement of oxidative burst by L-012-enhanced chemiluminescence in whole blood was also successfully applied in clinical studies and correlated with the walking distance as well as critical limb ischemia in patients with peripheral artery disease [49,50]. The combination of advanced HPLC-based fluorescence assays for the quantification of ROS and RNS formation was described for cell culture [51] that can be also used for specific detection of cellular superoxide generation [52]. In addition there are excellent reviews available discussing the detection of ROS and RNS formation in biological samples [53,54], including statements of the American Heart Association defining first and second choice assays [55].

Other experimental techniques comprise ROS-induced formation of microbubbles that are formed from liposome-encapsulated allylhydrazine, a liquid compound that yields nitrogen and propylene gas, which may be detected by ultrasound by in vivo application to animals [56] and in the future potentially in humans (reviewed in Ref. [9]). Also, L-band electron paramagnetic resonance (EPR) spectroscopy in combination with the most advanced spin traps represents a cutting-edge technique for the in vivo detection of ROS and RNS, which is currently translated from animal to human application [57]. Furthermore, the combination of different techniques such as immuno-spin trapping as reported for trapping of thyl radicals in proteins or other radicals in DNA by 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and formation of stable spin-adducts with subsequent immunological detection of DMPO-bound proteins or DNA molecules is already used for the detection of ROS and RNS formation, or more precisely their footprints in biological samples [58]. Additionally, the targeting of specific chemiluminescent or fluorescent dyes and/or ROS and RNS scavengers by linking them to site specific antibodies or loading them into nanoparticles might provide the next step in oxidative stress and redox biology research and may be used in humans in the near future (reviewed in Refs. [57,59]). For preclinical research also genetically encoded fluorescent enzymatic probes, e.g. HyPer for hydrogen peroxide detection [60,61] and geNOps for nitric oxide [62,63], will in the future allow the highly specific and timely as well as spatially resolved monitoring of ROS and RNS formation in isolated cells. More recently, the technique of genetically-encoded redox sensors was also translated to the animal level allowing the in vivo monitoring of redox changes in organs, even distinguishing between cytosolic and mitochondrial ROS/RNS formation by encoding a cytosolic or mitochondrial glutaredoxin/green fluorescent protein construct (Grx1-roGFP2) [64, 65]. Redox changes in tissues of these animals can be even conserved by alkylation of reduced thiols in the Grx1-roGFP2 construct for later “redox histology” using fluorescence microscopy [64,66].

## 3. Classical “oxidative stress” biomarkers in human cardiovascular disease

Mounting evidence indicates classical biomarkers of oxidative stress to be a prominent feature and suggestive of disease severity in several CVD phenotypes by displaying increased expression of these markers in CVD compared with control groups. Importantly, these markers are also



**Fig. 3. Prognostic value of classical oxidative stress markers for cardiovascular risk.** (A) Association of markers of lipid peroxidation (isoprostane [8-iso-PGF $_{2\alpha}$ ] and malondialdehyde [MDA]) [75,83,85], nitro-oxidative stress (3-nitrotyrosine [3-NT]) [96], oxidative mitochondrial DNA damage (8-hydroxy-deoxyguanosine [8-OHdG]) [120] and derivatives of reactive oxygen metabolites (d-ROMs) [125] with cardiovascular risk in the comparison of healthy subjects with cardiovascular disease patients. (B) Associations of d-ROM levels and total thiol levels (TTL) with cardiovascular disease-specific mortality (adjusted for age and sex). d-ROMs groups [Carratelli Units]: reference, <340; T1, 341–400; T2, 401–500; T3, >500. Graph was generated from tabular data in Ref. [124] with permission and reused from Ref. [48]. Copyright © 2015, Schöttker et al. \* indicates significant differences ( $p < 0.05$ ) versus reference groups.

partly predictive of future cardiovascular events and mortality in initially healthy subjects after adjustment for established cardiovascular risk factors, thereby demonstrating an independent crucial role of oxidative stress in the development and progression of CVD and mortality. However, future studies should address the incremental value of oxidative stress markers beyond established cardiovascular risk factors for the prediction of incident cardiovascular events and related outcomes.

### 3.1. Lipid peroxidation products 8-isoprostane, 4-hydroxynonenal and malondialdehyde

Malondialdehyde (MDA) is one of the best studied and most abundant (10–20  $\mu\text{M}$  in plasma) endproducts of lipid peroxidation chain reactions [10,43]. Other members of this class of redox biomarkers comprise peroxidation products of cholesterol, phospholipid and polyunsaturated fatty acids including ketones such as 4-HNE [67,68], alcohols such as isoprostanes [69], hydroperoxides such as 1-palmitoyl-2-(5'-oxo-valeroyl)-sn-glycero-3-phosphocholine, oxidized low-density lipoprotein (LDL) [70] and cyclic endoperoxides. Polyunsaturated fatty acids (free or in phospholipids) containing unconjugated olefinic bonds are easily oxidized by ROS/RNS and derived free radicals [71]. Commonly reported examples of oxidized cholesterol (oxysterols) include 6-cholesten-5 $\alpha$ -hydroperoxide, 7-ketocholesterol, 7-dehydrocholesterol and 25-hydroxycholesterol [72]. We here focus on 8-isoprostane, 4-HNE and MDA as they are most widely used in the clinical routine, fairly specific ELISAs are commercially available and their formation mechanism is fully characterized [10,43,67]. The gold standard for detection of these products are gas and liquid chromatography in combination with mass spectrometry [73], which can at least be used to validate the results obtained with ELISA in selected samples. Of note, due to their reactivity 4-HNE and MDA (and other reactive lipid

peroxidation products) can bind to thiol or amino groups in proteins (or other biomolecules) and thereby modulate the activity of enzymes and may act as toxifiers in disease pathomechanisms or confer redox hormesis via electrophilic activation of antioxidant defense systems. As a note of caution, we would like to mention that MDA and 4-HNE can also be formed by ROS-independent mechanisms such as the fragmentation of prostaglandin endoperoxide (PGH $_2$ ) or diendoperoxide (generated by oxygenation of arachidonic acid by cyclooxygenase-2 and 5-lipoxygenase) during platelet aggregation or inflammation [74].

A nested case-cohort study in postmenopausal women by Roest et al. showed that elevated levels of urinary 8-iso-prostaglandin F $_{2\alpha}$  (8-iso-PGF $_{2\alpha}$ ) were associated with increased cardiovascular mortality due to coronary artery disease (CAD) (141 deaths) and stroke (109 deaths) after a follow-up of 18 years [75]. An odds ratio (OR) of 1.8 (95% CI 1.1–3.1) was observed for the highest quartile of 8-iso-PGF $_{2\alpha}$  levels (Fig. 3A), independent of age, while further adjustment for systolic blood pressure, history of CVD, diabetes, smoking, and body mass index did not attenuate this association. A more recent meta-analysis from 2017, aimed at classifying oxidative damages across 50 human health conditions including 242 studies, displayed small increases in 8-iso-PGF $_{2\alpha}$  levels for hypertension (standardized mean difference (SMD) between affected and control group - Hedges'g 0.4, 95% CI 0.2–0.6), chronic heart failure (g 1.4, 95% CI 1.1–1.7), CAD (g 0.4, 95% CI 0.3–0.5), and ischemic stroke (g 1.2, 95% CI 1.0–1.4) compared to control groups [76]. A case-control study on 93 CAD patients and 93 age- and sex-matched control subjects demonstrated increased levels of urinary 8-iso-PGF $_{2\alpha}$  in CAD patients with multivariable analyses revealing an OR of 19.3 (95% CI 5.4–68.8) for the highest tertile (vs. lowest) of 8-iso-PGF $_{2\alpha}$  after adjustment for CAD risk factors [77]. Similar results were obtained by Wolfram et al. presenting increased levels of plasma, serum, salivary and urinary 8-iso-PGF $_{2\alpha}$  in CAD patients ( $n = 20$ ) with subgroup analyses indicating positive and negative

correlations of 8-iso-PGF2 $\alpha$  with New York Heart Association (NYHA) classification stages and left ventricular ejection fraction, respectively [78]. Contrarily, a study from Woodward et al. found no association between 8-iso-PGF2 $\alpha$  levels and risk of fatal or non-fatal CAD (OR 1.08, 95% CI 0.91–1.29 per 1-standard deviation (SD) increase in 8-iso-PGF2 $\alpha$  levels) for the comparison of 227 coronary cases and 420 control subjects [79].

Nakamura et al. showed 5-fold increased 4-HNE levels in 23 patients with dilated cardiomyopathy compared with 13 control subjects with normal cardiac function [80]. The treatment of 11 subjects for 9  $\pm$  4 months with carvedilol (22  $\pm$  8 mg/d), a beta-blocker with antioxidant capacity, reduced 4-HNE levels by 40% along with functional amelioration of heart failure symptoms. In a study by Mak et al. comparison of 8 patients with chronic heart failure with 8 age-matched patients with normal left ventricular function revealed elevated levels of total aldehydes including 4-HNE in heart failure patients [81]. Barrios et al. reported increased 4-HNE levels in 22 patients with acute MI compared with 20 control subjects without presence of acute CAD. Infarct size was strongly correlated with 4-HNE levels [82].

In 774 chronic heart failure patients, MDA was a significant predictor of death and a combined endpoint of death and heart transplantation at one-year follow-up [83]. After adjustment for a range of clinical and laboratory variables, elevated levels of serum MDA were associated with a two-fold increased risk of death (hazard ratio (HR) 2.103, 95% CI 1.330–3.325) as well as a two-fold increased risk of the combined endpoint (HR 2.000, 95% CI 1.366–2.928 per 1  $\mu$ mol/L). For the comparison of the highest vs. lowest quartile of baseline MDA levels the relative risk (RR) of death was 3.64 (95% CI, 1.917–6.926) and the RR for the combined endpoint was 2.71 (95% CI 1.551–4.739) (Fig. 3A). In line with this, Radovanovic et al. reported that high plasma MDA levels (>8  $\mu$ mol/L) in 120 chronic heart failure patients independently increased risk of death in median 13-month follow-up [84]. Patients with MDA levels above the cut-off had an eight times higher risk of death. A large prospective study of 634 patients with stable CAD from the PREVENT trial was able to demonstrate that serum MDA increased the risk of cardiovascular events [85]. For the comparison of the highest vs. lowest quartile of MDA, a RR of 3.30 (95% CI 1.47–7.42) for major vascular events (fatal/non-fatal MI and stroke) (Fig. 3A), a RR of 4.10 (95% CI 2.55–6.60) for non-fatal vascular events (unstable angina) and a RR of 3.84 (95% CI 2.56–5.76) for major vascular procedures (percutaneous interventions and coronary artery bypass grafting) were observed.

A meta-analysis of 12 observational studies aimed at investigating the association between oxidized LDL and risk of atherosclerotic CVD revealed an overall effect size of 1.79 (95% 1.56–2.05) [86]. Among 1371 patients with acute MI, increased levels of oxidized LDL were associated with a combined endpoint of death due to CVD, occurrence of unstable angina pectoris and MI at 6-month follow-up (HR 1.66, 95% CI 1.07–2.57 for highest vs. lowest tertile) [87]. Another study has shown a 215% (95% CI 47–576) increased risk of a major adverse coronary event in 246 patients with CAD in dependence of oxidized LDL levels (for highest vs. lowest quartile) [88]. An association between oxidized LDL and cardiovascular events was also reported in a study of 425 patients with acute coronary syndrome by demonstrating an HR of 2.88 (95% CI 1.93–4.32 per 1 mmol/L increase in oxidized LDL) for acute MI recurrence or acute coronary syndrome-related death at 5-year follow-up [89].

Ethylene in the breathing air of patients undergoing cardiopulmonary bypass surgery allowed real-time monitoring of endogenous lipid peroxidation in the intraoperative setting and was indicative of regional myocardial ischemia and reperfusion [90]. The authors suggest ethylene monitoring as a non-invasive method of risk stratification and prevention of intraoperative complications during cardiopulmonary bypass surgery. However, so far this method requires expensive instruments (mass spectrometry) that may not be affordable and applicable in every small hospital.

### 3.2. 3-Nitrotyrosine as a footprint of peroxynitrite in vivo formation (or inflammatory activation of the myeloperoxidase/hydrogen peroxide/nitrite pathway) and other NO-related markers

The major nitro-oxidative protein modifications elicited by peroxynitrite (ONOO $^-$ ) are S-glutathiolation and S-oxidation of cysteine as well as nitration of tyrosine residues. Other modifications are zinc finger oxidation, methionine sulfoxidation and nitration of tryptophan residues by ROS as well as RNS [10,43]. We here focus on 3-NT as there is substantial clinical evidence for its association with disease pathogenesis, fairly specific ELISAs are commercially available and its formation mechanism is well understood. Importantly, most other oxidative protein modifications are not specific for ONOO $^-$ . Regarding a summary of the clinical evidence for other protein-based oxidative stress markers, also including protein carbonyl groups, we refer to previous overviews [10,43]. The immunological detection methods can be validated by mass spectrometry-based methods. Importantly, also 3-NT can act as a toxifier in disease pathogenesis by triggering autoimmune reactions (IgGs against 3-NT) that also correlate with CAD health outcomes [91], inactivating important ROS scavenging and vasodilator-producing enzymes such as Mn-SOD [92] and prostacyclin synthase [93], or aggravating the prothrombotic effects of fibrinogen [94,95].

A case-control study (100 cases with established CAD and 108 controls with no clinical history or symptoms suggestive of CAD) by Shishehbor et al. aimed at investigating the association of systemic 3-NT levels and the presence of CAD [96]. The authors demonstrated that in patients with CAD, 3-NT levels were markedly higher compared with control subjects (median values, 9.1 vs. 5.2  $\mu$ mol/mol). Likewise, prevalence of CAD increased with higher 3-NT quartiles (26% in the lowest vs. 58% in the highest quartile). After multivariable adjustment for the Framingham Global Risk Score and levels of C-reactive protein, increased 3-NT levels were associated with higher odds of CAD (OR 6.1, 95% confidence interval (CI) 2.6–14.0 for highest vs. lowest quartile) (Fig. 3A). Moreover, statin therapy in 35 participants (atorvastatin 10 mg/d) for 12 weeks reduced 3-NT levels by 25%. Concordantly, Pirro et al. were able to demonstrate that the combination of a 4-week rosuvastatin therapy (10 mg/d) and low-fat diet was associated with a pronounced decrease in 3-NT levels as well as arterial stiffness, a well-established marker of CVD risk, in 35 patients with primary hypercholesterolemia (vs. 36 patients with a low-fat diet only) [97]. A study of 120 prediabetic patients by Chu et al. demonstrated increased levels of 2-h 3-NT levels after 75g oral glucose tolerance test in patients with concomitant CAD compared with patients having no CAD [98]. After multivariable adjustment for risk factors and glucose tolerance status, 2-h 3-NT levels in the highest quartile (OR 3.1, 95% CI 2.2–5.3 vs. lowest quartile) remained independently associated with the presence of CAD. Conversely, a recent study by Quidim et al. found that 3-NT levels were not predictive of mortality rates during a follow-up period of four years in a cohort of 342 patients with CAD [99].

Also, other products of nitric oxide-dependent reactions or even NO gas itself were used in the past or may be used in the future to test their diagnostic and prognostic value in CVD. Although nitrotryptophan derivatives were not used so far as markers of nitro-oxidative stress in the clinical setting, their measurement may provide superior insights in the RNS being formed and analytical methods to determine their levels in tissues and biological fluids have been established [100]. Another emerging marker of nitro-oxidative stress that was not applied in clinical studies so far is 8-nitroguanosine-3',5'-cyclic monophosphate (8-nitro-cGMP), which itself can act as an electrophile and contribute to cellular redox regulation [101,102]. Nitrated fatty acids represent another footprint of RNS reactions in vivo, display potent electrophilic properties and contribute to cellular redox signaling [103,104]. Of note, nitro-fatty acids could not only serve as redox biomarkers but also represent potent therapeutic compounds [105] and analytical methods are in place to detect and quantify these species in biological samples [106]. Recently, the urinary nitrolipidome was quantified using high



resolution mass spectrometry with fragmentation analysis [107].

The use of gaseous NO in exhaled air as a redox biomarker is inconclusive so far, as increased NO may be indicative of higher iNOS activity and an inflammatory phenotype, but vice versa may also mirror improved eNOS function and higher endogenous vasodilatory NO production. The exhaled NO concentrations showed a moderate trend of being lower in patients with stable CAD as compared to healthy subjects and was further decreased in both groups among active smokers [108]. Of note, physical exercise decreased exhaled NO concentrations in CAD patients but had only minor effects in healthy subjects, which may indicate dysregulated exercise-induced eNOS activation in the setting of CAD. The link between exhaled NO concentrations and endogenous NO production was supported by a dramatic increase in NO in the breathing air and plasma cGMP levels after infusion of L-arginine in patients with preeclampsia [109]. Therapy of hypertensive patients with the calcium antagonist amlodipine increased exhaled levels of NO in response to exercise [110] and exhaled NO concentrations correlated with exercise intolerance in patients with heart disease [111]. Whereas the measurement of exhaled NO levels as a diagnostic and prognostic tool for assessment of cardiovascular risk is still in its infancy, the application of this assay was already tested on a broader basis in patients with asthma and chronic obstructive pulmonary disease (COPD) to determine the inflammatory status in the lung [112].

The plasma or serum levels of the NO degradation products nitrite and nitrate have only limited diagnostic and prognostic value as they are largely affected by dietary uptake of these anions, especially by certain vegetables. However, in 2885 Framingham Offspring Study subjects, nitrate levels were associated with increased all-cause mortality (HR 1.21, 95% CI 1.03–1.40 per unit increase) at median follow-up of 17.3 years after adjustment common risk factors, whereas no association was observed for incident CVD (HR 1.08, 95% CI 0.89–1.31) [113]. Importantly, in an analysis of 2443 subjects without CVD at baseline, nitrate/nitrite levels were predictive of incident CVD (OR 1.35, 95% CI 1.01–1.80), further showing that nitrate/nitrite measurement added to a better reclassification of individuals at risk of CVD beyond classical cardiovascular risk factors [114].

### 3.3. Oxidative DNA/RNA damage

Nucleotide oxidation is a highly abundant DNA modification [115] and represents a pre-mutagenic lesion that induces CG:TA transversion mutations, which can also contribute to cancer [116]. The guanine moiety is particularly prone to oxidation under formation of 8-hydroxy-2-deoxyguanosine (8-OHdG) or 8-OHG (8-oxoGuo) and can be used as a marker of DNA and RNA oxidation by Fenton chemistry-derived hydroxyl radicals or peroxynitrite. Levels of free 8-OH(d)G can be measured upon DNA/RNA digestion and hydrolysis from tissues or directly in urine and serum/plasma [10,43]. Whereas commercial ELISAs are available but critically discussed [117], the gold standard for measurement is based on mass spectrometry methods.

A meta-analysis on the association between 8-OHdG levels and CVD (CAD, stroke, peripheral artery disease and carotid atherosclerosis) including five case-control studies and nine prospective studies (810 CVD patients and 1106 control subjects) showed higher levels of 8-OHdG in CVD patients compared with controls (SMD 1.04, 95% CI 0.61–1.47) [118]. In good agreement, a further meta-analysis of six studies (446 heart failure patients and 140 controls) from the same author group showed higher levels of 8-OHdG in heart failure patients than in controls (SMD 0.89, 95% CI 0.68–1.10) with a progressive increase for higher NYHA class [119]. A recent study by Wang et al. from 2020 aimed to investigate whether 8-OHdG may contribute to the incidence of CAD in 1920 patients with type 2 diabetes mellitus [120]. After multivariable adjustment, the authors reported elevated levels of 8-OHdG to be cross-sectionally related to increased odds of obstructive CAD (OR 1.38, 95% CI 1.24–1.52), higher number of diseased vessels with  $\geq 50\%$  stenosis (OR 1.29, 95% CI 1.19–1.41), modified Gensini

score (OR 1.28, 95% CI 1.18–1.39) and higher levels of C-reactive protein ( $\beta$  0.18, 95% CI 0.06–0.31). Importantly, increased levels of 8-OHdG were associated with a 59% higher risk of a major adverse cardiovascular and cerebral event in 701 diabetic patients subjected to coronary revascularization at one-year follow-up (hazard ratio (HR) 1.59, 95% CI 1.33–1.90 per 1-SD increase in 8-OHdG levels) (Fig. 3A). A study by Liu et al. found the extent of vascular complications to be associated with elevated RNA damage by measuring 8-HOG (8-oxoGuo) levels in 633 type 2 diabetes mellitus patients compared to 683 controls [121]. Levels of 8-HOG were highest among type 2 diabetes mellitus patients with vascular complications followed by type 2 diabetes mellitus patients without vascular complications and healthy controls, increasing overall with age. In good agreement, in 936 patients with type 2 diabetes mellitus, higher levels of 8-HOG were associated with increased risk of diabetes-related mortality including death from MI, stroke, and peripheral vascular disease at follow-up (HR 1.72, 95% CI 1.11–2.66 for quartile 4 vs. quartile 1) [122]. Furthermore, Broedbaek et al. demonstrated that 8-HOG levels predicted incident CVD in 1381 type 2 diabetes mellitus patients [123].

### 3.4. Other, less specified classical redox biomarkers such as ROM or TTL

In a meta-analysis from Schöttker et al. serum derivatives of reactive oxygen metabolite (D-ROM) and total thiol (TTL) levels were measured to determine their impact on all-cause and CVD mortality in 10,622 subjects from population-based cohorts from Germany, Poland, Czech Republic and Lithuania [124]. The results revealed that both D-ROM and TTL were independently associated with all-cause (D-ROM: RR 1.20, 95% CI 1.12–1.29 per 1-SD increase; TTL: RR 1.12, 95% CI 1.04–1.21 per 1-SD decrease) and CVD mortality (D-ROM: RR 1.30, 95% CI 1.12–1.51 per 1-SD increase; TTL: RR 1.25, 95% CI 1.09–1.45 per 1-SD decrease) after adjustment for established risk factors (Fig. 3B). A pooled analysis of the same cohorts from Xuan et al. also indicated D-ROM levels to be associated with MI (OR 1.21, 95% CI 1.05–1.40 per 100 Carr units increase) (Fig. 3A) and stroke incidence (OR 1.17, 95% CI 1.01–1.35), while TTL levels were only associated with the incidence of stroke (OR 0.79, 95% CI 0.63–0.99 for quartiles 2–4 vs. quartile 1) [125]. Masaki et al. confirmed the prognostic value of D-ROM by showing that high levels of D-ROM (highest vs. lowest quartile) in 265 patients with treated CVD were associated with increased risk of all-cause mortality (HR 10.791, 95% CI 1.032–112.805) and cardiovascular events (HR 2.651, 95% CI 1.138–6.177) at follow-up [126].

## 4. Redox-regulated microRNAs in human cardiovascular disease

Beyond the classical oxidative stress biomarkers, epigenetic profiling may represent a promising redox diagnostic strategy in the future, especially as the costs per assay continue to decrease. A group of naturally occurring, small and noncoding RNAs, microRNAs (miRNAs), has been identified as regulators of gene expression that are known to regulate approximately 30% of human genes [127,128]. In the clinical setting, miRNAs are regarded as advantageous biomarkers as they can be easily obtained from peripheral blood samples and are stable at room temperature as well as after being frozen and thawed [129]. A number of miRNA expression levels were shown to change in response to oxidative stress via their respective redox interaction partners comprising NRF2 and SIRT1, both detailed in this review, as well as NF- $\kappa$ B [130,131]. Due to the sheer number of miRNAs described in oxidative-stress-induced heart disease, a selection of prominent miRNAs involved in the onset and progression of CVD will be presented in the next section.

While miRNAs have been recognized as a diagnostic tool which still has to undergo robust clinical evaluation, the future may see multiple ways to therapeutically influence miRNA expression. Antagomirs, oligonucleotides designed to bind and inhibit miRNA [132], as well as miRNA mimics, which are designed to therapeutically re-establish miRNA levels [133], are currently being investigated within clinical



**Table 1**  
Studies on the association of oxidative stress biomarkers with cardiovascular disease/markers or mortality.

Biomarker	Study type	Quality level	Sampling/method of detection	Major outcome	Reference
3-NT	Case-control study (100 CAD patients, 108 CTR)	+++	Plasma/stable isotope dilution liquid chromatography–electrospray ionization tandem mass spectrometry-based method using an ion trap mass spectrometer	Increased 3-NT in CAD patients. OR of 4.4 (95% CI 1.8–10.6 for highest vs. lowest quartile) for CAD.	[96]
3-NT	Prospective cohort study (342 CAD patients)	+++	Serum/colorimetric ELISA	No relationship between 3-NT and mortality rates during 4-year follow-up.	[99]
3-NT	Clinical trial (120 prediabetic patients)	+++	Plasma/ELISA	2-h 3-NT after 75 g oral glucose tolerance tests is related to the presence of CAD (OR 3.1, 95% CI 2.2–5.3 for highest vs. lowest quartile).	[98]
3-NT	Animal study	+	Immunohistochemical analysis of rat hearts	Increased 3-NT after ischemia/reperfusion injury in rat hearts.	[243]
3-NT	Animal study	+	Dot blot method analysis of rat hearts	Increased 3-NT after MI in rats.	[244]
HNE	Clinical trial (23 patients with dilated cardiomyopathy, 13 CTR)	++	Immunohistochemical analysis of endomyocardial biopsies	5-fold increased HNE in cardiomyopathy patients. Treatment with carvedilol reduces HNE by 40%.	[80]
HNE	Clinical trial (22 patients with acute MI, 20 CTR)	++	Plasma/high-performance liquid chromatography	Increased HNE in MI patients. Infarct size strongly correlates with HNE.	[245]
HNE	Animal study	+	High-performance liquid chromatography analysis of rat hearts	Generation and release of HNE in isolated perfused hearts of normotensive and spontaneously hypertensive rats with cardiac hypertrophy and signs of HF during ischemia and reperfusion.	[246]
HNE	Animal study	+	Immunohistochemical and image analysis of rat hearts	Presence of HNE-modified proteins in ischemic rats hearts after transplantation and reperfusion.	[247]
8-iso PGF2 $\alpha$	Case-cohort study (250 cases of death due to CAD and stroke, 142 CTR)	+++	Urine/liquid chromatography/tandem mass spectrometry	Increased 8-iso PGF2 $\alpha$ predicts cardiovascular mortality in postmenopausal women (OR 1.8, 95% CI 1.1–3.1 for highest vs. lowest quartile).	[75]
8-iso PGF2 $\alpha$	Meta-analysis (242 studies)	++++	Mixed (plasma, urine, sputum)	Increased 8-iso PGF2 $\alpha$ in patients with hypertension (g 0.4, 95% CI 0.2–0.6), chronic HF (g 1.4, 95% CI 1.1–1.7), CAD (g 0.4, 95% CI 0.3–0.5) and ischemic stroke (g 1.2, 95% CI 1.0–1.4).	[76]
8-iso PGF2 $\alpha$	Animal study	+	Serum/enzyme immuno-assay	Increased 8-iso PGF2 $\alpha$ in the myocardium of rabbits with HF. 8-iso PGF2 $\alpha$ correlates with cardiac function.	[248]
8-iso PGF2 $\alpha$	Animal study	+	Plasma and myocardial tissue/ELISA	Increased 8-iso PGF2 $\alpha$ in rats with acute myocardial ischemia.	[249]
8-OHdG	Meta-analysis (14 studies)	++++	Mixed (urine and blood)	Increased 8-OHdG in CVD patients (SMD 1.04, 95% CI 0.61–1.47).	[118]
8-OHdG	Meta-analysis (6 studies)	++++	Mixed (urine and plasma)	Increased 8-OHdG in HF patients (SMD 0.89, 95% CI 0.68–1.10).	[119]
8-OHdG	Animal study	+	Immunohistochemical and image analysis of rat hearts as well as DNA analysis using ELISA	8-OHdG is related to cardiac dysfunction after ischemia/reperfusion injury in isolated rat hearts.	[250]
8-OHdG	Animal study	+	Immunohistochemical myocard analysis	Left ventricular dilatation and systolic dysfunction is associated increased 8-OHdG in rabbits after MI.	[251]
MDA	Prospective cohort study (774 chronic HF patients)	+++	Serum/Ohkawa's method	Increased MDA predicts death in chronic HF patients (RR 3.64, 95% CI 1.917–6.926 for highest vs. lowest quartile).	[83]
MDA	Prospective cohort study (634 stable CAD patients)	+++	Serum/TBARS	Increased MDA predicts cardiovascular events in patients with stable CAD (RR 3.30, 95% CI 1.47–7.42 for major vascular events, RR 4.10, 95% CI 2.55–6.60 for non-fatal vascular events and RR 3.84, 95% CI 2.56–5.76 for major vascular procedures).	[85]
MDA	Animal study	+	Plasma/ELISA	Increased MDA after myocardial injury induced by sepsis in rats.	[252]
MDA	Animal study	+	Immunohistochemical analysis of cardiac tissues	Increased MDA after HF induced by left anterior descending coronary artery ligation rats.	[253]
D-ROM	Meta-analysis (10,622 subjects)	++++	Serum/FRAP assay	Increased D-ROM predicts all-cause (RR 1.20, 95% CI 1.12–1.29 per 1-SD increase) and CVD mortality (RR 1.30, 95% CI 1.12–1.51) in 10,622 subjects from population-based cohorts.	[124]
D-ROM	Pooled case-control study (476 cases of MI and 2380 CTR, 454 cases of stroke and 2270 CTR)	+++	Serum/FRAP assay	Increased D-ROM predicts MI (OR 1.21, 95% CI 1.05–1.40 per 100 Carr units increase) and stroke (OR 1.17, 95% CI 1.01–1.35).	[125]
D-ROM	Animal study	+	Plasma/photometric test	Increased D-ROM in the myocardium of hypoxic rats.	[254]
D-ROM	Animal study	+	Serum/fras4 test		[255]

(continued on next page)

**Table 1** (continued)

Biomarker	Study type	Quality level	Sampling/method of detection	Major outcome	Reference
TTL	Meta-analysis (10,622 subjects)	++++	Serum/FRAP assay	Increased D-ROM is related to ventricular remodeling and arrhythmia in rats. Increased TTL predicts all-cause (RR 1.12, 95% CI 1.04–1.21 per 1-SD decrease) and CVD mortality (RR 1.25, 95% CI 1.09–1.45) in 10,622 subjects from population-based cohorts.	[124]
TTL	Pooled case-control study (476 cases of MI and 2380 CTR, 454 cases of stroke and 2270 CTR)	+++	Serum/FRAP assay	Increased TTL predicts stroke (OR 0.79, 95% CI 0.63–0.99 for quartiles 2–4 vs. quartile 1).	[125]
TTL	Animal study	+	Serum/spectrophotometry	Induction of MI in rats is associated with a decrease in TTL.	[256]
TTL	Animal study	+	Cardiac tissue/spectrophotometry	Induction of MI in rats is associated with a decrease in TTL.	[257]

+: Medium quality (animal study), ++: Good quality (clinical trial/cohort study <100 subjects), +++: Strong quality (clinical trial/cohort study >100 subjects), ++++: Very strong quality (large scale meta-analysis), 3-NT: 3-nitrotyrosine, CAD: Coronary artery disease, CTR: Controls, OR: Odds ratio, CI: Confidence interval, TBARS: Thiobarbituric Acid Reactive Substances, FRAP: Ferric Reducing Antioxidant Power, FRAS: Free Radical Analytical System 4, HNE: 4-Hydroxynonenal, MI: Myocardial infarction, HF: Heart failure, 8-iso PGF<sub>2</sub>α: 8-iso-prostaglandin F<sub>2</sub>α, g: standardized mean difference - Hedges'g, 8-OHdG: 8-Hydroxy-2-deoxyguanosine, CVD: Cardiovascular disease, MDA: Malondialdehyde, RR: Relative risk, D-ROM: Derivatives of reactive oxygen metabolite, SD: Standard deviation, TTL: Total thiol.

**Table 2**  
Selected microRNAs in human CVD.

miRNA	CVD	Clinical use	Molecular mechanism	Reference
199	CAD	Associated with MACE reduction	SIRT-1 induction	[140]
21	Heart failure	Screening parameter	Upregulation of natriuretic peptide B	[147, 148]
1254 and 1306-5p	Heart failure	Association with mortality	Unknown	[145]
26b-5p, 660-5p and 320a	STEMI	MACE prediction	Unknown	[141]
107, 22-3p and 146a-5p	AF	MACE prediction	Unknown	[160]

MACE: major adverse cardiovascular events; SIRT-1: sirtuin 1; STEMI: ST-elevation myocardial infarction; AF: atrial fibrillation.

trials [134]. Notably, none of the trials involve patients suffering from CVD.

MicroRNAs display a huge potential as a screening marker in CVD while at the same time suffering from an enormous gap of evidence. On the one hand, certain miRNAs were shown to independently predict cardiovascular events in certain pathological conditions (Table 2) while on the other, only a fraction of them has so far been investigated regarding their molecular targets. Further research on the molecular mechanisms of miRNAs in CVD seems warranted as their redox-sensitivity can be assumed from the available data [135], from their own contribution to ROS/RNS formation [136] and from the fact that their levels change in diseases that are associated with oxidative stress.

#### 4.1. Coronary artery disease and miRNAs 24, 92a and 199a

miRNA-24 is highly expressed in the vasculature [137] and is upregulated in response to vascular injury which in turn indirectly activates the NRF2-pathway by regulating KEAP1 resulting in an overall protective effect [138]. While miRNA-92a overexpression leads to impaired endothelial function by way of reducing heme oxygenase-1 (HO-1) expression, thus promoting the formation of oxidative stress in a murine model for diabetic vasculopathy [139], miRNA-199a expression was found to be lowered in patients with CAD [140]. In these patients, who had undergone coronary artery bypass surgery, miRNA-199a expression levels were found to be reduced in myocardial tissue in those who would

be less likely to suffer from major adverse cardiac and cerebrovascular events over the course of three years following surgery [140]. This predictive effect was attributed to the upregulation of SIRT1 in response to lowered miRNA-199a levels.

#### 4.2. Myocardial infarction and miRNAs 26b-5p, 660-5p and 320a

In patients presenting with ST-segment elevation MI (STEMI), a study by Jakob and colleagues found three miRNAs, 26b-5p, 660-5p and 320a, which were associated with cardiac death or recurrent MI within the next year [141]. In this study, miRNA levels in blood samples were shown to independently predict MACE while their predictive value was improved via combination with the clinically used GRACE score. While the miRNAs associated with cardiovascular events in this study are known to be involved in the development of cardiac hypertrophy [142] and cardiomyocyte apoptosis [143], no experimental data are available regarding their redox-sensitivity.

#### 4.3. Heart failure and miRNAs 1254, 1306 and 21

Amongst the myriad of microRNAs screened in the serum of patients suffering from heart failure (reviewed in detail by Zhou and colleagues [144]), miRNAs 1254 and 1306 have recently been identified as prognostic markers in the blood of approximately 2200 patients [145]. While in this study, higher levels of miRNAs 1254 and 1306 were associated with the risk for all-cause mortality and hospitalization due to heart failure, the mechanism behind this effect remains unknown [145]. MiRNA-1254 was also predictive of ventricular remodeling in patients with ST-segment-elevation MI [146]. MiRNA-21 was found to be upregulated in murine models for heart failure by Vettori and colleagues [147] as well as in patients suffering from heart failure [148–150], subclinical diabetic cardiomyopathy [151] and diastolic dysfunction [152]. MiRNA-21 is upregulated in multiple types of human cancer [153] and cell-growth in general, and consequently linked to cardiac hypertrophy [154] and vascular neointimal lesion formation [155]. MiRNA-21 was also implicated in suppression of angiogenic progenitor cell function in CAD patients via inhibition of eNOS by asymmetric dimethyl arginine [156]. Of note, treatment with the antagomir anti-miRNA-21 prevented myocardial damage and normalized cardiac function in a pig model of I/R injury [157].

#### 4.4. Atrial fibrillation and miRNAs 107, 22-3p and 146a-5p

Within the last two years, miRNAs in atrial fibrillation (AF) have

received increased attention. While a number of studies suggest a relationship of certain miRNAs with incident or recurrent AF [158,159], a recent study by Rivera-Caravanca et al. was able to show a correlation between miRNAs 107, 22-3p and 146a-5p and a higher risk of major cardiovascular events during a mean follow-up of 7.6 years [160]. While miRNAs 22-3p and 107 correlated positively with risk (HR 1.07 and 3.66, respectively), a negative correlation was found regarding miRNA 146a-5p (HR 0.86) [160]. For these miRNAs, however, data is lacking on whether they are regulated by oxidative stress.

## 5. Advanced redox biomarkers

### 5.1. sNox2-dp as a read-out of NOX2 activation in human cardiovascular/metabolic disease

Although NADPH oxidases are already investigated for therapeutic targeting since more than a decade, there are still no NOX inhibitors in clinical use for the therapy of CVD [161,162]. So far, there are several promising drug candidates for NOX inhibition in clinical phase II and III trials, although not directly for the therapy of CVD but associated comorbidities [27,163].

The molecular proof to demonstrate the direct association of soluble nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) 2-derived peptide (sNox2-dp) with NOX-2 activity was conducted in patients with chronic granulomatous disease<sup>4</sup> displaying better endothelial function (measured by flow-mediated dilation, FMD) and lower sNOX2-dp and 8-iso-PGF2 $\alpha$  levels [164]. Mechanistically, a burst of ROS was detected in collagen-stimulated platelets and in phorbol ester-stimulated human neutrophils, which was accompanied by a maximum of sNox2-dp release and could be blocked by matrix metalloproteinase-2 inhibition or the NOX-2 inhibitor NOX2ds-tat [165]. The presence of two comorbidities in children, hypercholesterolemia and obesity, resulted in an additive aggravation of 8-isoprostanes and sNox2-dp as well as impairment of endothelial function (FMD) [166]. Higher sNox2-dp as well as oxidized LDL levels were also observed in children with only one risk factor, hypercholesterolemia [167]. Patients with peripheral artery disease had enhanced sNOX2-dp and isoprostane levels but lower NO-derived metabolites and impaired endothelial function (measured by flow-mediated dilation), all of which correlated with more pronounced p47phox translocation to the membrane in patient platelets or white blood cells [168]. Non-alcoholic steatohepatitis (NASH) showed an association with higher levels of 8-isoprostanes and sNox2-dp, which also showed a correlation with histological grading of steatosis and liver inflammation, ballooning and fibrosis [169]. Non-alcoholic fatty liver disease (NAFLD) was associated with higher sNox2-dp and 8-isoprostane levels in patients, also correlating with more severe steatosis and portal inflammation [170] or with markers of infection [171]. Likewise, peripheral endothelial dysfunction (FMD) in patients with NASH also correlated with sNox2-dp and isoprostane levels, all of which was prevented by dietary uptake of polyphenol-rich dark chocolate [172]. Weight loss ( $\geq 5\%$ ) in patients with metabolic syndrome significantly diminished the serum sNOX2-dp and urinary 8-iso-PGF2 $\alpha$  levels and improved the antioxidant status [173].

Patients with COPD and AF showed higher levels of sNox2-dp and 8-isoprostane, which were additively increased in the presence of both risk factors [174]. Patients with obstructive sleep apnoea syndrome (OSAS) had higher urinary 8-iso-PGF2 $\alpha$  and serum sNox2-dp levels but lower NO-derived metabolites and impaired endothelial function (measured by flow-mediated dilation), all of which was ameliorated by continuous positive airway pressure therapy (CPAP) [175]. Similar observations were made in children with sleep disordered breathing who showed

higher sNox2-dp and 8-iso-PGF2 $\alpha$  levels but impaired endothelial function (measured by flow-mediated dilation) [176]. Also, active smokers displayed serum sNox2-dp levels but lower NO-derived metabolites and impaired endothelial function (measured by flow-mediated dilation) [177]. Children exposed to passive smoke also showed similar effects as the active adult smokers [178]. Workers exposed to metal-rich particulate matter with an aerodynamic diameter  $< 10 \mu\text{m}$  (PM<sub>10</sub>) had higher urinary 8-iso-PGF2 $\alpha$  and 8-OHdG as well as plasma sNOX2-dp levels [179].

### 5.2. Advanced glycation end products

Advanced glycation end products (AGEs) are a group of highly reactive compounds that are formed by reaction of lipids and proteins with AGE precursors such as (methyl)glyoxal [180]. AGE precursors are generated in the presence of high glucose/fructose levels under oxidative stress conditions as observed in diabetic patients but may also accumulate during the aging process [181] and by dietary uptake [182]. Glycation of lipids and proteins can change the activity and biological properties of these biomolecules but AGEs can also promote crosslinking and further oxidation of biomolecules. AGEs can also bind to their receptor (RAGE) and thereby induce inflammation and oxidative stress [183]. In an elderly population of 2111 U.S. subjects free of CVD at baseline, increased levels of carboxymethyl-lysine, a major AGE, were associated with elevated risk of incident cardiovascular events (comprising stroke and/or CAD) after multivariable adjustment (HR 1.11, 95% CI 1.03–1.19 per 1-SD increase) [184]. Likewise, Semba et al. demonstrated increased levels of carboxymethyl-lysine to be associated with increased risk of all-cause (HR 1.84, 95% CI 1.30–2.60 for highest vs. lowest two tertiles) and CVD mortality (HR 2.11, 95% CI 1.27–3.49) in a cohort of 1013 elderly Italian subjects after multivariable adjustment [185]. These observations were also confirmed for subjects without diabetes mellitus. Of note, there are pharmacological inhibitors of the AGE/receptor of AGE (RAGE) axis that may be translated to the clinical setting [186].

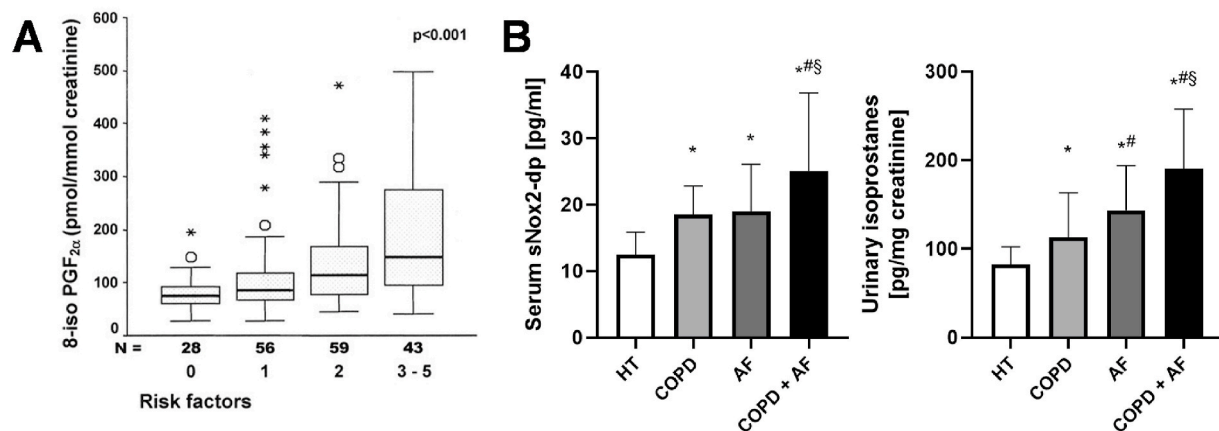
### 5.3. Myeloperoxidase expression and activity

Myeloperoxidase (MPO) expression and activity were proposed as suitable redox biomarkers describing a toxifying mechanism of H<sub>2</sub>O<sub>2</sub> with rather low reactivity in the presence of chloride compared to the more reactive hypochlorite (OCl<sup>-</sup>) [43,161]. In 1090 patients with acute coronary syndrome, MPO levels were assessed to determine its association with death and MI at 6-month follow-up [187]. The results displayed that elevated MPO levels ( $> 350 \mu\text{g/L}$ ) predicted an increased risk of cardiovascular events (HR 2.25, 95% CI 1.32–3.82), which were also shown to extend the prognostic value derived from traditional biochemical markers. In good agreement with these previous observations, MPO levels predicted increased odds of a major adverse cardiac event at 6-month follow-up in 490 subjects with acute chest pain [188]. Likewise, the activity of MPO was previously measured by 3-chlorotyrosine (or 3-bromotyrosine) concentrations in patients with MI by HPLC with electrochemical detection [189], as well as 2-chloroethidium using liquid chromatography with mass spectrometry quantification [190]. Although the clinical evidence for prognostic value of MPO in CVD is so far moderate, this redox biomarker was proposed as a reliable candidate for future routine use (reviewed in Refs. [161,191]). The importance of MPO as a redox biomarker is supported by several pharmacological MPO inhibitors that are currently investigated within phase II trials [27].

### 5.4. Redox profiling

In principle any protein involved in ROS/RNS generation or degradation can be used as a redox biomarker and measurement of its expression or activity will provide limited mechanistic insights and will

<sup>4</sup> Patients suffering from inborn defects in NADPH function - in the mentioned study gp91phox (x chromosomal recessive CGD) and p47phox (autosomal recessive CGD Type 1).



**Fig. 4. Additivity of comorbidities on redox biomarkers levels in patients with cardiovascular disease.** (A) Urinary levels of 8-iso-PGF<sub>2α</sub> showed additive accumulation with risk factors for heart disease (comorbidities such as obesity, diabetes mellitus, hypercholesterolemia, hypertension, and smoking). Median, interquartile range, outliers and extremes of urinary excretion are presented; p-value was calculated for the trend. Reused from Ref. [77] with permission. Copyright © 2004, Wolters Kluwer Health. (B) Levels of sNox2-dp and urinary isoprostanes in patients with hypertension (HT) and chronic obstructive pulmonary disease (COPD) and/or atrial fibrillation (AF) on top. Data are presented as mean ± SD (n = 33–49). \*, p < 0.05 vs. HT; #, p < 0.05 vs. COPD; §, p < 0.05 vs. AF. Graphs were generated from data in Ref. [174] with permission. Copyright © 2019, Mary Ann Liebert Inc.

probably also correlate with disease severity or cardiovascular risk. As the number of publications on this topic is huge and even discussion of selected examples would exceed the frame of the present review, we will only add some general considerations here. The expression and activity of antioxidant enzymes such as SODs, catalase, glutathione peroxidases or heme oxygenase-1 represent controversial redox biomarkers as they may be upregulated in one disease and down-regulated in the other. In principle, most antioxidant enzymes are under the control of redox-regulated transcription factors implying that under oxidative stress conditions their expression should be upregulated. However, also expression-suppressing mechanisms may come into play at the epigenetic level or by proteasomal degradation as well as activity of these antioxidant enzymes may be oxidatively inhibited as in the case of Mn-SOD [92]. Indeed, SODs, catalase, glutathione peroxidases are often found down-regulated or inactivated under disease conditions serving as a potential explanation of the observed oxidative stress condition. In contrast, heme oxygenase-1 is often upregulated due to the nature of its regulation via NRF2/KEAP1 that are activated by electrophiles such as ROS, although other regulatory mechanisms exist as well [26]. NRF2/KEAP1 and their antioxidant down-stream targets are therefore suitable redox biomarkers that characterize part of the antioxidant defense system and the process of redox hormesis. Also the activity of dysfunctional redox-sensitive enzymes, e.g. in mitochondria, can be used as a redox biomarker read-out: oxidative inactivation of mitochondrial aldehyde dehydrogenase (ALDH-2) correlated with larger infarct size in mouse models of MI [192] and aconitase activity was associated with lipid oxidation in the human failing heart [193], although both enzymatic activities are so far not regularly used as redox biomarkers in the clinical setting.

In addition, proteasomal activity may represent an attractive readout of redox dysregulation as the proteasomal system is subject to redox regulation [194] and changes in proteasomal activity will ultimately lead to the accumulation of oxidatively modified proteins [195]. Therefore, the determination of markers of proteasome activity by measurement of the concentrations of typical oxidized targets for proteasomal degradation (e.g. protein carbonyls) may provide additional mechanistic insights into dysregulated redox processes. Also impairment of the circadian rhythm is a well-accepted cardiovascular risk factor [196]. Oxidative stress and adverse redox signalling play a central role for the control of the circadian rhythm (also termed “redox control of cellular timekeeping”) [197]. A detailed description of harmful redox modifications within the circadian clock system and the impact of environmental stressors therein can be found in Ref. [198].

Also thiol-based enzymes play an important role for antioxidant defense and cellular redox signaling. Thioredoxins, peroxiredoxins and glutaredoxins play an important role for hydrogen peroxide and organic peroxide degradation but also their expression or activity shows no consistent regulatory readout (up- or down-regulation) for different CVD [199]. Therefore, assessment of a larger number of these redox markers, e.g. by (redox) proteomics [200,201] or transcriptomics, will provide more meaningful insights. The resulting “redox profiles” may show a characteristic pattern of up- and down-regulated antioxidant proteins or mRNA for a specific disease condition. A general description of redox analytical approaches can be found in Refs. [202,203], for redox proteomics of S-glutathionylated proteins in Ref. [204], for H<sub>2</sub>S-modified proteome (known as the sulfhydrylome) in Ref. [205] and for a systems biology approach in Ref. [206].

Redox profiling can be also based on assessment of a metabolic pattern. Determination of the GSH/GSSG ratio is the simplest readout of the cellular or serum/plasma redox state, however, with only limited prognostic implications [43]. Assessment of the trans-sulfuration pathway may be a more meaningful approach in the future [207]. Redox lipidomics (or oxidative phospholipidomics) [208,209] and phosphoproteomics [210] represent smaller subsets of the metabolome and proteome that are optimized to assess altered redox processes or oxidative stress conditions. In addition, the content of extracellular vesicles can provide important information on dysregulated redox processes and represent a useful prognostic tool to assess cardiovascular risk [211].

Finally, redox regulation of epigenetic profiles is a well-known process in the cancer field [212,213]. Epigenetic pathways involve a plethora of redox switches such as direct DNA modification by ROS (e.g. 5-hydroxy-methylcytosine or 8-OHdG), redox control of DNA methyltransferases (DNMTs) or DNA demethylases (e.g. TET) via oxygen/H<sub>2</sub>O<sub>2</sub> or redox-sensitive transcription factors, redox control of histone methyl/acetyltransferases (e.g. SMYD1) as well as demethylases/deacetylases (e.g. Jumoni, sirtuins) [37]. The known redox changes of enzymes involved in epigenetic control in heart disease were also previously summarized in full detail [214]. The above mentioned redox regulation of miRNAs and ncRNAs expression by various mechanisms also adds to this concept [39,136]. In general, the genome stability is redox regulated by various epigenetic and classical pathways [38]. The classical genome integrity maintaining mechanisms comprise the redox control of DNA repair mechanisms, mRNA stability (via GAPDH, AP-1 and HuR) and control of gene expression by redox-sensitive transcription factors such as NRF2, HIF-1α, OxyR and NFκB. Of note, epigenetic changes are



implicated in the pathogenesis of CVD [215,216]. In the future, the complete characterization of epigenetic profiles in heart disease may allow conclusions on the contribution of redox signaling pathways or specific ROS/RNS to these epigenetic changes and thereby disease severity/progression.

## 6. Additivity of redox biomarkers in the presence of multiple comorbidities

According to the proposed concept in Fig. 1, patients with different comorbidities should have additive accumulation of redox biomarkers. As a proof of concept, we here present selected examples. Patients with COPD and AF had elevated sNox2-dp and 8-isoprostane levels that showed an additive increase in the presence of both risk factors (Fig. 4) [174]. 3-NT levels in both abnormal glucose tolerance status and presence of CAD increased compared with the combination of normal glucose tolerance status and the presence of CAD [98]. Smoking, diabetes and body mass index were highly associated with systemic oxidative stress as determined by creatinine-indexed urinary 8-epi-PGF<sub>2α</sub> levels and a history of CVD [217]. Urinary 8-epi-PGF<sub>2α</sub> concentrations (normalized to creatinine) were also indicative of the presence of insulin resistance and obesity as envisaged by a dramatic increase in insulin-resistant patients with a body mass index  $\geq 30$  [218]. There was also an additive accumulation of 8-iso-PGF<sub>2α</sub> levels in the urine of CAD patients with multiple comorbidities such as obesity, diabetes mellitus, hypercholesterolemia, hypertension and smoking (Fig. 4) [77]. Levels of the AGE marker, carboxymethyl-lysine, in a population-based study were higher in the presence of the comorbidity diabetes and this additive increase was also associated with higher all-cause and CVD mortality of non-diabetic subjects [185]. Plasma levels of 8-iso-PGF<sub>2α</sub> gradually increased in individuals that were healthy, underwent coronary angiography without coronary artery atherosclerotic lesions or with proven CAD ( $123.2 \pm 9.5$ ,  $314.6 \pm 40$  and  $389.6 \pm 36.2$  pg/ml, respectively) [219]. Importantly, in the patient group, there was a substantial increase in 8-iso-PGF<sub>2α</sub> concentration in the presence of hypertension as compared to the normotensive group ( $394.2 \pm 42.7$  and  $232.7 \pm 25.1$  pg/ml, respectively) and also patients with dyslipidemia displayed higher 8-iso-PGF<sub>2α</sub> levels ( $359.1 \pm 35.6$  and  $240.3 \pm 34.3$  pg/ml). Finally, this study revealed a positive correlation between 8-iso-PGF<sub>2α</sub> concentrations and age. At the functional level, the presence of the typical cardiovascular comorbidities smoking and hypercholesterolemia aggravated endothelial dysfunction (measured by impaired acetylcholine-dependent forearm blood flow increases by plethysmography) in an additive manner [220]. This trend was also mirrored by the plasma levels of autoantibodies against oxidized LDL, which was significantly elevated in smokers with hypercholesterolemia.

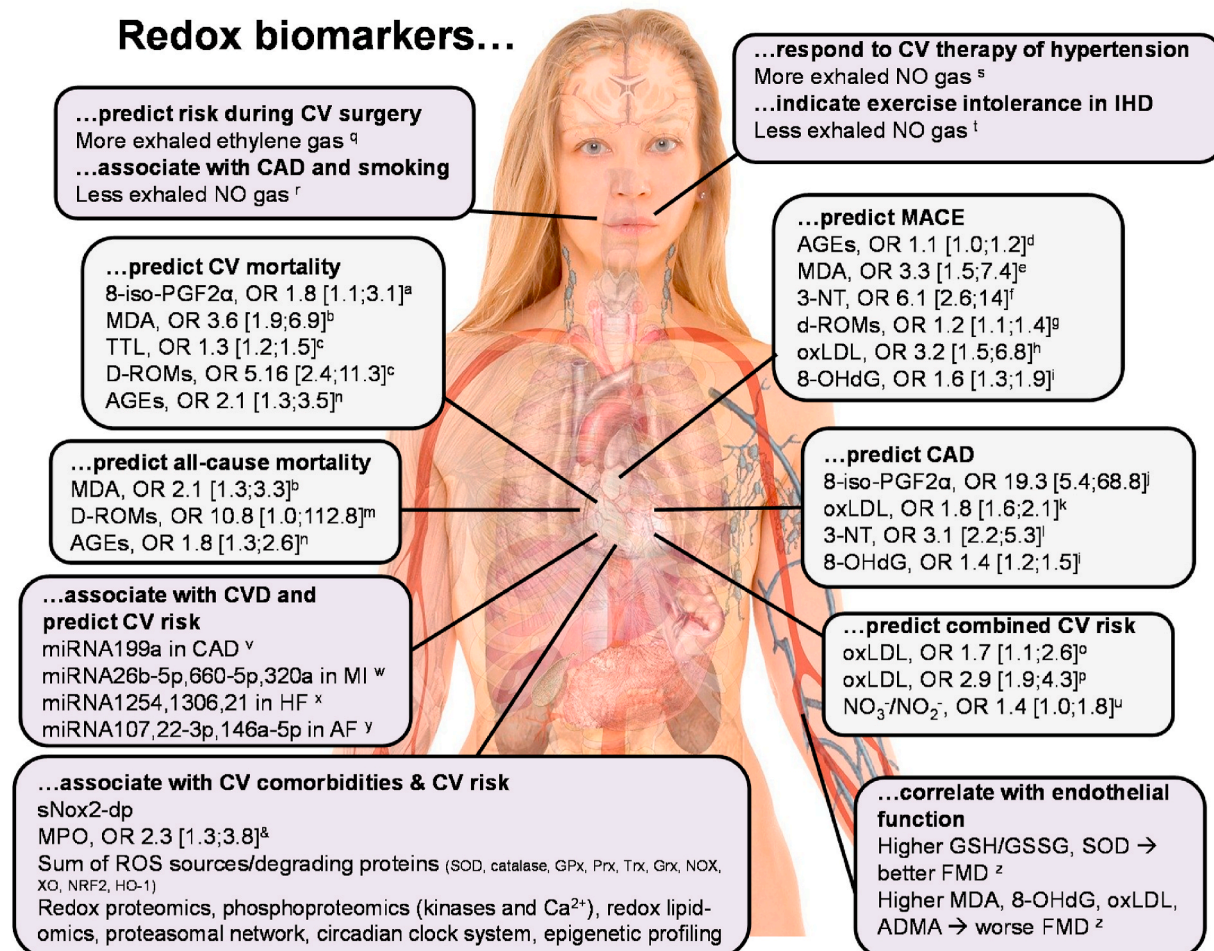
In a study with 46 patients with rheumatoid arthritis endothelial function, as assessed by FMD and coronary flow reserve, and nitro-oxidative stress, as assessed by 3-NT and MDA, were shown to be related to left ventricular myocardial deformation in rheumatoid arthritis patients [221]. In a double-blind crossover trial in 80 patients with rheumatoid arthritis (60 with CAD and 20 without) patients with CAD had 3-fold higher protein carbonyl, 3-NT, and MDA levels compared to the ones without CAD [222]. In a study of 100 poorly-controlled diabetic patients the reduction of oxidative stress was associated with improved arterial elasticity and myocardial deformation after 12 months of intensive antidiabetic treatment indicating that a decrease of oxidative stress burden was associated with a greater improvement of vascular function and myocardial deformation [223]. In 115 newly diagnosed untreated patients with primary systemic amyloidosis, increased FMD was found to be an independent predictor of all-cause mortality. Increased 3-NT levels were observed in high-risk amyloidosis patients with elevated FMD and hypotension, which may be secondary to increased inflammation and iNOS activity, as exhaled NO gas as well as 3-NT levels were also higher in these patients [224].

A major cardiovascular comorbidity is the aging process and

increased (mitochondrial) oxidative stress, impaired endothelial function as well as higher cardiovascular risk in the elderly is well documented [225]. The accumulating oxidative damage with increasing age is also supported by exacerbated lipofuscin concentrations in the lysosomal system of the elderly, where lipofuscin represents a highly aggregated mixture of cross-linked proteins, peroxidized lipids and carbohydrates [226]. Of note, lipofuscins themselves represent a source of ROS and trigger apoptosis, inhibit the proteasomal system and cause mitochondrial dysfunction [227], all of which leads to dysregulated proteostasis and thereby to a loss of a functional proteome due to insufficient removal of damaged/inactive proteins [226]. Despite this appealing concept, at present there are no large scale clinical studies or meta-analyses that link the aging process with accumulation of lipofuscin, whereas accumulation of lipofuscin in the retina seems to be associated with age-related macular degeneration [228]. However, support for an association of oxidative stress and aging comes from a study of 2190 subjects undergoing a health care examination by showing substantial relations to total antioxidant status, total peroxides and autoantibodies against oxidized LDL with age and body mass index [229]. These results were confirmed in smaller studies that demonstrated aging to be correlated with increased oxidative stress or impaired antioxidant defense [230–236]. Moreover, the MARK-AGE project revealed rather moderate associations of markers of oxidative stress or antioxidants with progressing age [237]. Only 3-NT showed a weak correlation with age and higher levels against the middle-aged control group, whereas no comparable trend was found for protein carbonyls and MDA. Only the antioxidant lycopene was lower in all higher age groups, whereas reduced cysteine levels even tended to increase with higher age. The authors concluded that lifestyle and environmental factors have a more pronounced impact on age-related redox biomarkers than previously anticipated [237]. Whereas the levels of most redox biomarkers seem to correlate with the progression or severity of a certain disease, so far there are no clear threshold values that could be used for early disease diagnosis, especially since most cardiovascular disease display a multi-comorbidity profile. Therefore, better redox biomarkers or even profiles are needed for a reliable cardiovascular risk stratification and the establishment of redox biomarkers in clinical routine use.

## 7. Conclusions and outlook

There are basically two major classes of redox biomarkers (Fig. 2). The classical markers of oxidative stress can neither provide specificity for the nature of ROS/RNS nor for certain disease/comorbidities but they have prognostic implications (Fig. 3 and Table 1) and show additivity in the presence of different disease/comorbidities (Fig. 4). The more meaningful redox profiling techniques (e.g. redox proteomics, lipidomics and epigenomics) may help in the future to add the missing specificity to redox diagnostics, to understand and better discriminate beneficial and harmful ROS/RNS processes (e.g. dysregulated kinase, proteasomal, circadian networks) and to identify highly specific redox targets for the development of novel redox drugs. The most promising non-classical redox biomarkers, even when measured as a single parameter, could be NOX enzymes, NRF2 and MPO as they are also subject therapeutic targeting within advanced clinical phase II-III trials on multiple disease categories [26,27]. Combining of these redox biomarkers with functional measurements, e.g. redox-sensitive endothelial function using flow-mediated dilation or acetylcholine-dependent increases in peripheral blood flow by plethysmography [9,238], may further improve their prognostic value. Endothelial function itself also correlates with markers of oxidative stress and redox biomarkers [239–241]. In addition, direct measurement of ROS/RNS formation using specific probes can provide valuable information on the nature, source and disease mechanisms but are hardly applicable to large clinical trials as storage of samples will usually modify the readout due to autoxidation of the probes or inactivation of enzymatic sources in a time-dependent manner. Other protocols for assessment of redox-related



**Fig. 5. Odds ratio (OR) or association of redox biomarkers with health risks or complications.** Grey boxes represent classical oxidative stress markers. Purple boxes represent advanced or experimental redox biomarkers. Numbers in square brackets represent the 95% confidence interval. Abbreviations: ADMA, asymmetric dimethylarginine; CV, cardiovascular; CAD, coronary artery disease; CVD, cardiovascular disease; FMD, flow-mediated dilation; IHD, ischemic heart disease; MACE, major cardiovascular events; oxLDL, oxidized low-density lipoprotein. All alphabetical cross references in this figure (<sup>a</sup>, <sup>b</sup>, <sup>c</sup> ...) are linked to literature reference numbers in the figure legend. The respective references are as follows: <sup>a</sup> [75], <sup>b</sup> [83], <sup>c</sup> [124], <sup>d</sup> [184], <sup>e</sup> [85], <sup>f</sup> [96], <sup>g</sup> [125], <sup>h</sup> [88], <sup>i</sup> [120], <sup>j</sup> [77], <sup>k</sup> [86], <sup>l</sup> [98], <sup>m</sup> [126], <sup>n</sup> [185], <sup>o</sup> [87], <sup>p</sup> [89], <sup>q</sup> [90], <sup>r</sup> [108], <sup>s</sup> [110], <sup>t</sup> [111], <sup>u</sup> [114], <sup>v</sup> [140], <sup>w</sup> [141], <sup>x</sup> [145,148–152], <sup>y</sup> [160], <sup>z</sup> [239–241], & [187]. Open access source for body image can be found at Pixabay (<https://pixabay.com/de/photos/anatomie-frau-mensch-k%C3%B6rper-haut-254120/>). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

biomarkers or biological changes with relevance for preclinical and clinical studies on cardioprotection (e.g. oxygen consumption or mitochondrial membrane potential) were previously summarized in a compendium of practical guidelines [242]. Fig. 5 summarizes the prognostic value of the classical markers of oxidative stress and features of other redox biomarkers. Further support for the prognostic value of redox biomarkers (mostly oxidative stress parameters) comes from multiple studies on pharmacological antioxidant interventions leading to a normalization of these redox biomarkers as well as functional parameters (reviewed in Ref. [18]).

### Funding

A.D., S.S. and T.M. were supported by vascular biology research grants from the Boehringer Ingelheim Foundation for the collaborative research group “Novel and neglected cardiovascular risk factors: molecular mechanisms and therapeutics”. Further support was provided by the Else-Kröner Fresenius Foundation (2019\_A110 to S.D. and 2017\_A106 to S.S.), the German Heart Foundation/German Foundation of Heart Research (F/51/19 to S.D.) and the Foundation Heart of Mainz (continuous support to A.D., O.H., S.S. and S.D.). I.A. acknowledges

support from the European Union (ERDF) and Greek national funds through the Operational Program “Competitiveness, Entrepreneurship and Innovation”, under the call “RESEARCH – CREATE - INNOVATE” (project code: 5048539). Our research was continuously supported by the European Cooperation in Science and Technology and EU-CARDIOPROTECTION COST-ACTION (CA16225), a funding scheme to enhance scientific networking in Europe. Thomas Münzel is PI of the DZHK (German Center for Cardiovascular Research), Partner Site Rhine-Main, Mainz, Germany.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### References

- S.S. Lim, T. Vos, A.D. Flaxman, G. Danaei, K. Shibuya, H. Adair-Rohani, M. Amann, H.R. Anderson, K.G. Andrews, M. Aryee, C. Atkinson, L.J. Bacchus, A. N. Bahalim, K. Balakrishnan, J. Balmes, S. Barker-Collo, A. Baxter, M.L. Bell, J. D. Blore, F. Blyth, C. Bonner, G. Borges, R. Bourne, M. Boussinesq, M. Brauer, P. Brooks, N.G. Bruce, B. Brunekreef, C. Bryan-Hancock, C. Bucello,

- R. Buchbinder, F. Bull, R.T. Burnett, T.E. Byers, B. Calabria, J. Carapetis, E. Carnahan, Z. Chafe, F. Charlson, H. Chen, J.S. Chen, A.T. Cheng, J.C. Child, A. Cohen, K.E. Colson, B.C. Cowie, S. Darby, S. Darling, A. Davis, L. Deegenhardt, F. Dentener, D.C. Des Jarlais, K. Devries, M. Dherani, E.L. Ding, E.R. Dorsey, T. Driscoll, K. Edmond, S.E. Ali, R.E. Engell, P.J. Erwin, S. Fahimi, G. Falder, F. Farzadfar, A. Ferrari, M.M. Finucane, S. Flaxman, F.G. Fowkes, G. Freedman, M.K. Freeman, E. Gakidou, S. Ghosh, E. Giovannucci, G. Gmel, K. Graham, R. Grainger, B. Grant, D. Gunnell, H.R. Gutierrez, W. Hall, H.W. Hoek, A. Hogan, H.D. Hosgood 3rd, D. Hoy, H. Hu, B.J. Hubbell, S.J. Hutchings, S.E. Ibeanusi, G. L. Jacklyn, R. Jasrasaria, J.B. Jonas, H. Kan, J.A. Kanis, N. Kassebaum, N. Kawakami, Y.H. Khang, S. Khatibzadeh, J.P. Khoo, C. Kok, F. Laden, R. Lalloo, Q. Lan, T. Lathlean, J.L. Leasher, J. Leigh, Y. Li, J.K. Lin, S.E. Lipschultz, S. Lozano, R. Lozano, Y. Lu, J. Mak, R. Malekzadeh, L. Mallinger, W. Marcesnes, L. March, R. Marks, R. Martin, P. McGale, J. McGrath, S. Mehta, G.A. Mensah, T. R. Merriman, R. Micha, C. Michaud, V. Mishra, K. Mohd Hanafiah, A.A. Mokdad, L. Morawwan, D. Mozaffarian, T. Murphy, M. Naghavi, B. Neal, P.K. Nelson, J. M. Nolla, R. Norman, C. Olives, S.B. Omer, J. Orchard, R. Osborne, B. Ostro, A. Page, K.D. Pandey, C.D. Parry, E. Passmore, J. Patra, N. Pearce, P.M. Pelizzari, M. Petzold, M.R. Phillips, D. Pope, C.A. Pope 3rd, J. Powles, M. Rao, H. Razavi, E. A. Rehfuss, J.T. Rehm, B. Ritz, F.P. Rivara, T. Roberts, C. Robinson, J. A. Rodriguez-Portales, I. Romieu, R. Room, L.C. Rosenfeld, A. Roy, L. Rushton, J. A. Salomon, U. Sampson, L. Sanchez-Riera, E. Sanman, A. Sapkota, S. Seedat, P. Shi, K. Shield, R. Shivakoti, G.M. Singh, D.A. Sleet, E. Smith, K.R. Smith, N. J. Stapelberg, K. Steenland, H. Stockl, L.J. Stovner, K. Straif, L. Straney, G. D. Thurston, J.H. Tran, R. Van Dingenen, A. van Donkelaar, J.L. Veerman, L. Vijayakumar, R. Weintraub, H.M. Weissman, R.A. White, H. Whiteford, S. T. Wiersma, J.D. Wilkinson, H.C. Williams, W. Williams, N. Wilson, A.D. Woolf, P. Yip, J.M. Zielinski, A.D. Lopez, C.J. Murray, M. Ezzati, M.A. AlMazroa, Z. A. Memish, A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010, *Lancet* 380 (2012) 2224–2260, [https://doi.org/10.1016/S0140-6736\(12\)61766-8](https://doi.org/10.1016/S0140-6736(12)61766-8).
- [2] C.J. Murray, M. Ezzati, A.D. Flaxman, S. Lim, R. Lozano, C. Michaud, M. Naghavi, J.A. Salomon, K. Shibuya, T. Vos, D. Wikler, A.D. Lopez, GBD 2010: design, definitions, and metrics, *Lancet* 380 (2012) 2063–2066, [https://doi.org/10.1016/S0140-6736\(12\)61899-6](https://doi.org/10.1016/S0140-6736(12)61899-6).
- [3] R. Lozano, M. Naghavi, K. Foreman, S. Lim, K. Shibuya, V. Aboyans, J. Abraham, T. Adair, R. Aggarwal, S.Y. Ahn, M. Alvarado, H.R. Anderson, L.M. Anderson, K. G. Andrews, C. Atkinson, L.M. Baddour, S. Barker-Collo, D.H. Bartels, M.L. Bell, E. J. Benjamin, D. Bennett, K. Bhalla, B. Bikbov, A. Bin Abdulhak, G. Birbeck, F. Blyth, I. Bolliger, S. Boufous, C. Bucello, M. Burch, P. Burney, J. Carapetis, H. Chen, D. Chou, S.S. Chugh, L.E. Coffey, S.D. Colan, S. Colquhoun, K. E. Colson, J. Condon, M.D. Connor, L.T. Cooper, M. Corriere, M. Cortinovis, K. C. de Vaccaro, W. Couser, B.C. Cowie, M.H. Criqui, M. Cross, K.C. Dabhadkar, N. Dahodwala, D. De Leo, L. Degenhardt, A. Delossantos, J. Denenberg, D.C. Des Jarlais, S.D. Dharmaratne, E.R. Dorsey, T. Driscoll, H. Duber, B. Ebel, P.J. Erwin, P. Espindola, M. Ezzati, V. Feigin, A.D. Flaxman, M.H. Forouzanfar, F.G. Fowkes, R. Franklin, M. Fransen, M.K. Freeman, S.E. Gabriel, E. Gakidou, F. Gaspari, R. F. Gillum, D. Gonzalez-Medina, Y.A. Halasa, D. Haring, J.E. Harrison, R. Havmoller, R.J. Hay, B. Hoen, P.J. Hotez, D. Hoy, K.H. Jacobsen, S.L. James, R. Jasrasaria, S. Jayaraman, N. Johns, G. Karthikeyan, N. Kassebaum, A. Keren, J. P. Khoo, L.M. Knowlton, O. Kobusingye, A. Koranteng, R. Krishnamurthi, M. Lipnick, S.E. Lipschultz, S.L. Ohno, J. Mabwajano, M.F. Maclntyre, L. Mallinger, L. March, G.B. Marks, R. Marks, A. Matsumori, R. Matzopoulos, B. M. Mayosi, J.H. McAnulty, M.M. McDermott, J. McGrath, G.A. Mensah, T. R. Merriman, C. Michaud, M. Miller, T.R. Miller, C. Mock, A.O. Mocosumbi, A. A. Mokdad, A. Moran, K. Mulholland, M.N. Nair, L. Naldi, K.M. Narayan, K. Nasser, P. Norman, M. O'Donnell, S.B. Omer, K. Ortblad, R. Osborne, D. Ozgediz, B. Pahari, J.D. Pandian, A.P. Rivero, R.P. Padilla, F. Perez-Ruiz, N. Perico, D. Phillips, K. Pierce, C.A. Pope 3rd, E. Porrini, F. Pourmalek, M. Raju, D. Ranganathan, J.T. Rehm, D.B. Rein, G. Remuzzi, F.P. Rivara, T. Roberts, F. R. De Leon, L.C. Rosenfeld, L. Rushton, R.L. Sacco, J.A. Salomon, U. Sampson, E. Sanman, D.C. Schwebel, M. Segui-Gomez, D.S. Shepard, D. Singh, J. Singleton, K. Sliwa, E. Smith, A. Steer, J.A. Taylor, B. Thomas, I.M. Tleyjeh, J.A. Towbin, T. Truelsen, E.A. Undurraga, N. Venketasubramanian, L. Vijayakumar, T. Vos, G. R. Wagner, M. Wang, W. Wang, K. Watt, M.A. Weinstock, R. Weintraub, J. D. Wilkinson, A.D. Woolf, S. Wulf, P.H. Yeh, P. Yip, A. Zabetian, Z.J. Zheng, A. D. Lopez, C.J. Murray, M.A. AlMazroa, Z. A. Memish, 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 (2012) 2095–2128, [https://doi.org/10.1016/S0140-6736\(12\)61728-0](https://doi.org/10.1016/S0140-6736(12)61728-0).
- [4] WHO, Projections of mortality and causes of death, 2016 to 2060. [https://www.who.int/healthinfo/global\\_burden\\_disease/projections/en/](https://www.who.int/healthinfo/global_burden_disease/projections/en/).
- [5] WHO, Global health observatory data on causes of non-communicable disease in 2016. [https://www.who.int/gho/ncd/mortality\\_morbidity/en/](https://www.who.int/gho/ncd/mortality_morbidity/en/).
- [6] H. Dai, A.A. Much, E. Maor, E. Asher, A. Younis, Y. Xu, Y. Lu, X. Liu, J. Shu, N. L. Bragazzi, 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), <https://doi.org/10.1093/ehjqcco/qcaa076>.
- [7] S. Ghoneim, A. Dhorepalli, A.R. Shah, G. Ram, S. Ahmad, C. Kim, I. Asaad, Non-alcoholic steatohepatitis and the risk of myocardial infarction: a population-based national study, *World J. Hepatol.* 12 (2020) 378–388, <https://doi.org/10.4254/wjh.v12.i7.378>.
- [8] K.K. Griendling, G.A. FitzGerald, Oxidative stress and cardiovascular injury: Part II: animal and human studies, *Circulation* 108 (2003) 2034–2040.
- [9] A. Daiber, S. Steven, A. Weber, V.V. Shuvaev, V.R. Muzykantor, I. Laher, H. Li, S. Lamas, T. Munzel, Targeting vascular (endothelial) dysfunction, *Br. J. Pharmacol.* 174 (2017) 1591–1619, <https://doi.org/10.1111/bph.13517>.
- [10] J. Egea, I. Fabregat, Y.M. Frapart, P. Ghezzi, A. Goralach, T. Kietzmann, K. Kubaichuk, U.G. Knaus, M.G. Lopez, G. Olaso-Gonzalez, A. Petry, R. Schulz, J. Vina, P. Winyard, K. Abbas, O.S. Ademowo, C.B. Afonso, I. Andreadou, H. Antelmann, F. Antunes, M. Aslan, M.M. Bachschmid, R.M. Barbosa, V. Belousov, C. Berndt, D. Bernlohr, E. Bertran, A. Bindoli, S.P. Bottari, P.M. Brito, G. Carrara, A.I. Casas, A. Chatzi, N. Chondrogianni, M. Conrad, M.S. Cooke, J. G. Costa, A. Cuadrado, P. My-Chan Dang, B. De Smet, B. Debele-Butuner, I.H. K. Dias, J.D. Dunn, A.J. Edson, M. El Assar, J. El-Benna, P. Ferdinandy, A. S. Fernandes, K.E. Fladmark, U. Forstermann, R. Giniatullin, Z. Giricz, A. Gorbe, H. Griffiths, V. Hampl, A. Hanf, J. Herget, P. Hernansanz-Agustin, M. Hillion, J. Huang, S. Ilikay, P. Jansen-Durr, V. Jaquet, J.A. Joles, B. Kalyanaraman, D. Kaminsky, M. Karbaschi, M. Kleanthous, L.O. Klotz, B. Korac, K.S. Korkmaz, R. Koziel, D. Kracun, K.H. Krause, V. Kren, T. Krieg, J. Laranjinha, A. Lazou, H. Li, A. Martinez-Ruiz, R. Matsui, G.J. McBean, S.P. Meredith, J. Messens, V. Miguel, Y. Mikhed, I. Milisav, L. Milkovic, A. Miranda-Vizuete, M. Mojoyov, M. Monsalve, P.A. Mouthuy, J. Mulvey, T. Munzel, V. Muzykantor, I.T.N. Nguyen, M. Oelze, N. G. Oliveira, C.M. Palmeira, N. Papaevgeniou, A. Pavicevic, B. Pedre, F. Peyrot, M. Phylactides, G.G. Pircalabioru, A.R. Pitt, H.E. Poulsen, I. Prieto, M. P. Rigobello, N. Robledinos-Anton, L. Rodriguez-Manas, A.P. Rolo, F. Rousset, T. Ruskovska, N. Saraiva, S. Sasson, K. Schroder, K. Semen, T. Seredenina, A. Shakirzyanova, G.L. Smith, T. Soldati, B.C. Sousa, C.M. Spickett, A. Stancic, M. J. Stasia, H. Steinbrenner, V. Stepanic, S. Steven, K. Tokatlidis, E. Tuncay, B. Turan, F. Ursini, J. Vacek, O. Vajnerova, K. Valentova, F. Van Breusegem, L. Varisli, E.A. Veal, A.S. Yalcin, O. Yelisseyeva, N. Zarkovic, M. Zatloukalova, J. Zielonka, R.M. Touyz, A. Papapetropoulos, T. Grune, S. Lamas, H. Schmidt, F. Di Lisa, A. Daiber, 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 biology* 13 (2017) 94–162, <https://doi.org/10.1016/j.redox.2017.05.007>.
- [11] K.K. Griendling, G.A. FitzGerald, Oxidative stress and cardiovascular injury: Part I: basic mechanisms and in vivo monitoring of ROS, *Circulation* 108 (2003) 1912–1916.
- [12] H. Sies (Ed.), *Oxidative Stress: Oxidants and Antioxidants*, Academic Press, London, UK, 1991.
- [13] E. Ho, K. Karimi Galougahi, C.C. Liu, R. Bhindi, G.A. Figtree, Biological markers of oxidative stress: applications to cardiovascular research and practice, *Redox biology* 1 (2013) 483–491, <https://doi.org/10.1016/j.redox.2013.07.006>.
- [14] D. Harman, Aging: a theory based on free radical and radiation chemistry, *J. Gerontol.* 11 (1956) 298–300.
- [15] P. Ghezzi, L. Floridi, D. Boraschi, A. Cuadrado, G. Manda, S. Levic, F. D'Acquisto, A. Hamilton, T.J. Athersuch, L. Selley, Oxidative stress and inflammation induced by environmental and psychological stressors: a biomarker perspective, *Antioxidants Redox Signal.* 28 (2018) 852–872, <https://doi.org/10.1089/ars.2017.7147>.
- [16] P. Ghezzi, V. Jaquet, F. Marcucci, H. Schmidt, The oxidative stress theory of disease: levels of evidence and epistemological aspects, *Br. J. Pharmacol.* 174 (2017) 1784–1796, <https://doi.org/10.1111/bph.13544>.
- [17] S. Steven, T. Munzel, A. Daiber, Exploiting the pleiotropic antioxidant effects of established drugs in cardiovascular disease, *Int. J. Mol. Sci.* 16 (2015) 18185–18223, <https://doi.org/10.3390/ijms160818185>.
- [18] A. Daiber, S. Chlopicki, Revisiting pharmacology of oxidative stress and endothelial dysfunction in cardiovascular disease: evidence for redox-based therapies, *Free Radic. Biol. Med.* 157 (2020) 15–37, <https://doi.org/10.1016/j.freeradbiomed.2020.02.026>.
- [19] A.F. Chen, D.D. Chen, A. Daiber, F.M. Faraci, H. Li, C.M. Rembold, I. Laher, Free radical biology of the cardiovascular system, *Clin. Sci. (Lond.)* 123 (2012) 73–91, <https://doi.org/10.1042/cs20110562> [pii] <https://doi.org/10.1042/CS20110562>.
- [20] T. Gori, T. Munzel, Oxidative stress and endothelial dysfunction: therapeutic implications, *Ann. Med.* 43 (2011) 259–272, <https://doi.org/10.3109/07853890.2010.543920>.
- [21] H.H. Schmidt, R. Stocker, C. Vollbracht, G. Paulsen, D. Riley, A. Daiber, A. Cuadrado, Antioxidants in translational medicine, *Antioxidants Redox Signal.* 23 (2015) 1130–1143, <https://doi.org/10.1089/ars.2015.6393>.
- [22] S. Karbach, P. Wenzel, A. Waisman, T. Munzel, A. Daiber, eNOS uncoupling in cardiovascular diseases—the role of oxidative stress and inflammation, *Curr. Pharmaceut. Des.* 20 (2014) 3579–3594.
- [23] T. Munzel, T. Gori, R.M. Bruno, S. Taddei, Is oxidative stress a therapeutic target in cardiovascular disease? *Eur. Heart J.* 31 (2010) 2741–2748, <https://doi.org/10.1093/eurheartj/ehq396>.
- [24] A. Daiber, M. Oelze, S. Daub, S. Steven, A. Schuff, S. Kroller-Schon, M. Hausding, P. Wenzel, E. Schulz, T. Gori, T. Munzel, Vascular redox signaling, redox switches in endothelial nitric oxide synthase and endothelial dysfunction, in: I. Laher (Ed.), *Systems Biology of Free Radicals and Antioxidants*, Springer-Verlag, Berlin Heidelberg, 2014, pp. 1177–1211.
- [25] A. Daiber, F. Di Lisa, M. Oelze, S. Kroller-Schon, S. Steven, E. Schulz, T. Munzel, Crosstalk of mitochondria with NADPH oxidase via reactive oxygen and nitrogen species signalling and its role for vascular function, *Br. J. Pharmacol.* 174 (2017) 1670–1689, <https://doi.org/10.1111/bph.13403>.
- [26] A. Cuadrado, G. Manda, A. Hassan, M.J. Alcaraz, C. Barbas, A. Daiber, P. Ghezzi, R. Leon, M.G. Lopez, B. Oliva, M. Pajares, A.I. Rojo, N. Robledinos-Anton, A. M. Valverde, E. Guney, H. Schmidt, Transcription factor NRF2 as a therapeutic target for chronic diseases: a systems medicine approach, *Pharmacol. Res.* 70 (2018) 348–383, <https://doi.org/10.1124/pr.117.014753>.



- [27] A.I. Casas, C. Nogales, H.A.M. Mucke, A. Petraina, A. Cuadrado, A.I. Rojo, P. Ghezzi, V. Jaquet, F. Augsburger, F. Dufrasne, J. Soubhye, S. Deshwal, M. Di Sante, N. Kaludercic, F. Di Lisa, H. Schmidt, On the clinical pharmacology of reactive oxygen species, *Pharmacol. Rev.* 72 (2020) 801–828, <https://doi.org/10.1124/pr.120.019422>.
- [28] C. Nogales, A.G.B. Gronning, S. Sadegh, J. Baumbach, H. Schmidt, Network medicine-based unbiased disease modules for drug and diagnostic target identification in ROSopathies. *Handbook of experimental pharmacology*. [https://doi.org/10.1007/164\\_2020\\_386](https://doi.org/10.1007/164_2020_386), 2020.
- [29] N.H. Schork, Personalized medicine: time for one-person trials, *Nature* 520 (2015) 609–611, <https://doi.org/10.1038/520609a>.
- [30] M.S. Lee, A.J. Flammer, L.O. Lerman, A. Lerman, Personalized medicine in cardiovascular diseases, *Korean Circ J* 42 (2012) 583–591, <https://doi.org/10.4070/kcj.2012.42.9.583>.
- [31] E. Schulz, P. Wenzel, T. Munzel, A. Daiber, Mitochondrial redox signaling: interaction of mitochondrial reactive oxygen species with other sources of oxidative stress, *Antioxidants Redox Signal.* 20 (2014) 308–324, <https://doi.org/10.1089/ars.2012.4609>.
- [32] B.H. Robinson, The role of manganese superoxide dismutase in health and disease, *J. Inherit. Metab. Dis.* 21 (1998) 598–603.
- [33] D.B. Zorov, C.R. Filburn, L.O. Klotz, J.L. Zweier, S.J. Sollott, Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes, *J. Exp. Med.* 192 (2000) 1001–1014.
- [34] D.B. Zorov, M. Juhaszova, S.J. Sollott, Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release, *Physiol. Rev.* 94 (2014) 909–950, <https://doi.org/10.1152/physrev.00026.2013>.
- [35] A. Goralach, K. Bertram, S. Hudecova, O. Krizanova, Calcium and ROS: a mutual interplay, *Redox biology* 6 (2015) 260–271, <https://doi.org/10.1016/j.redox.2015.08.010>.
- [36] A. Corcoran, T.G. Cotter, Redox regulation of protein kinases, *FEBS J.* 280 (2013) 1944–1965, <https://doi.org/10.1111/febs.12224>.
- [37] T. Kietzmann, A. Petry, A. Shvetsova, J.M. Gerhold, A. Goralach, The epigenetic landscape related to reactive oxygen species formation in the cardiovascular system, *Br. J. Pharmacol.* 174 (2017) 1533–1554, <https://doi.org/10.1111/bph.13792>.
- [38] Y. Mikhed, A. Goralach, U.G. Knaus, A. Daiber, Redox regulation of genome stability by effects on gene expression, epigenetic pathways and DNA damage/repair, *Redox biology* 5 (2015) 275–289, <https://doi.org/10.1016/j.redox.2015.05.008>.
- [39] V. Miguel, S. Lamas, C. Espinosa-Diez, Role of non-coding-RNAs in response to environmental stressors and consequences on human health, *Redox biology* (2020) 101580, <https://doi.org/10.1016/j.redox.2020.101580>.
- [40] R.J. Gryglewski, R.M. Palmer, S. Moncada, Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor, *Nature* 320 (1986) 454–456.
- [41] C. Espinosa-Diez, V. Miguel, D. Mennerich, T. Kietzmann, P. Sanchez-Perez, S. Cadenas, S. Lamas, Antioxidant responses and cellular adjustments to oxidative stress, *Redox biology* 6 (2015) 183–197, <https://doi.org/10.1016/j.redox.2015.07.008>.
- [42] D. Hilfiker-Kleiner, K. Sliwa, Pathophysiology and epidemiology of peripartum cardiomyopathy, *Nat. Rev. Cardiol.* 11 (2014) 364–370, <https://doi.org/10.1038/nrcardio.2014.37>.
- [43] J. Frijhoff, P.G. Winyard, N. Zarkovic, S.S. Davies, R. Stocker, D. Cheng, A. R. Knight, E.L. Taylor, J. Oettrich, T. Ruskovska, A.C. Gasparovic, A. Cuadrado, D. Weber, H.E. Poulsen, T. Grune, H.H. Schmidt, P. Ghezzi, Clinical relevance of biomarkers of oxidative stress, *Antioxidants Redox Signal.* 23 (2015) 1144–1170, <https://doi.org/10.1089/ars.2015.6317>.
- [44] WHO, Biomarkers in Risk Assessment: Validity and Validation, WHO, Geneva, 2001.
- [45] P. Ghezzi, Environmental risk factors and their footprints in vivo - a proposal for the classification of oxidative stress biomarkers, *Redox biology* (2020) 101442, <https://doi.org/10.1016/j.redox.2020.101442>.
- [46] A. Daiber, M. Oelze, S. Steven, S. Kroller-Schon, T. Munzel, Taking up the cudgels for the traditional reactive oxygen and nitrogen species detection assays and their use in the cardiovascular system, *Redox biology* 12 (2017) 35–49, <https://doi.org/10.1016/j.redox.2017.02.001>.
- [47] R. Schnabel, S. Blankenberg, E. Lubos, K.J. Lackner, H.J. Rupprecht, C. Espinola-Klein, N. Jachmann, F. Post, D. Peetz, C. Bickel, F. Cambien, L. Tiret, T. Munzel, Asymmetric dimethylarginine and the risk of cardiovascular events and death in patients with coronary artery disease: results from the AtheroGene Study, *Circ. Res.* 97 (2005) e53–59.
- [48] A. Daiber, N. Xia, S. Steven, M. Oelze, A. Hanf, S. Kroller-Schon, T. Munzel, H. Li, New therapeutic implications of endothelial nitric oxide synthase (eNOS) function/dysfunction in cardiovascular disease, *Int. J. Mol. Sci.* 20 (2019), <https://doi.org/10.3390/ijms20010187>.
- [49] J.F. Doppeide, C. Doppler, M. Scheer, V. Obst, M.C. Radmacher, M.P. Radsack, L. Gori, A. Warnholtz, C. Fottner, T. Munzel, A. Daiber, C. Espinola-Klein, Critical limb ischaemia is characterised by an increased production of whole blood reactive oxygen species and expression of TREM-1 on neutrophils, *Atherosclerosis* 229 (2013) 396–403, <https://doi.org/10.1016/j.atherosclerosis.2013.05.029>.
- [50] S. Steven, A. Daiber, J.F. Doppeide, T. Munzel, C. Espinola-Klein, Peripheral artery disease, redox signaling, oxidative stress - basic and clinical aspects, *Redox biology* 12 (2017) 787–797, <https://doi.org/10.1016/j.redox.2017.04.017>.
- [51] J. Zielonka, G. Cheng, M. Zielonka, T. Ganesh, A. Sun, J. Joseph, R. Michalski, W. J. O'Brien, J.D. Lambeth, B. Kalyanaram, High-throughput assays for superoxide and hydrogen peroxide design OF A screening workflow to identify inhibitors OF NADPH oxidases, *J. Biol. Chem.* 289 (2014) 16176–16189, <https://doi.org/10.1074/jbc.M114.548693>.
- [52] J. Zielonka, J. Vasquez-Vivar, B. Kalyanaram, Detection of 2-hydroxyethidium in cellular systems: a unique marker product of superoxide and hydroethidine, *Nat. Protoc.* 3 (2008) 8–21, <https://doi.org/10.1038/nprot.2007.473>.
- [53] S.I. Dikalov, D.G. Harrison, Methods for detection of mitochondrial and cellular reactive oxygen species, *Antioxidants Redox Signal.* 20 (2014) 372–382, <https://doi.org/10.1089/ars.2012.4886>.
- [54] K. Debowska, D. Debski, M. Hardy, M. Jakubowska, B. Kalyanaram, A. Marcinek, R. Michalski, B. Michalowski, O. Ouari, A. Sikora, R. Smulik, J. Zielonka, Toward selective detection of reactive oxygen and nitrogen species with the use of fluorogenic probes—Limitations, progress, and perspectives, *Pharmacol. Rep. : PR* 67 (2015) 756–764, <https://doi.org/10.1016/j.pharep.2015.03.016>.
- [55] J.K. Griendling, R.M. Touyz, J.L. Zweier, S. Dikalov, W. Chilian, Y.R. Chen, D. G. Harrison, A. Bhatnagar, S American heart association council on basic cardiovascular, Measurement of reactive oxygen species, reactive nitrogen species, and redox-dependent signaling in the cardiovascular system: a scientific statement from the American heart association, *Circ. Res.* 119 (2016) e39–75, <https://doi.org/10.1161/RES.000000000000110>.
- [56] J.K. Perng, S. Lee, K. Kundu, C.F. Caskey, S.F. Knight, S. Satir, K.W. Ferrara, W. R. Taylor, F.L. Degertekin, D. Sorescu, N. Murthy, Ultrasound imaging of oxidative stress in vivo with chemically-generated gas microbubbles, *Annu. Biomed. Eng.* 40 (2012) 2059–2068, <https://doi.org/10.1007/s10439-012-0573-9>.
- [57] G. Maulucci, G. Bacic, L. Bridal, H.H. Schmidt, B. Tavitian, T. Viel, H. Utsumi, A. S. Yalcin, M. De Spirito, Imaging reactive oxygen species-induced modifications in living systems, *Antioxidants Redox Signal.* 24 (2016) 939–958, <https://doi.org/10.1089/ars.2015.6415>.
- [58] R.P. Mason, Imaging free radicals in organelles, cells, tissue, and in vivo with immuno-spin trapping, *Redox biology* 8 (2016) 422–429, <https://doi.org/10.1016/j.redox.2016.04.003>.
- [59] M.D. Howard, E.D. Hood, B. Zern, V.V. Shuvaev, T. Grosser, V.R. Muzykantov, Nanocarriers for vascular delivery of anti-inflammatory agents, *Annu. Rev. Pharmacol. Toxicol.* 54 (2014) 205–226, <https://doi.org/10.1146/annurev-pharmtox-011613-140002>.
- [60] D.S. Bilan, V.V. Belousov, HyPer family probes: state of the Art, *Antioxidants Redox Signal.* 24 (2016) 731–751, <https://doi.org/10.1089/ars.2015.6586>.
- [61] Y.G. Ermakova, D.S. Bilan, M.E. Matlashov, N.M. Mishina, K.N. Markvicheva, O. M. Subach, F.V. Subach, I. Bogeski, M. Hoth, G. Enikolopov, V.V. Belousov, Red fluorescent genetically encoded indicator for intracellular hydrogen peroxide, *Nat. Commun.* 5 (2014) 5222, <https://doi.org/10.1038/ncomms6222>.
- [62] E. Eroglu, B. Gottschalk, S. Charoensin, S. Blass, H. Bischof, R. Rost, C. T. Madreiter-Sokolowski, B. Pelzmann, E. Bernhart, W. Sattler, S. Hallstrom, T. Malinski, M. Waldeck-Weiermair, W.F. Graier, R. Malli, Development of novel FP-based probes for live-cell imaging of nitric oxide dynamics, *Nat. Commun.* 7 (2016) 10623, <https://doi.org/10.1038/ncomms10623>.
- [63] E. Eroglu, S. Charoensin, H. Bischof, J. Ramadani, B. Gottschalk, M.R. Depaoli, M. Waldeck-Weiermair, W.F. Graier, R. Malli, Genetic biosensors for imaging nitric oxide in single cells, *Free Radic. Biol. Med.* 128 (2018) 50–58, <https://doi.org/10.1016/j.freeradbiomed.2018.01.027>.
- [64] L. Swain, A. Kesemeyer, S. Meyer-Roxlau, C. Vettel, A. Zieseniss, A. Guntsch, A. Jatho, A. Becker, M.S. Nanadikar, B. Morgan, S. Dennerlein, A.M. Shah, A. El-Armouche, V.O. Nikolaev, D.M. Katschinski, Redox imaging using cardiac myocyte-specific transgenic biosensor mice, *Circ. Res.* 119 (2016) 1004–1016, <https://doi.org/10.1161/CIRCRESAHA.116.309551>.
- [65] A.G. Shokhina, A.I. Kostyuk, Y.G. Ermakova, A.S. Panova, D.B. Staroverov, E. S. Egorov, M.S. Baranov, G.J. van Belle, D.M. Katschinski, V.V. Belousov, D. S. Bilan, Red fluorescent redox-sensitive biosensor Grx1-roCherry, *Redox biology* 21 (2019) 101071, <https://doi.org/10.1016/j.redox.2018.10.1071>.
- [66] L. Swain, M.S. Nanadikar, S. Borowik, A. Zieseniss, D.M. Katschinski, Transgenic organisms meet redox bioimaging: one step closer to physiology, *Antioxidants Redox Signal.* 29 (2018) 603–612, <https://doi.org/10.1089/ars.2017.7469>.
- [67] J.P. Castro, T. Jung, T. Grune, W. Siems, 4-Hydroxynonenal (HNE) modified proteins in metabolic diseases, *Free Radic. Biol. Med.* 111 (2017) 309–315, <https://doi.org/10.1016/j.freeradbiomed.2016.10.497>.
- [68] K. Zarkovic, A. Jakovcovic, N. Zarkovic, Contribution of the HNE-immunohistochemistry to modern pathological concepts of major human diseases, *Free Radic. Biol. Med.* 111 (2017) 110–126, <https://doi.org/10.1016/j.freeradbiomed.2016.12.009>.
- [69] S.S. Davies, L.J. Roberts, 2nd. F2-isoprostanes as an indicator and risk factor for coronary heart disease, *Free Radic. Biol. Med.* 50 (2011) 559–566, <https://doi.org/10.1016/j.freeradbiomed.2010.11.023>.
- [70] B.M. Winkhofer-Roob, G. Faustmann, J.M. Roob, Low-density lipoprotein oxidation biomarkers in human health and disease and effects of bioactive compounds, *Free Radic. Biol. Med.* 111 (2017) 38–86, <https://doi.org/10.1016/j.freeradbiomed.2017.04.345>.
- [71] E. Gianazza, M. Brioschi, A.M. Fernandez, C. Banfi, Lipoxidation in cardiovascular diseases, *Redox biology* 23 (2019) 101119, <https://doi.org/10.1016/j.redox.2019.101119>.
- [72] L. Iuliano, Pathways of cholesterol oxidation via non-enzymatic mechanisms, *Chem. Phys. Lipids* 164 (2011) 457–468, <https://doi.org/10.1016/j.chemphyslip.2011.06.006>.
- [73] L. Li, S. Zhong, X. Shen, Q. Li, W. Xu, Y. Tao, H. Yin, Recent development on liquid chromatography-mass spectrometry analysis of oxidized lipids, *Free Radic.*



- Biol. Med. 144 (2019) 16–34, <https://doi.org/10.1016/j.freeradbiomed.2019.06.006>.
- [74] M. Griesser, W.E. Boeglin, T. Suzuki, C. Schneider, Convergence of the 5-LOX and COX-2 pathways: heme-catalyzed cleavage of the 5S-HETE-derived di-endoperoxide into aldehyde fragments, *J. Lipid Res.* 50 (2009) 2455–2462, <https://doi.org/10.1194/jlr.M900181-JLR200>.
- [75] M. Roest, H.A. Voorbij, Y.T. Van der Schouw, P.H. Peeters, T. Teerlink, P. G. Scheffer, High levels of urinary F2-isoprostanes predict cardiovascular mortality in postmenopausal women, *J Clin Lipidol* 2 (2008) 298–303, <https://doi.org/10.1016/j.jacl.2008.06.004>.
- [76] T.J. van 't Erve, M.B. Kadiiska, S.J. London, R.P. Mason, Classifying oxidative stress by F2-isoprostane levels across human diseases: a meta-analysis, *Redox biology* 12 (2017) 582–599, <https://doi.org/10.1016/j.redox.2017.03.024>.
- [77] E. Schwedhelm, A. Bartling, H. Lenzen, D. Tsikas, R. Maas, J. Brummer, F. M. Gutzki, J. Berger, J.C. Frolich, R.H. Boger, Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: a matched case-control study, *Circulation* 109 (2004) 843–848, <https://doi.org/10.1161/01.CIR.0000116761.93647.30>.
- [78] R. Wolfgram, A. Oguogho, B. Palumbo, H. Sinzinger, Enhanced oxidative stress in coronary heart disease and chronic heart failure as indicated by an increased 8-epi-PGF(2alpha), *Eur. J. Heart Fail.* 7 (2005) 167–172, <https://doi.org/10.1016/j.ejheart.2004.05.007>.
- [79] M. Woodward, Kevin D. Croft, Trevor A. Mori, H. Headlam, Xiao S. Wang, C. Suarna, Mark J. Raftery, Stephen W. MacMahon, R. Stocker, Association between both lipid and protein oxidation and the risk of fatal or non-fatal coronary heart disease in a human population, *Clin. Sci.* 116 (2008) 53–60, <https://doi.org/10.1042/cs20070404>.
- [80] K. Nakamura, K. Kusano, Y. Nakamura, M. Kakishita, K. Ohta, S. Nagase, M. Yamamoto, K. Miyaji, H. Saito, H. Morita, T. Emori, H. Matsubara, S. Toyokuni, T. Ohe, Carvedilol decreases elevated oxidative stress in human failing myocardium, *Circulation* 105 (2002) 2867–2871, <https://doi.org/10.1161/01.cir.0000018605.14470.dd>.
- [81] S. Mak, D.C. Lehotay, M. Yazdanpanah, E.R. Azevedo, P.P. Liu, G.E. Newton, Unsaturated aldehydes including 4-OH-nonenal are elevated in patients with congestive heart failure, *J. Card. Fail.* 6 (2000) 108–114.
- [82] N. Haramaki, H. Ikeda, Y. Takajo, A. Katoh, S. Kanaya, S. Shintani, R. Haramaki, T. Murohara, T. Imaizumi, Long-term smoking causes nitroglycerin resistance in platelets by depletion of intraplatelet glutathione, *Arterioscler. Thromb. Vasc. Biol.* 21 (2001) 1852–1856.
- [83] E. Romuk, C. Wojciechowska, W. Jachec, A. Zemla-Woszek, A. Momot, M. Buczkowska, P. Rozenytr, Malondialdehyde and uric acid as predictors of adverse outcome in patients with chronic heart failure, *Oxidative medicine and cellular longevity* (2019) 9246138, <https://doi.org/10.1155/2019/9246138>, 2019.
- [84] S. Radovanovic, A. Savic-Radojevic, M. Pljesa-Ercegovac, T. Djukic, S. Suvakov, M. Krotin, D.V. Simic, M. Matic, Z. Radojicic, T. Pekmezovic, T. Simic, Markers of oxidative damage and antioxidant enzyme activities as predictors of morbidity and mortality in patients with chronic heart failure, *J. Card. Fail.* 18 (2012) 493–501, <https://doi.org/10.1016/j.cardfail.2012.04.003>.
- [85] M.F. Walter, R.F. Jacob, B. Jeffers, M.M. Ghdanfar, G.M. Preston, J. Buch, R. P. Mason, P. study, Serum levels of thiobarbituric acid reactive substances predict cardiovascular events in patients with stable coronary artery disease: a longitudinal analysis of the PREVENT study, *J. Am. Coll. Cardiol.* 44 (2004) 1996–2002, <https://doi.org/10.1016/j.jacc.2004.08.029>.
- [86] S. Gao, D. Zhao, M. Wang, F. Zhao, X. Han, Y. Qi, J. Liu, Association between circulating oxidized LDL and atherosclerotic cardiovascular disease: a meta-analysis of observational studies, *Can. J. Cardiol.* 33 (2017) 1624–1632, <https://doi.org/10.1016/j.cjca.2017.07.015>.
- [87] M. Gomez, V. Valle, F. Aros, G. Sanz, J. Sala, M. Fiol, J. Bruguera, R. Elosua, L. Molina, H. Marti, M.I. Covas, A. Rodriguez-Llorian, M. Fito, M.A. Suarez-Pinilla, R. Amezaga, J. Marrugat, F.g.o. researchers, L.D.L. Oxidized, Lipoprotein (a) and other emergent risk factors in acute myocardial infarction (FORTIAM study), *Rev. Esp. Cardiol.* 62 (2009) 373–382, [https://doi.org/10.1016/s1885-5857\(09\)71664-0](https://doi.org/10.1016/s1885-5857(09)71664-0).
- [88] K. Shimada, H. Mokuno, E. Matsunaga, T. Miyazaki, K. Sumiyoshi, K. Miyauchi, H. Daida, Circulating oxidized low-density lipoprotein is an independent predictor for cardiac event in patients with coronary artery disease, *Atherosclerosis* 174 (2004) 343–347, <https://doi.org/10.1016/j.atherosclerosis.2004.01.029>.
- [89] Y.C. Zhang, Y. Tang, Y. Chen, X.H. Huang, M. Zhang, J. Chen, Y.G. Sun, Y.G. Li, Oxidized low-density lipoprotein and C-reactive protein have combined utility for better predicting prognosis after acute coronary syndrome, *Cell Biochem. Biophys.* 68 (2014) 379–385, <https://doi.org/10.1007/s12013-013-9718-1>.
- [90] S.M. Cristescu, R. Kiss, S. Hekker, M. Dalby, F.J. Harren, T.H. Risby, N. Marczin, B.s.i. Harefield, Real-time monitoring of endogenous lipid peroxidation by exhaled ethylene in patients undergoing cardiac surgery, *Am. J. Physiol. Lung Cell Mol. Physiol.* 307 (2014) L509–L515, <https://doi.org/10.1152/ajplung.00168.2014>.
- [91] L. Thomson, M. Tenopoulou, R. Lightfoot, E. Tsika, I. Parastatidis, M. Martinez, T. M. Greco, P.T. Doulias, Y. Wu, W.H. Tang, S.L. Hazen, H. Ischiropoulos, Immunoglobulins against tyrosine-nitrated epitopes in coronary artery disease, *Circulation* 126 (2012) 2392–2401, <https://doi.org/10.1161/CIRCULATIONAHA.112.103796>.
- [92] L.A. MacMillan-Crow, J.P. Crow, J.D. Kerby, J.S. Beckman, J.A. Thompson, Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 11853–11858.
- [93] B. Davis, M.H. Zou, CD40 ligand-dependent tyrosine nitration of prostacyclin synthase in vivo, *Circulation* 112 (2005) 2184–2192, <https://doi.org/10.1161/CIRCULATIONAHA.105.553206>.
- [94] M. Martinez, A. Cuker, A. Mills, R. Lightfoot, Y. Fan, W.H. Tang, S.L. Hazen, H. Ischiropoulos, Nitrated fibrinogen is a biomarker of oxidative stress in venous thromboembolism, *Free Radic. Biol. Med.* 53 (2012) 230–236, <https://doi.org/10.1016/j.freeradbiomed.2012.05.004>.
- [95] C. Vadseth, J.M. Souza, L. Thomson, A. Seagraves, C. Nagaswami, T. Scheiner, J. Torbet, G. Vilair, J.S. Bennett, J.C. Murciano, V. Muzykantov, M.S. Penn, S. L. Hazen, J.W. Weisel, H. Ischiropoulos, Pro-thrombotic state induced by post-translational modification of fibrinogen by reactive nitrogen species, *J. Biol. Chem.* 279 (2004) 8820–8826, <https://doi.org/10.1074/jbc.M306101200>.
- [96] M.H. Shishehbor, R.J. Aviles, M.L. Brennan, X. Fu, M. Goormastic, G.L. Pearce, N. S.H. Sheu, J.F. Keane Jr., M.S. Penn, D.L. Sprecher, J.A. Vita, S.L. Hazen, Association of nitrotyrosine levels with cardiovascular disease and modulation by statin therapy, *JAMA* 289 (2003) 1675–1680, <https://doi.org/10.1001/jama.289.13.1675>.
- [97] M. Pirro, G. Schillaci, M.R. Mannarino, G. Savarese, G. Vaudo, D. Siepi, R. Paltricia, E. Mannarino, Effects of rosuvastatin on 3-nitrotyrosine and aortic stiffness in hypercholesterolemia, *Nutr. Metabol. Cardiovasc. Dis.* 17 (2007) 436–441, <https://doi.org/10.1016/j.numecd.2006.02.009>.
- [98] C.S. Chu, K.T. Lee, K.H. Cheng, M.Y. Lee, H.F. Kuo, T.H. Lin, H.M. Su, W.C. Voon, S.H. Sheu, W.T. Lai, 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 (2012) 21, <https://doi.org/10.1186/1475-2840-11-21>.
- [99] A.V.L. Quidim, T. Bruno, P.C.L. Leocadio, I.S. Santos, J.I. Alvarez-Leite, P.L. Dos Reis Menta, P.A. Lotufo, I.M. Bensor, A.C. Goulart, The prognostic value of nitrotyrosine levels in coronary heart disease: long-term evaluation in the Acute Coronary Syndrome Registry Strategy (ERICO study), *Clin. Biochem.* 66 (2019) 37–43, <https://doi.org/10.1016/j.clinbiochem.2019.02.006>.
- [100] T. Nuriel, A. Hansler, S.S. Gross, Protein nitrotyrosine: formation, significance and identification, *Journal of proteomics* 74 (2011) 2300–2312, <https://doi.org/10.1016/j.jpropt.2011.05.032>.
- [101] M. Nishida, A. Nishimura, T. Matsunaga, H. Motohashi, S. Kasamatsu, T. Akaike, Redox regulation of electrophilic signaling by reactive persulfides in cardiac cells, *Free Radic. Biol. Med.* 109 (2017) 132–140, <https://doi.org/10.1016/j.freeradbiomed.2017.01.024>.
- [102] S. Fujii, T. Akaike, Redox Signaling by 8-nitro-cyclic guanosine monophosphate: nitric oxide- and reactive oxygen species-derived electrophilic messenger, *Antioxidants Redox Signal.* 19 (2013) 1236–1246, <https://doi.org/10.1089/ars.2012.5067>.
- [103] B.A. Freeman, P.R. Baker, F.J. Schopfer, S.R. Woodcock, A. Napolitano, M. d'Ischia, Nitro-fatty acid formation and signaling, *J. Biol. Chem.* 283 (2008) 15515–15519, <https://doi.org/10.1074/jbc.R800004200>.
- [104] A. Trostchansky, H. Rubbo, Nitrated fatty acids: mechanisms of formation, chemical characterization, and biological properties, *Free Radic. Biol. Med.* 44 (2008) 1887–1896, <https://doi.org/10.1016/j.freeradbiomed.2008.03.006>.
- [105] A. Trostchansky, L. Bonilla, L. Gonzalez-Perilli, H. Rubbo, Nitro-fatty acids: formation, redox signaling, and therapeutic potential, *Antioxidants Redox Signal.* 19 (2013) 1257–1265, <https://doi.org/10.1089/ars.2012.5023>.
- [106] T. Melo, J.F. Montero-Bullon, P. Domingues, M.R. Domingues, Discovery of bioactive nitrated lipids and nitro-lipid-protein adducts using mass spectrometry-based approaches, *Redox biology* 23 (2019) 101106, <https://doi.org/10.1016/j.redox.2019.101106>.
- [107] S.R. Salvatore, P. Rowart, F.J. Schopfer, Mass spectrometry-based study defines the human urine nitro-lipidome, *Free Radic. Biol. Med.* (2020), <https://doi.org/10.1016/j.freeradbiomed.2020.10.305>.
- [108] P. Nadziakiewicz, P. Knapiak, D. Ziora, D. Nowak, Moderate exercise decreases nitric oxide exhalation in patients with stable coronary artery disease, *J. Physiol. Pharmacol.* 57 (Suppl 4) (2006) 213–221.
- [109] C. Grunewald, K. Carlstrom, G. Kumlien, A. Ringqvist, J. Lundberg, Exhaled oral and nasal nitric oxide during L-arginine infusion in preeclampsia, *Gynecol. Obstet. Invest.* 46 (1998) 232–237, <https://doi.org/10.1159/000010040>.
- [110] M. Kato, A. Matsumoto, T. Nakajima, K. Hirose, K. Iwasawa, K. Takenaka, H. Yamashita, S. Sugiura, Y. Hirata, R. Nagai, Amlodipine increases nitric oxide production in exhaled air during exercise in patients with essential hypertension, *Am. J. Hypertens.* 17 (2004) 729–733, <https://doi.org/10.1016/j.amjhyper.2004.05.025>.
- [111] H. Adachi, S. Oshima, S. Sakurai, T. Toyama, H. Hoshizaki, K. Taniguchi, H. Ito, Nitric oxide exhalation correlates with ventilatory response to exercise in patients with heart disease, *Eur. J. Heart Fail.* 5 (2003) 639–643, [https://doi.org/10.1016/s1388-9842\(03\)00107-7](https://doi.org/10.1016/s1388-9842(03)00107-7).
- [112] E. Tufvesson, E. Nilsson, T.A. Popov, R. Hesselstrand, L. Bjermer, Fractional exhaled breath temperature in patients with asthma, chronic obstructive pulmonary disease, or systemic sclerosis compared to healthy controls, *Eur Clin Respir J* 7 (2020) 1747014, <https://doi.org/10.1080/20018525.2020.1747014>.
- [113] R. Maas, V. Xanthakis, T. Goen, J. Muller, E. Schwedhelm, R.H. Boger, R.S. Vasan, Plasma nitrate and incidence of cardiovascular disease and all-cause mortality in the community: the Framingham offspring study, *Journal of the American Heart Association* 6 (2017), <https://doi.org/10.1161/JAHA.117.006224>.
- [114] F. Hadaegh, S. Asgari, M. Bozorganesh, S. Jeddji, F. Azizi, A. Ghasemi, Added value of total serum nitrate/nitrite for prediction of cardiovascular disease in

- middle east caucasian residents in Tehran, *Nitric Oxide* 54 (2016) 60–66, <https://doi.org/10.1016/j.niox.2016.02.004>.
- [115] H. Kasai, P.F. Crain, Y. Kuchino, S. Nishimura, A. Ootsuyama, H. Tanooka, Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair, *Carcinogenesis* 7 (1986) 1849–1851.
- [116] E.L. Larsen, A. Weimann, H.E. Poulsen, Interventions targeted at oxidatively generated modifications of nucleic acids focused on urine and plasma markers, *Free Radic. Biol. Med.* 145 (2019) 256–283, <https://doi.org/10.1016/j.freeradbiomed.2019.09.030>.
- [117] H.E. Poulsen, A. Weimann, T. Henriksen, L.K. Kjaer, E.L. Larsen, E.R. Carlsson, C. K. Christensen, I. Brandslund, M. Fenger, Oxidatively generated modifications to nucleic acids in vivo: measurement in urine and plasma, *Free Radic. Biol. Med.* 145 (2019) 336–341, <https://doi.org/10.1016/j.freeradbiomed.2019.10.001>.
- [118] A. Di Minno, L. Turnu, B. Porro, I. Squellierio, V. Cavalca, E. Tremoli, M.N. Di Minno, 8-Hydroxy-2-Deoxyguanosine levels and cardiovascular disease: a systematic review and meta-analysis of the literature, *Antioxidants Redox Signal.* 24 (2016) 548–555, <https://doi.org/10.1089/ars.2015.6508>.
- [119] A. Di Minno, L. Turnu, B. Porro, I. Squellierio, V. Cavalca, E. Tremoli, M.N. Di Minno, 8-Hydroxy-2-deoxyguanosine levels and heart failure: a systematic review and meta-analysis of the literature, *Nutr. Metabol. Cardiovasc. Dis.* 27 (2017) 201–208, <https://doi.org/10.1016/j.numecd.2016.10.009>.
- [120] X.B. Wang, N.H. Cui, X. Liu, X. Liu, Mitochondrial 8-hydroxy-2'-deoxyguanosine and coronary artery disease in patients with type 2 diabetes mellitus, *Cardiovasc. Diabetol.* 19 (2020) 22, <https://doi.org/10.1186/s12933-020-00998-6>.
- [121] X. Liu, W. Gan, Y. Zou, B. Yang, Z. Su, J. Deng, L. Wang, J. Cai, Elevated levels of urinary markers of oxidative DNA and RNA damage in type 2 diabetes with complications, *Oxidative medicine and cellular longevity* (2016) 4323198, <https://doi.org/10.1155/2016/4323198>, 2016.
- [122] K. Broedbaek, V. Siersma, T. Henriksen, A. Weimann, M. Petersen, J.T. Andersen, E. Jimenez-Solem, L.J. Hansen, J.E. Henriksen, S.J. Bonnema, N. de Fine Olivarius, H.E. Poulsen, Association between urinary markers of nucleic acid oxidation and mortality in type 2 diabetes: a population-based cohort study, *Diabetes Care* 36 (2013) 669–676, <https://doi.org/10.2337/dc12-0998>.
- [123] K. Broedbaek, R. Koster-Rasmussen, V. Siersma, F. Persson, H.E. Poulsen, N. de Fine Olivarius, Urinary albumin and 8-oxo-7,8-dihydroguanosine as markers of mortality and cardiovascular disease during 19 years after diagnosis of type 2 diabetes - a comparative study of two markers to identify high risk patients, *Redox biology* 13 (2017) 363–369, <https://doi.org/10.1016/j.redox.2017.06.005>.
- [124] B. Schottker, H. Brenner, E.H. Jansen, J. Gardiner, A. Peasey, R. Kubinova, A. Pajak, R. Topor-Madry, A. Tamosiunas, K.U. Saum, B. Holleczek, H. Pikhart, M. Bobak, Evidence for the free radical/oxidative stress theory of ageing from the CHANCES consortium: a meta-analysis of individual participant data, *BMC Med.* 13 (2015) 300, <https://doi.org/10.1186/s12916-015-0537-7>.
- [125] Y. Xuan, M. Bobak, A. Anusriti, E. Jansen, A. Pajak, A. Tamosiunas, K.U. Saum, B. Holleczek, X. Gao, H. Brenner, B. Schottker, Association of serum markers of oxidative stress with myocardial infarction and stroke: pooled results from four large European cohort studies, *Eur. J. Epidemiol.* 34 (2019) 471–481, <https://doi.org/10.1007/s10654-018-0457-x>.
- [126] N. Masaki, A. Sato, S. Horii, T. Kimura, T. Toya, R. Yasuda, T. Namba, H. Yada, A. Kawamura, T. Adachi, Usefulness of the d-ROMs test for prediction of cardiovascular events, *Int. J. Cardiol.* 222 (2016) 226–232, <https://doi.org/10.1016/j.ijcard.2016.07.225>.
- [127] V. Ambros, microRNAs: tiny regulators with great potential, *Cell* 107 (2001) 823–826, [https://doi.org/10.1016/s0092-8674\(01\)00616-x](https://doi.org/10.1016/s0092-8674(01)00616-x).
- [128] T. Treiber, N. Treiber, G. Meister, Regulation of microRNA biogenesis and its crosstalk with other cellular pathways, *Nat. Rev. Mol. Cell Biol.* 20 (2019) 5–20, <https://doi.org/10.1038/s41580-018-0059-1>.
- [129] S. Gilad, E. Meiri, Y. Yogeve, S. Benjamin, D. Lebanony, N. Yerushalmi, H. Benjamin, M. Kushnir, H. Cholak, N. Melamed, Z. Bentwich, M. Hod, Y. Goren, A. Chajut, Serum microRNAs are promising novel biomarkers, *PLoS One* 3 (2008) e3148, <https://doi.org/10.1371/journal.pone.0003148>.
- [130] Y. Wan, R. Cui, J. Gu, X. Zhang, X. Xiang, C. Liu, K. Qu, T. Lin, Identification of four oxidative stress-responsive MicroRNAs, miR-34a-5p, miR-1915-3p, miR-638, and miR-150-3p, in hepatocellular carcinoma, *Oxidative medicine and cellular longevity* 2017 (2017) 5189138, <https://doi.org/10.1155/2017/5189138>.
- [131] J. Banerjee, S. Khanna, A. Bhattacharya, MicroRNA regulation of oxidative stress, *Oxidative medicine and cellular longevity* (2017) 2872156, <https://doi.org/10.1155/2017/2872156>, 2017.
- [132] J. Krutzfeldt, N. Rajewsky, R. Braich, K.G. Rajeev, T. Tuschl, M. Manoharan, M. Stoffel, Silencing of microRNAs in vivo with 'antagomirs', *Nature* 438 (2005) 685–689, <https://doi.org/10.1038/nature04303>.
- [133] H.R. Wang, M. Wu, H. Yu, S. Long, A. Stevens, D.W. Engers, H. Sackin, J. S. Daniels, E.S. Dawson, C.R. Hopkins, C.W. Lindsley, M. Li, O.B. McManus, Selective inhibition of the Kir2 family of inward rectifier potassium channels by a small molecule probe: the discovery, SAR, and pharmacological characterization of ML133, *ACS Chem. Biol.* 6 (2011) 845–856, <https://doi.org/10.1021/cb200146a>.
- [134] E. Bonneau, B. Neveu, E. Kostantin, G.J. Tsongalis, V. De Guire, How close are miRNAs from clinical practice? A perspective on the diagnostic and therapeutic market, *EJIFCC* 30 (2019) 114–127.
- [135] B. Kura, B. Szeiffova Bacova, B. Kalocayova, M. Sykora, J. Slezak, Oxidative stress-responsive MicroRNAs in heart injury, *Int. J. Mol. Sci.* 21 (2020), <https://doi.org/10.3390/ijms21010358>.
- [136] V. Miguel, J.Y. Cui, L. Daimiel, C. Espinosa-Diez, C. Fernandez-Hernando, T. J. Kavanagh, S. Lamas, The role of MicroRNAs in environmental risk factors, noise-induced hearing loss, and mental stress, *Antioxidants Redox Signal.* 28 (2018) 773–796, <https://doi.org/10.1089/ars.2017.7175>.
- [137] X.M. Fu, Y.Z. Zhou, Z. Cheng, X.B. Liao, X.M. Zhou, MicroRNAs: novel players in aortic aneurysm, *BioMed Res. Int.* 2015 (2015) 831641, <https://doi.org/10.1155/2015/831641>.
- [138] J. Zhang, W. Cai, Z. Fan, C. Yang, W. Wang, M. Xiong, C. Ma, J. Yang, MicroRNA-24 inhibits the oxidative stress induced by vascular injury by activating the Nrf2/Ho-1 signaling pathway, *Atherosclerosis* 290 (2019) 9–18, <https://doi.org/10.1016/j.atherosclerosis.2019.08.023>.
- [139] L. Gou, L. Zhao, W. Song, L. Wang, J. Liu, H. Zhang, Y. Huang, C.W. Lau, X. Yao, X.Y. Tian, W.T. Wong, J.Y. Luo, Y. Huang, Inhibition of miR-92a suppresses oxidative stress and improves endothelial function by upregulating heme oxygenase-1 in db/db mice, *Antioxidants Redox Signal.* 28 (2018) 358–370, <https://doi.org/10.1089/ars.2017.7005>.
- [140] A.H. Yamac, M.A. Huyut, E. Yilmaz, I. Celikkale, A. Bacaksiz, Y. Demir, A. R. Demir, M. Erturk, N. Bakhshaliyev, R. Ozdemir, U. Kilic, MicroRNA 199a is downregulated in patients after coronary artery bypass graft surgery and is associated with increased levels of sirtuin 1 (SIRT 1) protein and major adverse cardiovascular events at 3-year follow-up, *Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. : international medical journal of experimental and clinical research* 24 (2018) 6245–6254, <https://doi.org/10.12659/MSM.912065>.
- [141] P. Jakob, T. Kacprowski, S. Briand-Schumacher, D. Heg, R. Klingenberg, B. E. Stahli, M. Jaguszewski, N. Rodondi, D. Nanchen, L. Raber, P. Vogt, F. Mach, S. Windecker, U. Volker, C.M. Matter, T.F. Luscher, U. Landmesser, Profiling and validation of circulating microRNAs for cardiovascular events in patients presenting with ST-segment elevation myocardial infarction, *Eur. Heart J.* 38 (2017) 511–515, <https://doi.org/10.1093/eurheartj/ehw563>.
- [142] M. Han, Z. Yang, D. Sayed, M. He, S. Gao, L. Lin, S. Yoon, M. Abdellatif, GATA4 expression is primarily regulated via a miR-26b-dependent post-transcriptional mechanism during cardiac hypertrophy, *Cardiovasc. Res.* 93 (2012) 645–654, <https://doi.org/10.1093/cvr/cvs001>.
- [143] X.P. Ren, J. Wu, X. Wang, M.A. Sartor, K. Jones, J. Qian, P. Nicolau, T. J. Pritchard, G.C. Fan, MicroRNA-320 is involved in the regulation of cardiac ischemia/reperfusion injury by targeting heat-shock protein 20, *Circulation* 119 (2009) 2357–2366, <https://doi.org/10.1161/CIRCULATIONAHA.108.814145>.
- [144] S.S. Zhou, J.P. Jin, J.Q. Wang, Z.G. Zhang, J.H. Freedman, Y. Zheng, L. Cai, miRNAs in cardiovascular diseases: potential biomarkers, therapeutic targets and challenges, *Acta Pharmacol. Sin.* 39 (2018) 1073–1084, <https://doi.org/10.1038/aps.2018.30>.
- [145] A. Bayes-Genis, D.E. Lanfear, M.W.J. de Ronde, J. Lupon, J.J. Leenders, Z. Liu, N. P.A. Zuihoff, M.J.C. Eijkemans, E. Zamora, M. De Antonio, A.H. Zwinderman, S. J. Pinto-Sietsma, Y.M. Pinto, Prognostic value of circulating microRNAs on heart failure-related morbidity and mortality in two large diverse cohorts of general heart failure patients, *Eur. J. Heart Fail.* 20 (2018) 67–75, <https://doi.org/10.1002/ehf.984>.
- [146] D. de Gonzalo-Calvo, G. Cediel, C. Bar, J. Nunez, E. Revuelta-Lopez, J. Gavara, C. Rios-Navarro, V. Llorente-Cortes, V. Bodi, T. Thum, A. Bayes-Genis, Circulating miR-1254 predicts ventricular remodeling in patients with ST-Segment-Elevation Myocardial Infarction: a cardiovascular magnetic resonance study, *Sci. Rep.* 8 (2018) 15115, <https://doi.org/10.1038/s41598-018-33491-y>.
- [147] S. Vettori, S. Gay, O. Distler, Role of MicroRNAs in fibrosis, *Open Rheumatol. J.* 6 (2012) 130–139, <https://doi.org/10.2174/1874312901206010130>.
- [148] G. Sytkiewicz, M. Tomaniak, O. Blaszczyk, L. Koltowski, K.J. Filipiak, D. Sitkiewicz, Circulating microribonucleic acids miR-1, miR-21 and miR-208a in patients with symptomatic heart failure: preliminary results, *Arch Cardiovasc Dis* 108 (2015) 634–642, <https://doi.org/10.1016/j.acvd.2015.07.003>.
- [149] H.A. Cakmak, E. Coskumpinar, B. Ikitimur, H.A. Barman, B. Karadag, N. O. Tiryakioglu, K. Kahraman, V.A. Vural, The prognostic value of circulating microRNAs in heart failure: preliminary results from a genome-wide expression study, *J. Cardiovasc. Med.* 16 (2015) 431–437, <https://doi.org/10.2459/JCM.0000000000000233>.
- [150] Y. Goren, M. Kushnir, B. Zafir, S. Tabak, B.S. Lewis, O. Amir, Serum levels of microRNAs in patients with heart failure, *Eur. J. Heart Fail.* 14 (2012) 147–154, <https://doi.org/10.1093/eurjhf/hfr155>.
- [151] L. Tao, X. Huang, M. Xu, Z. Qin, F. Zhang, F. Hua, X. Jiang, Y. Wang, Value of circulating miRNA-21 in the diagnosis of subclinical diabetic cardiomyopathy, *Mol. Cell. Endocrinol.* (2020) 110944, <https://doi.org/10.1016/j.mce.2020.110944>.
- [152] B. Dai, H. Li, J. Fan, Y. Zhao, Z. Yin, X. Nie, D.W. Wang, C. Chen, MiR-21 protected against diabetic cardiomyopathy induced diastolic dysfunction by targeting gelsolin, *Cardiovasc. Diabetol.* 17 (2018) 123, <https://doi.org/10.1186/s12933-018-0767-z>.
- [153] A.M. Krichevsky, G. Gabriely, miR-21: a small multi-faceted RNA, *J. Cell Mol. Med.* 13 (2009) 39–53, <https://doi.org/10.1111/j.1582-4934.2008.00556.x>.
- [154] T. Thum, P. Galuppo, C. Wolf, J. Fiedler, S. Kneitz, L.W. van Laake, P. A. Doevendans, C.L. Mummery, J. Borlak, A. Haverich, C. Gross, S. Engelhardt, G. Ertl, J. Bauersachs, MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure, *Circulation* 116 (2007) 258–267, <https://doi.org/10.1161/CIRCULATIONAHA.107.687947>.
- [155] R. Ji, Y. Cheng, J. Yue, J. Yang, X. Liu, H. Chen, D.B. Dean, C. Zhang, MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation, *Circ. Res.* 100 (2007) 1579–1588, <https://doi.org/10.1161/CIRCRESAHA.106.141986>.
- [156] F. Fleissner, V. Jazbutyte, J. Fiedler, S.K. Gupta, X. Yin, Q. Xu, P. Galuppo, S. Kneitz, M. Mayr, G. Ertl, J. Bauersachs, T. Thum, Short communication: asymmetric dimethylarginine impairs angiogenic progenitor cell function in

- patients with coronary artery disease through a microRNA-21-dependent mechanism, *Circ. Res.* 107 (2010) 138–143, <https://doi.org/10.1161/CIRCRESAHA.110.216770>.
- [157] R. Hinkel, D. Ramanujam, V. Kaczmarek, A. Howe, K. Klett, C. Beck, A. Dueck, T. Thum, K.L. Laugwitz, L. Maegdefessel, C. Weber, C. Kupatt, S. Engelhardt, AntimiR-21 prevents myocardial dysfunction in a pig model of ischemia/reperfusion injury, *J. Am. Coll. Cardiol.* 75 (2020) 1788–1800, <https://doi.org/10.1016/j.jacc.2020.02.041>.
- [158] A. Briasoulis, S. Sharma, T. Telila, S. Mallikethi-Reddy, N. Papageorgiou, E. Oikonomou, D. Tousoulis, MicroRNAs in atrial fibrillation, *Curr. Med. Chem.* 26 (2019) 855–863, <https://doi.org/10.2174/0929867324666170920151024>.
- [159] N. Kapodistrias, G. Theocharopoulou, P. Vlamos, A hypothesis of circulating MicroRNAs' implication in high incidence of atrial fibrillation and other electrocardiographic abnormalities in cancer patients, *Adv. Exp. Med. Biol.* 1196 (2020) 1–9, [https://doi.org/10.1007/978-3-030-32637-1\\_1](https://doi.org/10.1007/978-3-030-32637-1_1).
- [160] J.M. Rivera-Caravaca, R. Teruel-Montoya, V. Roldan, R. Cifuentes-Riquelme, J. A. Crespo-Matas, A.M. de Los Reyes-Garcia, S. Aguila, M.P. Fernandez-Perez, L. Reguilon-Gallego, L. Zapata-Martinez, N. Garcia-Barbera, V. Vicente, F. Marin, C. Martinez, R. Gonzalez-Conejero, Pilot study on the role of circulating miRNAs for the improvement of the predictive ability of the 2MACE score in patients with atrial fibrillation, *J. Clin. Med.* 9 (2020), <https://doi.org/10.3390/jcm9113645>.
- [161] A.I. Casas, V.T. Dao, A. Daiber, G.J. Maghzal, F. Di Lisa, N. Kaludercic, S. Leach, A. Cuadrado, V. Jaquet, T. Seredenina, K.H. Krause, M.G. Lopez, R. Stocker, P. Ghezzi, H.H. Schmidt, Reactive oxygen-related diseases: therapeutic targets and emerging clinical indications, *Antioxidants Redox Signal.* 23 (2015) 1171–1185, <https://doi.org/10.1089/ars.2015.6433>.
- [162] K. Winkler, J.J. Hermans, P. Schiffers, A. Moens, M. Paul, H.H. Schmidt, NOX1, 2, 4, 5: counting out oxidative stress, *Br. J. Pharmacol.* 164 (2011) 866–883, <https://doi.org/10.1111/j.1476-5381.2011.01249.x>.
- [163] V.T. Dao, A.I. Casas, G.J. Maghzal, T. Seredenina, N. Kaludercic, N. Robledinos-Anton, F. Di Lisa, R. Stocker, P. Ghezzi, V. Jaquet, A. Cuadrado, H.H. Schmidt, Pharmacology and clinical drug candidates in redox medicine, *Antioxidants Redox Signal.* 23 (2015) 1113–1129, <https://doi.org/10.1089/ars.2015.6430>.
- [164] L. Loffredo, R. Carnevale, V. Sanguigni, A. Plebani, P. Rossi, C. Pignata, D. De Mattia, A. Finocchi, B. Martire, M.C. Pietrogrande, S. Martino, E. Gambineri, G. Giardino, A.R. Soresina, F. Martino, P. Pignatelli, F. Violi, Does NADPH oxidase deficiency cause artery dilatation in humans? *Antioxidants Redox Signal.* 18 (2013) 1491–1496, <https://doi.org/10.1089/ars.2012.4987>.
- [165] C. Nocella, V. Cammisotto, S. Bartimoccia, V. Castellani, L. Loffredo, D. Pastori, P. Pignatelli, V. Sanguigni, F. Violi, R. Carnevale, A novel role of MMP2 in regulating platelet NOX2 activation, *Free Radic. Biol. Med.* 152 (2020) 355–362, <https://doi.org/10.1016/j.freeradbiomed.2020.03.033>.
- [166] L. Loffredo, F. Martino, R. Carnevale, P. Pignatelli, E. Catasca, L. Perri, C. M. Calabrese, M.M. Palumbo, F. Baratta, M. Del Ben, F. Angelico, F. Violi, Obesity and hypercholesterolemia are associated with NOX2 generated oxidative stress and arterial dysfunction, *J. Pediatr.* 161 (2012) 1004–1009, <https://doi.org/10.1016/j.jpeds.2012.05.042>.
- [167] L. Loffredo, P. Pignatelli, F. Martino, R. Carnevale, S. Bartimoccia, E. Catasca, C. Colantoni, C. Zanoni, L. Perri, F. Violi, Early increase of NOX2-derived oxidative stress in children: relationship with age, *Pediatr. Res.* 73 (2013) 788–793, <https://doi.org/10.1038/pr.2013.55>.
- [168] L. Loffredo, R. Carnevale, R. Cangemi, F. Angelico, T. Augelletti, S. Di Santo, C. M. Calabrese, L. Della Volpe, P. Pignatelli, L. Perri, S. Basili, F. Violi, NOX2 up-regulation is associated with artery dysfunction in patients with peripheral artery disease, *Int. J. Cardiol.* 165 (2013) 184–192, <https://doi.org/10.1016/j.ijcard.2012.01.069>.
- [169] L. Loffredo, A.M. Zicari, L. Perri, R. Carnevale, C. Nocella, F. Angelico, M. Del Ben, A. Mosca, S. Zaffina, N. Panera, A. Alisi, M. Duse, F. Violi, V. Nobili, Does Nox2 overactivate in children with nonalcoholic fatty liver disease? *Antioxidants Redox Signal.* 30 (2019) 1325–1330, <https://doi.org/10.1089/ars.2018.7596>.
- [170] G. Carpino, D. Pastori, F. Baratta, D. Overi, G. Labbadia, L. Polimeni, A. Di Costanzo, G. Pannitteri, R. Carnevale, M. Del Ben, M. Arca, F. Violi, F. Angelico, E. Gaudio, PNPLA3 variant and portal/periportal histological pattern in patients with biopsy-proven non-alcoholic fatty liver disease: a possible role for oxidative stress, *Sci. Rep.* 7 (2017) 15756, <https://doi.org/10.1038/s41598-017-15943-z>.
- [171] F. Baratta, D. Pastori, S. Bartimoccia, V. Cammisotto, N. Cocomello, A. Colantoni, C. Nocella, R. Carnevale, D. Ferro, F. Angelico, F. Violi, M. Del Ben, Poor adherence to mediterranean diet and serum lipopolysaccharide are associated with oxidative stress in patients with non-alcoholic fatty liver disease, *Nutrients* 12 (2020), <https://doi.org/10.3390/nu12061732>.
- [172] L. Loffredo, F. Baratta, P. Ludovica, S. Battaglia, R. Carnevale, C. Nocella, M. Novo, G. Pannitteri, F. Ceci, F. Angelico, F. Violi, M. Del Ben, Effects of dark chocolate on endothelial function in patients with non-alcoholic steatohepatitis, *Nutr. Metabol. Cardiovasc. Dis.* 28 (2017) 143–149, <https://doi.org/10.1016/j.numecd.2017.10.027>.
- [173] M. Del Ben, F. Angelico, R. Cangemi, L. Loffredo, R. Carnevale, T. Augelletti, F. Baratta, L. Polimeni, P. Pignatelli, F. Violi, Moderate weight loss decreases oxidative stress and increases antioxidant status in patients with metabolic syndrome, *ISRN Obes* (2012) 960427, <https://doi.org/10.5402/2012/960427>, 2012.
- [174] D. Pastori, P. Andreozzi, R. Carnevale, S. Bartimoccia, S. Limaj, S. Melandri, M. Brunori, G. Spallacci, F. Violi, P. Pignatelli, Does the coexistence of chronic obstructive pulmonary disease and atrial fibrillation affect Nox2 activity and urinary isoprostanes excretion? *Antioxidants Redox Signal.* 31 (2019) 786–790, <https://doi.org/10.1089/ars.2019.7811>.
- [175] M. Del Ben, M. Fabiani, L. Loffredo, L. Polimeni, R. Carnevale, F. Baratta, M. Brunori, F. Albanese, T. Augelletti, F. Violi, F. Angelico, Oxidative stress mediated arterial dysfunction in patients with obstructive sleep apnoea and the effect of continuous positive airway pressure treatment, *BMC Pulm. Med.* 12 (2012) 36, <https://doi.org/10.1186/1471-2466-12-36>.
- [176] L. Loffredo, A.M. Zicari, F. Occasi, L. Perri, R. Carnevale, F. Angelico, M. Del Ben, F. Martino, C. Nocella, V. Savastano, A. Cesoni Marcelli, M. Duse, F. Violi, Endothelial dysfunction and oxidative stress in children with sleep disordered breathing: role of NADPH oxidase, *Atherosclerosis* 240 (2015) 222–227, <https://doi.org/10.1016/j.atherosclerosis.2015.03.024>.
- [177] L. Loffredo, R. Carnevale, L. Perri, E. Catasca, T. Augelletti, R. Cangemi, F. Albanese, C. Piccheri, C. Nocella, P. Pignatelli, F. Violi, NOX2-mediated arterial dysfunction in smokers: acute effect of dark chocolate, *Heart* 97 (2011) 1776–1781, <https://doi.org/10.1136/heartjnl-2011-300304>.
- [178] L. Loffredo, A.M. Zicari, F. Occasi, L. Perri, R. Carnevale, S. Battaglia, F. Angelico, M. Del Ben, F. Martino, C. Nocella, A. Farcomeni, G. De Castro, M. Duse, F. Violi, Passive smoking exacerbates nicotinamide-adenine dinucleotide phosphate oxidase isoform 2-induced oxidative stress and arterial dysfunction in children with persistent allergic rhinitis, *J. Pediatr.* 202 (2018) 252–257, <https://doi.org/10.1016/j.jpeds.2018.06.053>.
- [179] P.A. Bertazzi, L. Cantone, P. Pignatelli, L. Angelici, V. Bollati, M. Bonzini, M. Carugno, P.M. Mannucci, F. Violi, Does enhancement of oxidative stress markers mediate health effects of ambient air particles? *Antioxidants Redox Signal.* 21 (2014) 46–51, <https://doi.org/10.1089/ars.2013.5694>.
- [180] E.D. Schleicher, A. Bierhaus, H.U. Haring, P.P. Nawroth, R. Lehmann, Chemistry and pathobiology of advanced glycation end products, *Contrib. Nephrol.* (2001) 1–9, <https://doi.org/10.1159/000060056>.
- [181] F. Akhter, D. Chen, A. Akhter, S.F. Yan, S. ShiDu Yan, Age-dependent accumulation of dicarbonyls and advanced glycation endproducts (AGEs) associates with mitochondrial stress, *Free Radic. Biol. Med.* (2020), <https://doi.org/10.1016/j.freeradbiomed.2020.12.021>.
- [182] C. Ott, K. Jacobs, E. Hauke, A. Navarrete Santos, T. Grune, A. Simm, Role of advanced glycation end products in cellular signaling, *Redox biology* 2 (2014) 411–429, <https://doi.org/10.1016/j.redox.2013.12.016>.
- [183] S. Steven, K. Frenis, M. Oelze, S. Kalinovic, M. Kuntic, M.T. Bayo Jimenez, K. Vujacic-Mirski, J. Helmstadter, S. Kroller-Schon, T. Munzel, A. Daiber, Vascular inflammation and oxidative stress: major triggers for cardiovascular disease, *Oxidative medicine and cellular longevity* (2019) 7092151, <https://doi.org/10.1155/2019/7092151>, 2019.
- [184] J.R. Kizer, D. Benkeser, A.M. Arnold, J.H. Ix, K.J. Mukamal, L. Djousse, R. P. Tracy, D.S. Siscovick, B.M. Psaty, S.J. Ziemann, Advanced glycation/ glycoxidation endproduct carboxymethyl-lysine and incidence of coronary heart disease and stroke in older adults, *Atherosclerosis* 235 (2014) 116–121, <https://doi.org/10.1016/j.atherosclerosis.2014.04.013>.
- [185] R.D. Semba, S. Bandinelli, K. Sun, J.M. Guralnik, L. Ferrucci, Plasma carboxymethyl-lysine, an advanced glycation end product, and all-cause and cardiovascular disease mortality in older community-dwelling adults, *J. Am. Geriatr. Soc.* 57 (2009) 1874–1880, <https://doi.org/10.1111/j.1532-5415.2009.02438.x>.
- [186] D. Sanajou, A. Ghorbani Haghjo, H. Argani, S. Aslani, AGE-RAGE axis blockade in diabetic nephropathy: current status and future directions, *Eur. J. Pharmacol.* 833 (2018) 158–164, <https://doi.org/10.1016/j.ejphar.2018.06.001>.
- [187] S. Baldus, C. Heeschen, T. Meinertz, A.M. Zeiher, J.P. Eiserich, T. Munzel, M. L. Simoons-Cw. Hamm, C. Investigators, Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes, *Circulation* 108 (2003) 1440–1445, <https://doi.org/10.1161/01.CIR.0000090690.67322.51>.
- [188] S.J. Nicholls, W.H. Tang, D. Brennan, M.L. Brennan, S. Mann, S.E. Nissen, S. L. Hazen, Risk prediction with serial myeloperoxidase monitoring in patients with acute chest pain, *Clin. Chem.* 57 (2011) 1762–1770, <https://doi.org/10.1373/clinchem.2011.166827>.
- [189] M.L. Cheng, C.M. Chen, P.W. Gu, H.Y. Ho, D.T. Chiu, Elevated levels of myeloperoxidase, white blood cell count and 3-chlorotyrosine in Taiwanese patients with acute myocardial infarction, *Clin. Biochem.* 41 (2008) 554–560, <https://doi.org/10.1016/j.clinbiochem.2008.02.006>.
- [190] J. Talib, G.J. Maghzal, D. Cheng, R. Stocker, Detailed protocol to assess in vivo and ex vivo myeloperoxidase activity in mouse models of vascular inflammation and disease using hydroethidine, *Free Radic. Biol. Med.* 97 (2016) 124–135, <https://doi.org/10.1016/j.freeradbiomed.2016.05.004>.
- [191] K. Karimi Galougahi, C. Antoniadis, S.J. Nicholls, K.M. Channon, G.A. Fritree, Redox biomarkers in cardiovascular medicine, *Eur. Heart J.* 36 (2015) 1576–1582, <https://doi.org/10.1093/eurheartj/ehv126>, 1582a–b.
- [192] C.H. Chen, G.R. Budas, E.N. Churchill, M.H. Disatnik, T.D. Hurley, D. Mochly-Rosen, Activation of aldehyde dehydrogenase-2 reduces ischemic damage to the heart, *Science* 321 (2008) 1493–1495, <https://doi.org/10.1126/science.1158554> [pii] 10.1126/science.1158554.
- [193] C. Maack, T. Kartes, H. Kilter, H.J. Schafers, G. Nickenig, M. Bohm, U. Laufs, Oxygen free radical release in human failing myocardium is associated with increased activity of rac1-GTPase and represents a target for statin treatment, *Circulation* 108 (2003) 1567–1574, <https://doi.org/10.1161/01.CIR.0000091084.46500.BB>.
- [194] T.J. Hohn, T. Grune, The proteasome and the degradation of oxidized proteins: part III-Redox regulation of the proteasomal system, *Redox biology* 2 (2014) 388–394, <https://doi.org/10.1016/j.redox.2013.12.029>.
- [195] T. Jung, A. Hohn, T. Grune, The proteasome and the degradation of oxidized proteins: Part II - protein oxidation and proteasomal degradation, *Redox biology* 2 (2014) 99–104, <https://doi.org/10.1016/j.redox.2013.12.008>.



- [196] S. Crnko, B.C. Du Pre, J.P.G. Sluijter, L.W. Van Laake, Circadian rhythms and the molecular clock in cardiovascular biology and disease, *Nat. Rev. Cardiol.* 16 (2019) 437–447, <https://doi.org/10.1038/s41569-019-0167-4>.
- [197] M. Putker, J.S. O'Neill, Reciprocal control of the circadian clock and cellular redox state - a critical appraisal, *Mol. Cell.* 39 (2016) 6–19, <https://doi.org/10.14348/molcells.2016.2323>.
- [198] H. Li, A.B. Kilgallen, T. Munzel, E. Wolf, S. Lecour, R. Schulz, A. Daiber, L.W. Van Laake, Influence of mental stress and environmental toxins on circadian clocks: implications for redox regulation of the heart and cardioprotection, *Br. J. Pharmacol.* 177 (2020) 5393–5412, <https://doi.org/10.1111/bph.14949>.
- [199] M.K. Ahsan, I. Lekli, D. Ray, J. Yodoi, D.K. Das, Redox regulation of cell survival by the thioredoxin superfamily: an implication of redox gene therapy in the heart, *Antioxidants Redox Signal.* 11 (2009) 2741–2758, <https://doi.org/10.1089/ARS.2009.2683>.
- [200] D.A. Butterfield, M. Perluigi, Redox proteomics: a key tool for new insights into protein modification with relevance to disease, *Antioxidants Redox Signal.* 26 (2017) 277–279, <https://doi.org/10.1089/ars.2016.6919>.
- [201] D.A. Butterfield, I. Dalle-Donne, Redox proteomics, *Antioxidants Redox Signal.* 17 (2012) 1487–1489, <https://doi.org/10.1089/ars.2012.4742>.
- [202] X. Chen, J. Lee, H. Wu, A.W. Tsang, C.M. Furdul, Mass spectrometry in advancement of redox precision medicine, *Adv. Exp. Med. Biol.* 1140 (2019) 327–358, [https://doi.org/10.1007/978-3-030-15950-4\\_19](https://doi.org/10.1007/978-3-030-15950-4_19).
- [203] V. Kumar, T.D. Calamaras, D. Haeussler, W.S. Colucci, R.A. Cohen, M.E. McComb, D. Pimentel, M.M. Bachschmid, Cardiovascular redox and ox stress proteomics, *Antioxidants Redox Signal.* 17 (2012) 1528–1559, <https://doi.org/10.1089/ars.2012.4706>.
- [204] M. Fratelli, H. Demol, M. Puype, S. Casagrande, I. Eberini, M. Salmona, V. Bonetto, M. Mengozzi, F. Duffieux, E. Miclet, A. Bachi, J. Vandekerckhove, E. Gianazza, P. Ghezzi, Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes, *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 3505–3510.
- [205] X.H. Gao, L. Li, M. Parisien, J. Wu, I. Bederman, Z. Gao, D. Krokowski, S. M. Chirieleison, D. Abbott, B. Wang, P. Arvan, M. Cameron, M. Chance, B. Willard, M. Hatzoglou, Discovery of a redox thiol switch: implications for cellular energy metabolism, *Mol. Cell. Proteomics : MCP* 19 (2020) 852–870, <https://doi.org/10.1074/mcp.RA119.001910>.
- [206] P. Ghezzi, K. Davies, A. Delaney, L. Floridi, Theory of signs and statistical approach to big data in assessing the relevance of clinical biomarkers of inflammation and oxidative stress, *Proc. Natl. Acad. Sci. U. S. A.* 115 (2018) 2473–2477, <https://doi.org/10.1073/pnas.1719807115>.
- [207] S. Rius-Perez, S. Perez, I. Torres-Cuevas, P. Marti-Andres, R. Talens-Visconti, A. Paradela, L. Guerrero, L. Franco, G. Lopez-Rodas, L. Torres, F. Corrales, J. Sastre, Blockade of the trans-sulfuration pathway in acute pancreatitis due to nitration of cystathionine beta-synthase, *Redox biology* 28 (2020) 101324, <https://doi.org/10.1016/j.redox.2019.101324>.
- [208] A. Reis, Oxidative Phospholipidomics in health and disease: achievements, challenges and hopes, *Free Radic. Biol. Med.* 111 (2017) 25–37, <https://doi.org/10.1016/j.freeradbiomed.2017.01.014>.
- [209] F. Gruber, C. Krenslhner, M.S. Narzt, The impact of recent advances in lipidomics and redox lipidomics on dermatological research, *Free Radic. Biol. Med.* 144 (2019) 256–265, <https://doi.org/10.1016/j.freeradbiomed.2019.04.019>.
- [210] K.L. Skeffington, A.R. Bond, M.G. Bigotti, S. AbdulGhani, D. Iacobazzi, S.L. Kang, K.J. Heesom, M.C. Wilson, S. Stoica, R. Martin, M. Caputo, M.S. Suleiman, M. T. Ghorbel, Changes in inflammation and oxidative stress signalling pathways in coarcted aorta triggered by bicuspid aortic valve and growth in young children, *Experimental and therapeutic medicine* 20 (2020) 48, <https://doi.org/10.3892/etm.2020.9171>.
- [211] M. Mallocci, L. Perdomo, M. Veerasamy, R. Andriantsitohaina, G. Simard, M. C. Martinez, Extracellular vesicles: mechanisms in human health and disease, *Antioxidants Redox Signal.* 30 (2019) 813–856, <https://doi.org/10.1089/ars.2017.7265>.
- [212] M.J. Hitchler, F.E. Domann, Redox regulation of the epigenetic landscape in cancer: a role for metabolic reprogramming in remodeling the epigenome, *Free Radic. Biol. Med.* 53 (2012) 2178–2187, <https://doi.org/10.1016/j.freeradbiomed.2012.09.028>.
- [213] P. Hayes, U.G. Knaus, Balancing reactive oxygen species in the epigenome: NADPH oxidases as target and perpetrator, *Antioxidants Redox Signal.* 18 (2013) 1937–1945, <https://doi.org/10.1089/ars.2012.4895>.
- [214] A. Hanf, M. Oelze, A. Manea, H. Li, T. Munzel, A. Daiber, The anti-cancer drug doxorubicin induces substantial epigenetic changes in cultured cardiomyocytes, *Chem. Biol. Interact.* 313 (2019) 108834, <https://doi.org/10.1016/j.cbi.2019.108834>.
- [215] J.M. Ordovas, C.E. Smith, Epigenetics and cardiovascular disease, *Nat. Rev. Cardiol.* 7 (2010) 510–519, <https://doi.org/10.1038/nrcardio.2010.104>.
- [216] P. van der Harst, L.J. de Windt, J.C. Chambers, Translational perspective on epigenetics in cardiovascular disease, *J. Am. Coll. Cardiol.* 70 (2017) 590–606, <https://doi.org/10.1016/j.jacc.2017.05.067>.
- [217] J.F. Keaney Jr., M.G. Larson, R.S. Vasan, P.W. Wilson, I. Lipinska, D. Corey, J. M. Massaro, P. Sutherland, J.A. Vita, E.J. Benjamin, S. Framingham, Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study, *Arterioscler. Thromb. Vasc. Biol.* 23 (2003) 434–439, <https://doi.org/10.1161/01.ATV.0000058402.34138.11>.
- [218] J.B. Meigs, M.G. Larson, C.S. Fox, J.F. Keaney Jr., R.S. Vasan, E.J. Benjamin, Association of oxidative stress, insulin resistance, and diabetes risk phenotypes: the Framingham Offspring Study, *Diabetes Care* 30 (2007) 2529–2535, <https://doi.org/10.2337/dc07-0817>.
- [219] C. Vassalle, N. Botto, M.G. Andreassi, S. Berti, A. Biagini, Evidence for enhanced 8-isoprostane plasma levels, as index of oxidative stress in vivo, in patients with coronary artery disease, *Coron. Artery Dis.* 14 (2003) 213–218, <https://doi.org/10.1097/01.mca.0000063504.13456.c3>.
- [220] T. Heitzer, S. Ylä-Herttuala, J. Luoma, S. Kurz, T. Munzel, H. Just, M. Olschewski, H. Drexler, Cigarette smoking potentiates endothelial dysfunction of forearm resistance vessels in patients with hypercholesterolemia: role of oxidized LDL, *Circulation* 93 (1996) 1346–1353.
- [221] I. Ikonomidis, S. Tzortzis, J. Lekakis, I. Paraskevaidis, I. Andreadou, M. Nikolaou, T. Kaplanoglou, P. Katsimbri, G. Skarantavos, P. Soucacos, D.T. Kremastinos, Lowering interleukin-1 activity with anakinra improves myocardial deformation in rheumatoid arthritis, *Heart* 95 (2009) 1502–1507, <https://doi.org/10.1136/hrt.2009.168971>.
- [222] I. Ikonomidis, S. Tzortzis, I. Andreadou, I. Paraskevaidis, C. Katseli, P. Katsimbri, G. Pavlidis, J. Parissis, D. Kremastinos, M. Anastasiou-Nana, J. Lekakis, Increased benefit of interleukin-1 inhibition on vascular function, myocardial deformation, and twisting in patients with coronary artery disease and coexisting rheumatoid arthritis, *Circulation. Cardiovascular imaging* 7 (2014) 619–628, <https://doi.org/10.1161/CIRCIMAGING.113.001193>.
- [223] V. Lambadiari, G. Pavlidis, F. Kousathana, E. Maratou, D. Georgiou, I. Andreadou, A. Kountouri, M. Varoudi, K. Balampanis, J. Parissis, H. Triantafyllidi, K. Katogiannis, D. Birba, J. Lekakis, G. Dimitriadis, I. Ikonomidis, Effects of different antidiabetic medications on endothelial glycoalkal, myocardial function, and vascular function in type 2 diabetic patients: one year follow-up study, *J. Clin. Med.* 8 (2019), <https://doi.org/10.3390/jcm8070983>.
- [224] K. Stamatelopoulos, G. Georgiopoulos, F. Athanasouli, P.E. Nikolaou, M. Lykka, M. Roussou, M. Gavriatopoulou, A. Laina, G. Trakada, M. Charakida, D. Delialis, I. Petropoulos, C. Pamboukas, E. Manios, M. Karakitsou, C. Papatimachal, A. Gatsiou, I. Lambrinouadaki, E. Terpos, K. Stellos, I. Andreadou, M. A. Dimopoulos, E. Kastritis, Reactive vasodilation predicts mortality in primary systemic light-chain amyloidosis, *Circ. Res.* 125 (2019) 744–758, <https://doi.org/10.1161/CIRCRESAHA.119.314862>.
- [225] Y. Mikhed, A. Daiber, S. Steven, Mitochondrial oxidative stress, mitochondrial DNA damage and their role in age-related vascular dysfunction, *Int. J. Mol. Sci.* 16 (2015) 15918–15953, <https://doi.org/10.3390/ijms160715918>.
- [226] I. Korovila, M. Hugo, J.P. Castro, D. Weber, A. Hohn, T. Grune, T. Jung Proteostasis, Oxidative stress and aging, *Redox biology* 13 (2017) 550–567, <https://doi.org/10.1016/j.redox.2017.07.008>.
- [227] S. Reeg, T. Grune, Protein oxidation in aging: does it play a role in aging progression? *Antioxidants Redox Signal.* 23 (2015) 239–255, <https://doi.org/10.1089/ars.2014.6062>.
- [228] J. Hanus, F. Zhao, S. Wang, Current therapeutic developments in atrophic age-related macular degeneration, *Br. J. Ophthalmol.* 100 (2016) 122–127, <https://doi.org/10.1136/bjophthalmol-2015-306972>.
- [229] W. Wonisch, A. Falk, I. Sundl, B.M. Winklhofer-Roob, M. Lindschinger, Oxidative stress increases continuously with BMI and age with unfavourable profiles in males, *Aging Male* 15 (2012) 159–165, <https://doi.org/10.3109/13685538.2012.669436>.
- [230] L. Gil, W. Siems, B. Mazurek, J. Gross, P. Schroeder, P. Voss, T. Grune, Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes, *Free Radic. Res.* 40 (2006) 495–505, <https://doi.org/10.1080/10715760600592962>.
- [231] K.B. Pandey, M.M. Mehdi, P.K. Maurya, S.I. Rizvi, Plasma protein oxidation and its correlation with antioxidant potential during human aging, *Dis. Markers* 29 (2010) 31–36, <https://doi.org/10.3233/DMA-2010-0723>.
- [232] K. Komosinska-Vashev, P. Olczyk, K. Winsz-Szczotka, K. Klimek, K. Olczyk, Plasma biomarkers of oxidative and AGE-mediated damage of proteins and glycosaminoglycans during healthy ageing: a possible association with ECM metabolism, *Mechanisms of ageing and development* 133 (2012) 538–548, <https://doi.org/10.1016/j.mad.2012.07.001>.
- [233] U. Cakatay, R. Kayali, H. Uzun, Relation of plasma protein oxidation parameters and paraoxonase activity in the ageing population, *Clin. Exp. Med.* 8 (2008) 51–57, <https://doi.org/10.1007/s10238-008-0156-0>.
- [234] P.S. Samiec, C. Drews-Botsch, E.W. Flagg, J.C. Kurtz, P. Sternberg Jr., R.L. Reed, D.P. Jones, Glutathione in human plasma: decline in association with aging, age-related macular degeneration, and diabetes, *Free Radic. Biol. Med.* 24 (1998) 699–704, [https://doi.org/10.1016/s0891-5849\(97\)00286-4](https://doi.org/10.1016/s0891-5849(97)00286-4).
- [235] D. Giustarini, I. Dalle-Donne, S. Lorenzini, A. Milzani, R. Rossi, Age-related influence on thiol, disulfide, and protein-mixed disulfide levels in human plasma, *The journals of gerontology. Series A, Biological sciences and medical sciences* 61 (2006) 1030–1038, <https://doi.org/10.1093/gerona/61.10.1030>.
- [236] D.P. Jones, V.C. Mody Jr., J.L. Carlson, M.J. Lynn, P. Sternberg Jr., Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses, *Free Radic. Biol. Med.* 33 (2002) 1290–1300, [https://doi.org/10.1016/s0891-5849\(02\)01040-7](https://doi.org/10.1016/s0891-5849(02)01040-7).
- [237] D. Weber, W. Stuetz, O. Toussaint, F. Debacq-Chainiaux, M.E.T. Dolle, E. Jansen, E.S. Gonos, C. Franceschi, E. Sikora, A. Hervonen, N. Breusing, T. Sindlinger, M. Moreno-Villanueva, A. Burkle, T. Grune, Associations between specific redox biomarkers and age in a large European cohort: the MARK-AGE project, *Oxidative medicine and cellular longevity* (2017) 1401452, <https://doi.org/10.1155/2017/1401452>.
- [238] T. Heitzer, T. Schlinzig, K. Krohn, T. Meinertz, T. Munzel, Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with



- coronary artery disease, *Circulation* 104 (2001) 2673–2678, <https://doi.org/10.1161/hc4601.099485>.
- [239] A. Fratta Pasini, A. Albiero, C. Stranieri, M. Cominacini, A. Pasini, C. Mozzini, P. Vallerio, L. Cominacini, U. Garbin, Serum oxidative stress-induced repression of Nrf2 and GSH depletion: a mechanism potentially involved in endothelial dysfunction of young smokers, *PLoS One* 7 (2012), e30291, <https://doi.org/10.1371/journal.pone.0030291>.
- [240] B. Jurado-Gamez, M.C. Fernandez-Marin, J.L. Gomez-Chaparro, L. Munoz-Cabrera, J. Lopez-Barea, F. Perez-Jimenez, J. Lopez-Miranda, Relationship of oxidative stress and endothelial dysfunction in sleep apnoea, *Eur. Respir. J.* 37 (2011) 873–879, <https://doi.org/10.1183/09031936.00027910>.
- [241] M.I. Yilmaz, M. Saglam, K. Caglar, E. Cakir, A. Sonmez, T. Ozgurtas, A. Aydin, T. Eyileten, O. Ozcan, C. Acikel, M. Tasar, G. Genctoy, K. Erbil, A. Vural, C. Zoccali, The determinants of endothelial dysfunction in CKD: oxidative stress and asymmetric dimethylarginine, *Am. J. Kidney Dis.* : the official journal of the National Kidney Foundation 47 (2006) 42–50, <https://doi.org/10.1053/j.ajkd.2005.09.029>.
- [242] H.E. Botker, D. Hausenloy, I. Andreadou, S. Antonucci, K. Boengler, S. M. Davidson, S. Deshwal, Y. Devaux, F. Di Lisa, M. Di Sante, P. Efentakis, S. Femmino, D. Garcia-Dorado, Z. Gircic, B. Ibanez, E. Ilodromitis, N. Kaludercic, P. Kleinbongard, M. Neuhauser, M. Ovize, P. Pagliaro, M. Rahbek-Schmidt, M. Ruiz-Meana, K.D. Schluter, R. Schulz, A. Skyschally, C. Wilder, D.M. Yellon, P. Ferdinandy, G. Heusch, Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection, *Basic Res. Cardiol.* 113 (2018) 39, <https://doi.org/10.1007/s00395-018-0696-8>.
- [243] Y. Zhang, Q. Sun, B. He, J. Xiao, Z. Wang, X. Sun, Anti-inflammatory effect of hydrogen-rich saline in a rat model of regional myocardial ischemia and reperfusion, *Int. J. Cardiol.* 148 (2011) 91–95, <https://doi.org/10.1016/j.ijcard.2010.08.058>.
- [244] M.S. Oliveira, L.Y. Tanaka, E.L. Antonio, L.I. Brandizzi, A.J. Serra, L. Dos Santos, J.E. Krieger, F.R.M. Laurindo, P.J.F. Tucci, Hyperbaric oxygenation improves redox control and reduces mortality in the acute phase of myocardial infarction in a rat model, *Mol. Med. Rep.* 21 (2020) 1431–1438, <https://doi.org/10.3892/mmr.2020.10968>.
- [245] M.J. Barrios, G. Munoz, R. T, 315 - 4-hydroxynonenal as oxidative damage index in acute myocardial infarction patients, *Free Radic. Biol. Med.* 100 (2016) S136.
- [246] I.E. Blasig, T. Grune, K. Schonheit, E. Rohde, M. Jakstadt, R.F. Haseloff, W. G. Siems, 4-Hydroxynonenal, a novel indicator of lipid peroxidation for reperfusion injury of the myocardium, *Am. J. Physiol.* 269 (1995) H14–H22, <https://doi.org/10.1152/ajpheart.1995.269.1.H14>.
- [247] A. Renner, M.R. Sagstetter, H. Harms, V. Lange, M.E. Gotz, O. Elert, Formation of 4-hydroxy-2-nonenal protein adducts in the ischemic rat heart after transplantation, *J. Heart Lung Transplant.* 24 (2005) 730–736, <https://doi.org/10.1016/j.healun.2004.02.021>.
- [248] X.Y. Wu, A.Y. Luo, Y.R. Zhou, J.H. Ren, N-acetylcysteine reduces oxidative stress, nuclear factor- $\kappa$ B activity and cardiomyocyte apoptosis in heart failure, *Mol. Med. Rep.* 10 (2014) 615–624, <https://doi.org/10.3892/mmr.2014.2292>.
- [249] Q.C. Yan, A.H. Xiong, X. Xiao, Y.T. Luo, [Significance of plasma 8-iso-prostaglandin F $_{2\alpha}$  level in acute myocardial ischemia and intervention effect of N-acetylcysteine: a study in rats], *Di Yi Jun Yi Da Xue Xue Bao* 23 (2003) 605–607.
- [250] H. Inafuku, Y. Kuniyoshi, S. Yamashiro, K. Arakaki, T. Nagano, Y. Morishima, Y. Kise, Determination of oxidative stress and cardiac dysfunction after ischemia/reperfusion injury in isolated rat hearts, *Ann. Thorac. Cardiovasc. Surg.* 19 (2013) 186–194, <https://doi.org/10.5761/atcs.0a.12.01896>.
- [251] F. Qin, M. Simeone, R. Patel, Inhibition of NADPH oxidase reduces myocardial oxidative stress and apoptosis and improves cardiac function in heart failure after myocardial infarction, *Free Radic. Biol. Med.* 43 (2007) 271–281, <https://doi.org/10.1016/j.freeradbiomed.2007.04.021>.
- [252] C. Yang, K. Wu, S.H. Li, Q. You, Protective effect of curcumin against cardiac dysfunction in sepsis rats, *Pharm. Biol.* 51 (2013) 482–487, <https://doi.org/10.3109/13880209.2012.742116>.
- [253] A.O. Sehirli, D. Koyun, S. Tetik, D. Ozsavci, O. Yiginer, S. Cetinel, O.E. Tok, Z. Kaya, M. Akkiprik, E. Kilic, G. Sener, Melatonin protects against ischemic heart failure in rats, *J. Pineal Res.* 55 (2013) 138–148, <https://doi.org/10.1111/jpi.12054>.
- [254] A. Gyongyosi, L. Terraneo, P. Bianciardi, A. Tosaki, I. Lekli, M. Samaja, The impact of moderate chronic hypoxia and hyperoxia on the level of apoptotic and autophagic proteins in myocardial tissue, *Oxidative medicine and cellular longevity* (2018) 5786742, <https://doi.org/10.1155/2018/5786742>, 2018.
- [255] S. Kurokawa, S. Niwano, H. Niwano, S. Ishikawa, J. Kishihara, Y. Aoyama, T. Kosukegawa, Y. Masaki, T. Izumi, Progression of ventricular remodeling and arrhythmia in the primary hyperoxidative state of glutathione-depleted rats, *Circ. J.* 75 (2011) 1386–1393, <https://doi.org/10.1253/circj.10-1089>.
- [256] P.M. Boarescu, I. Chirila, A.E. Bulboaca, I.C. Bocsan, R.M. Pop, D. Gheban, S. D. Bolboaca, Effects of curcumin nanoparticles in isoproterenol-induced myocardial infarction, *Oxidative medicine and cellular longevity* (2019) 7847142, <https://doi.org/10.1155/2019/7847142>, 2019.
- [257] P.M. Boarescu, I. Boarescu, I.C. Bocsan, R.M. Pop, D. Gheban, A.E. Bulboaca, C. Nicula, R.M. Rajnoveanu, S.D. Bolboaca, Curcumin nanoparticles protect against isoproterenol induced myocardial infarction by alleviating myocardial tissue oxidative stress, electrocardiogram, and biological changes, *Molecules* 24 (2019), <https://doi.org/10.3390/molecules24152802>.