Influence of cardiometabolic comorbidities on myocardial function, infarction, and cardioprotection: Role of cardiac redox signaling

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Abstract:	The morbidity and mortality from cardiovascular diseases (CVD) remain high. Metabolic diseases such as obesity, hyperlipidemia, diabetes mellitus (DM), non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) as well as hypertension are the most common comorbidities in patients with CVD. These comorbidities result in increased myocardial oxidative stress, mainly from increased activity of nicotinamide adenine dinucleotide phosphate oxidases, uncoupled endothelial nitric oxide synthase, mitochondria as well as downregulation of antioxidant defense systems. Oxidative and nitrosative stress play an important role in ischemia/reperfusion injury and may account for increased susceptibility of the myocardium to infarction with one or several of the above comorbidities. On the other hand, controlled release of reactive oxygen species is also important for cardioprotective signaling. In this review we summarize the current data on the effect of hypertension and major cardiometabolic comorbidities such as obesity, hyperlipidemia, DM, NAFLD/NASH on cardiac redox homeostasis as well as on ischemia/reperfusion injury and cardioprotection. We also review and discuss the therapeutic interventions that may restore the redox imbalance in the diseased myocardium in the presence of these comorbidities.
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To the Editorial Office Free Radical Biology & Medicine
Prof. Giovanni Mann

Dear Prof. Mann,
Thank you very much for inviting us to contribute a review paper to Special Issue "Implications of oxidative stress and redox biochemistry for heart disease and cardioprotection" in Free Radical Biology & Medicine, edited by Dr. Andreas Daiber, Dr. Derek J. Hausenloy, Dr. Ioanna Andreadou, and Dr. Rainer Schulz. With this letter we would like to submit our review manuscript with the title " **INFLUENCE OF CARDIOMETABOLIC COMORBIDITIES ON MYOCARDIAL FUNCTION, INFARCTION, AND CARDIOPROTECTION: ROLE OF CARDIAC REDOX SIGNALING**" for consideration for publication in Special Issue "Implications of oxidative stress and redox biochemistry for heart disease and cardioprotection" in Free Radical Biology & Medicine.

Hereby we declare that 1) the paper is not under consideration elsewhere 2) all authors have read and approved the manuscript 3) the full disclosure of any potential conflict of interest is provided 4) we will accept the publication costs

As three of the guest editors of the special issue are involved as authors on the manuscript, we ask for your support to handle the review process. Hoping that our manuscript is suitable for publication in *Free Radical Biology & Medicine,* we look forward to receiving your comments.

Sincerely yours,

Prof. Ioanna Andreadou
Prof. Andreas Daiber
Prof. Rainer Schulz

Highlights

- Evaluation of the impact of ischemic heart disease for the global burden of disease
- The role of oxidative stress in cardiovascular diseases and cardiometabolic comorbidities
- Specific role of ROS and adverse redox signaling in ischemia/reperfusion damage and heart failure
- Summary of redox targeting in cardiovascular disease in general
- Summary of redox targeting in ischemia/reperfusion damage and heart failure in particular

Special issue "Implications of oxidative stress and redox biochemistry for heart disease and cardioprotection"

Review article

INFLUENCE OF CARDIOMETABOLIC COMORBIDITIES ON MYOCARDIAL FUNCTION, INFARCTION, AND CARDIOPROTECTION: ROLE OF CARDIAC REDOX SIGNALING

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Abstract

The morbidity and mortality from cardiovascular diseases (CVD) remain high. Metabolic diseases such as obesity, hyperlipidemia, diabetes mellitus (DM), non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) as well as hypertension are the most common comorbidities in patients with CVD. These comorbidities result in increased myocardial oxidative stress, mainly from increased activity of nicotinamide adenine dinucleotide phosphate oxidases, uncoupled endothelial nitric oxide synthase, mitochondria as well as downregulation of antioxidant defense systems. Oxidative and nitrosative stress play an important role in ischemia/reperfusion injury and may account for increased susceptibility of the myocardium to infarction with one or several of the above comorbidities. On the other hand, controlled release of reactive oxygen species is also important for cardioprotective signaling. In this review we summarize the current data on the effect of hypertension and major cardiometabolic comorbidities such as obesity, hyperlipidemia, DM, NAFLD/NASH on cardiac redox homeostasis as well as on ischemia/reperfusion injury and cardioprotection. We also review and discuss the therapeutic interventions that may restore the redox imbalance in the diseased myocardium in the presence of these comorbidities.

Keywords: cardiovascular comorbidities; oxidative stress; myocardial infarction; redox therapeutic strategies.

Abbreviations

AGE	advanced glycation end-products
AMPK	AMP-activated protein kinase
Apo	apoprotein
BH4	tetrahydrobiopterin
BMI	body mass index
CAMK-II	calmodulin-dependent kinase-II
CAT	catalase
CR	caloric restriction
CVD	cardiovascular disease
DAMP	damage-associated molecular patterns
DHA	docosahexaenoic acid
DM	diabetes mellitus
DPP	dipeptidyl protease
eNOS	endothelial nitric oxide synthase
EPA	eicosapentaenoic acid
ERK	extracellular signal regulated kinase
ETC	electron transport chain
FMD	flow-mediated dilatation
FOXO	forkhead box protein O
GLP-1	glucagon-like peptide-1
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	reduced glutathione
GSK-3β	glycogen synthase kinase-3β
GSSG	oxidized glutathione
GST	glutathione transferase
HF	heart failure
HIF	hypoxia inducible factor
HKII	hexokinase-II

H_2S	hydrogen sulfide
HSP	heat shock protein
IHD	ischemic heart disease
IL	interleukin
iNOS	inducible nitric oxide synthase
IRI	ischemia/reperfusion injury
KO	(gene) knockout (mouse strain)
LDL	low-density lipoprotein
LV	left ventricle
LVH	left ventricular hypertrophy
MAO	monoamine oxidase
MAPK	mitogen-activated protein kinase
MI	myocardial infarction
mPTP	mitochondrial permeability transition pore
3-MST	3-mercaptopyruvate sulfurtransferase
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NFAT	nuclear factor of activated T-cells
NFκB	nuclear factor-kappa B
NNT	nicotinamide nucleotide transhydrogenase
NO	nitric oxide
NOX	nicotinamide adenine dinucleotide phosphate (NADPH) oxidase
Nrf-2	nuclear factor erythroid 2-related factor
NSTEMI	non-ST elevation myocardial infarction
OSE	oxidation specific epitopes
Ox-LDL	oxidised low-density lipoprotein (LDL)
PCSK9	proprotein convertase subtilisin/kexin type 9
PDE5	phosphodiesterase-5
PGC coactivator	peroxisome proliferator activated receptor-gamma (PPAR-γ)

PKC	protein kinase C
PKG	cyclic guanosine monophosphate (cGMP)-dependent kinase
Pon	paraoxonase
PPAR	peroxisome proliferator activated receptor
Prdx	peroxiredoxin
PUFA	polyunsaturated fatty acid
RAGE	receptor of advanced glycation end-products (AGE)
RNS	reactive nitrogen species
ROS	reactive oxygen species
SGLT2	sodium-glucose cotransporter-2
SIRT	sirtuin
sNox2-dp	soluble NOX2-derived peptide
SOD	superoxide dismutase
SphK1	sphingosine kinase-1
STEMI	ST-elevation myocardial infarction
STZ	streptozotocin
T2DM	type-2 diabetes mellitus
TNFα	tumor necrosis factor-alpha
Trx	thioredoxin
UCP	uncoupling protein
VSMC	vascular smooth muscle cells
XO	xanthine oxidase
ZDF	Zucker diabetic fatty (rat strain)

1. Introduction

Cardiovascular diseases (CVD) are the leading causes of disease burden and the primary causes of death worldwide [1]. CVD are systemic diseases, rarely occurring alone so it is common to find multiple comorbid conditions in the setting of CVD, particularly in the elderly population. Comorbidities, the presence of one or more chronic diseases among patients with CVD, are increasing due to reduced case fatality of ischemic heart disease (IHD) and prolonged life expectancy [2, 3]. However, the rising prevalence of diabetes mellitus (DM) worldwide, linked to the almost ubiquitous increase of obesity and non-alcoholic fatty liver disease and steatohepatitis (NAFLD and NASH) globally, is mitigating reductions in the burden of CVD by effective cardiological interventions (cholesterol and blood pressure lowering, coronary interventions, etc.). Metabolic diseases such as obesity, hyperlipidemia, DM, NAFLD and NASH as well as hypertension are common comorbidities in patients with IHD and heart failure (HF) and affect the clinical outcomes profoundly [4].

Obesity and DM synergistically cause myocardial dysfunction independent of coronary artery disease and hypertension since both conditions share similar pathophysiological mechanisms [5, 6]. Similarly, hyperlipidemia *per se* is able to negatively affect myocardial function. These metabolic heart diseases (myocardial dysfunction caused by obesity, hyperlipidemia, and DM) are characterized by altered myocardial energetics with mitochondrial dysfunction, nitro-oxidative stress, abnormal cellular metabolism leading to lipotoxicity in the myocytes, cardiac autonomic neuropathy, as well as increased inflammation and interstitial collagen deposition [7-9]. These pathological changes 1. are further aggravated by the parallel development of coronary atherosclerosis; 2. result in subclinical myocardial dysfunction (initially diastolic) and eventually the development of overt HF with preserved ejection fraction that may over time progress into HF with reduced ejection fraction [10]; and 3. exert numerous biochemical effects on the heart that negatively affect the development of ischemia/reperfusion injury (IRI) and interfere with cardioprotective interventions, notably ischemic conditioning. However, the exact mechanism by which the remarkable cardioprotective effect of ischemic conditioning is attenuated or abolished in the presence of major cardiovascular risk factors and comorbidities is not fully understood [11]. Accentuated myocardial oxidative stress has been reported in the

presence of major comorbidities (**Figure 1**); therefore, it is plausible that redox signaling-dependent changes profoundly contribute to the pathological phenotypes.

In this review we summarize the current data on the effect of major cardiovascular comorbidities on cardiac redox homeostasis, focusing on metabolic diseases such as obesity, hyperlipidemia, DM, hypertension and NAFLD/NASH. We will also review the therapeutic interventions that may restore the redox imbalance in the diseased myocardium in presence of these comorbidities.

2. Obesity

According to WHO data for 2014, 11% of men and 15% of women (>18 years old) were obese (body mass index [BMI] > 30 kg/m^2) [12]. More than 42 million children under the age of 5 years were reported to be overweight in 2013. Obesity increases the risk of myocardial infarction (MI) by 20-40% (odds ratio 1.2-1.4 in different studies). High BMI is ranked fifth among the leading risk factors for disability-adjusted life years (years lived with severe illness) based on the global burden of disease data for 2019 [13]. Obesity is strongly associated with the development of atherosclerosis, but it may also have direct effects on the heart [14]. Results from the Framingham Heart Study indicated that increased BMI correlates well with greater risk for developing HF both in men and women [10]. Data from patients and animal models clearly indicate that the heart undergoes structural and functional changes in obesity [14]. Hearts from obese subjects have increased left and right ventricular wall thickness, increased left atrium dimensions, fibrosis and accumulation of intracellular triglycerides [14]. Subclinical contractile alterations have been detected in obese patients, along with diastolic dysfunction [14, 15]. Similar results have been observed in experimental models of obesity, suggesting that obesity alone does not cause impairment in systolic function, although it does affect cardiac relaxation properties [16-18].

Increased circulating free fatty acids trigger a vicious cycle harming the antioxidant response in overweight and obese individuals [19, 20]. This is particularly true for the myocardium under stress conditions; glucose, compared with fatty acids, is the more efficient substrate to boost high energy products with respect to oxygen consumption

[21]. Despite these evident repercussions on cardiac structure and function in obesity, it is challenging to distinguish between the effects deriving directly from obesity and the effects of other comorbidities strongly associated with obesity, such as atherosclerosis, hypertension, hyperlipidemia and DM.

2.1. Obesity and Redox signaling in myocardial infarction

A number of studies have highlighted an increased myocardial susceptibility to IRI in experimental models of obesity and in patients [22-26]. However, other studies report conflicting results, such as normal or even enhanced functional recovery following ischemia/reperfusion in obese animals [27-29]. The reason for this discrepancy is not clear. One possibility is that changes in hemodynamics (i.e. preload and afterload) may confound contractile defects *in vivo* [14]. In addition, obesity is associated with elevated circulating concentrations of insulin and fatty acids that might affect the extent of myocardial damage after IRI [30-32]. Indeed, one study found that obesity led to increased infarct size and reduced functional recovery after ischemia/reperfusion performed *ex vivo* with the classic Krebs-Henseleit perfusion buffer, but the presence of insulin and fatty acids in the buffer completely abolished these differences between obese and non-obese hearts [22]. An additional factor that may affect the outcome is age, since aged obese hearts show reduced functional recovery when subjected to preconditioning [33].

From a molecular standpoint, changes in substrate utilization, mitochondrial function, redox signaling and inflammation occur much earlier and precede measurable changes in cardiac function in obese hearts. The functional recovery of the heart after ischemia/reperfusion can be improved by increasing glucose oxidation during reperfusion [32]. Unsurprisingly, increased delivery of fatty acids in obese hearts and activation of related pathways, such as peroxisome proliferator-activated receptor alpha (PPARα), contribute to myocardial degeneration [14]. A common denominator in these metabolic alterations is oxidative stress. BMI was directly correlated with several oxidative stress parameters, positively with p47phox expression and hydroethidium oxidation, but negatively correlated with endothelial nitric oxide synthase (eNOS) phosphorylation and dihydrofolate reductase expression in patients undergoing coronary artery bypass graft surgery [34]. In patients with IHD, BMI also correlated with leptin levels and oxidative stress markers, with an impact on cardiovascular and

operative risk profiles [35]. The coexistence of hypercholesterolemia and obesity in children caused additive increase of 8-isoprostanes and soluble nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) 2-derived peptide (sNox2-dp, a marker of NOX2 activation) and additive impairment of endothelial function measured by flow-mediated dilation (FMD) [36]. Obesity is associated with alterations in mitochondrial function, number and turnover [37-40]; thus, impairment in mitochondrial oxidative capacity observed in ob/ob mice inevitably results in increased superoxide formation [14]. Indeed, the mitochondrial respiratory chain (i.e. complexes I and III) is considered the most relevant source of reactive oxygen species (ROS) in obese or diabetic hearts [41]. An additional mechanism for mitochondrial ROS formation is represented by $p66^{Shc}$ that, upon phosphorylation by protein kinase C (PKC), translocates to mitochondria to induce hydrogen peroxide (H_2O_2) formation [42]. $p66^{Shc}$ is critical for insulin signaling and glucose uptake and its phosphorylation is increased in obesity and DM [43, 44]. Moreover, its deletion reduces oxidative stress and atherogenesis in mice fed with high-fat diet [45].

Besides mitochondria, other enzymes within the cell contribute to the alteration of redox equilibrium and there may be crosstalk between them. For instance, $p66^{Shc}$ inhibits forkhead-box-protein O (FOXO) transcription factors in the nucleus thereby affecting the expression of antioxidant enzymes [46]. Importantly, $p66^{Shc}$ can also activate ras-related C3 botulinum toxin substrate 1 (rac1) and trigger NOX mediated ROS formation [46]. Indeed, NOX activity is enhanced in obese animals and its inhibition prevents oxidative stress and impairment in cardiac function in these hearts [47, 48]. Both mitochondrial and NOX-dependent ROS formation play a major role in lipotoxicity. Obese patients have higher circulating levels of saturated fatty acid palmitate that can trigger mitochondrial ROS formation. This can in turn be amplified by NOX2 causing mitochondrial dysfunction and further amplifying oxidative stress in a vicious cycle [49]. Furthermore, the inability of cardiomyocytes to respond to an increased fatty acid load results in the generation of toxic lipid intermediates, such as ceramide, that promote mitochondrial dysfunction and cell death [48, 50]. Lipotoxicity further aggravates cardiac IRI and mitochondrial ROS play a major role in this mechanism [51, 52]. Indeed, it has been demonstrated that ROS produced by the mitochondrial flavoenzyme monoamine oxidase A (MAO-A) inhibit sphingosine kinase-1 (SphK1) and are associated with generation of proapoptotic ceramide. It is

noteworthy that SphK1 inhibition, ceramide accumulation, infarct size and cardiomyocyte apoptosis were significantly decreased in MAO-A deficient animals subjected to IRI [52]. MAO plays a major role in the oxidative stress in diabetic cardiomyopathy [53] and it remains to be elucidated whether these mechanisms also apply to changes observed in obese hearts. Interestingly, the selective MAO-B inhibitor, selegiline, was able to reduce adiposity and improve metabolic parameters in a rat model of diet-induced obesity [54].

Among other sources of ROS in the heart, xanthine oxidase (XO) has been shown to promote oxidative stress, inflammation and alterations in cardiac structure and function in mice fed a Western diet [55]. On the other hand, antioxidant enzymes also play a major role in obese hearts. For instance, expression and/or activity of many antioxidant enzymes is reduced in cardiac tissue or in the circulation of obese animals [48]. Moreover, mitochondrial peroxidases involved in ROS removal use NADPH provided mostly by nicotinamide nucleotide transhydrogenase (NNT). A recent study showed that, in conditions of high nutrient availability and low energy demand, NNT activity maintains low ROS levels through a fine modulation of mitochondrial oxygen utilization [56]. In failing hearts, NNT activity can be reversed resulting in the depletion of mitochondrial antioxidant capacity and oxidative stress [57]. Yet, whether alterations in NNT activity may be responsible for altered redox equilibrium in obese and ischemic hearts has not been investigated to date.

2.2. Pharmacological redox modulation in obesity and cardioprotection

Lifestyle intervention, caloric restriction (CR), exercise training and different pharmaceutics/nutraceuticals have been proposed to limit the inflammatory response and ROS generation and to improve the antioxidant machinery in obesity. ω-3-polyunsaturated fatty acids (PUFAs) are broadly used as a secondary interventional approach in CVD and have been extensively investigated in the setting of obesity. *In vitro* studies have shown that PUFAs interfere with eicosanoid generation [58] and decrease NOX activity [59]. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) administration increased the expression of heme oxygenase-1 by a mechanism dependent on nuclear factor erythroid 2-related factor 2 (Nrf-2) [60]. Moreover, PUFA supplementation in humans resulted in increased expression of antioxidants such as catalase (CAT), heme oxygenase-2, glutathione transferases (GST) and glutathione

reductase (GR) and in the down regulation of pro-oxidant genes such as the glutathione peroxidases [61]. In addition to PUFAs, polyphenols were found to boost nitric oxide (NO) bioavailability by inducing eNOS activity, while reducing NOX1 in obese animals [62]. Long term resveratrol administration, besides increasing the expression of eNOS in white adipose tissues, reduces the systemic inflammatory response by increasing the circulating levels of adiponectin and lowering the release of tumor necrosis factor-alpha (TNFα) [63]. Mechanistically, these compounds were found to exert their anti-inflammatory and cardioprotective effects by activating the adenosine monophosphate (AMP)-activated protein kinase (AMPK), peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α- and PPARγ-mediated pathways in Zucker Diabetic Fatty (ZDF) rats [63]-[64].

A number of primary and secondary interventional studies have reported the benefit of CR, indicating that CR is effective in reducing the anti-inflammatory response and in improving the antioxidant response in obese individuals [65]. In particular, CR-mediated protection relies on the decrease of oxidative stress markers via sirtuins (SIRT), NAD^+-dependent deacetylases [66]-[67], FOXO [68] and PGC-1α-mediated mitochondrial bioenergetics [69]. CR can additionally exert cardioprotection by induction of antioxidant adaptive genes associated to the increased expression of adiponectin and the activation of the AMPK [70]. It was also noticed that CR-mediated cardioprotection occurs via SIRT1 and PGC-1α in obese animals [71]. Polyphenols and exercise training were reported to induce stress response genes and mitochondrial biogenesis via AMPK and SIRT mediated reduction of FOXO activity [72]-[73]. Of note, prebiotics, probiotics, and synbiotics were found to induce cardioprotection by restoring mitochondrial dysfunction via the improvement of the electromechanical proton gradient in obese animals [74].

An improvement of mitochondrial biogenesis and myocardial function in obese transgenic mice overexpressing mitochondrial-CAT has been demonstrated, an effect relying on the decrease of ROS generation in the heart [75]. Lowell et al. first demonstrated the role of mitochondrial uncoupling in driving obesity [76]. Partial mitochondrial uncoupling improving post-ischemic functional recovery via a ROS-dependent pathway has been observed [77]. SIRT1 [78] and PPARγ pathways were recently found to drive white-to-brown adipose tissue remodelling via uncoupling proteins (UCP) such as UCP1 [79]. Mild uncoupling of oxidative phosphorylation is

one of the mechanisms suggested to be cardioprotective as chemical uncoupling mimics ischemic preconditioning and of note, chemical uncouplers acting on mitochondrial H_2O_2 production in the heart, share common cardioprotective mechanisms with low concentrations of dietary polyphenols [80]. Mechanistically the UCP3-mediated cardio-protection against IRI may involve the inhibition of the mitochondrial permeability transition pore (mPTP) opening, mitochondrial calcium overload and ROS generation [81].

Obesity abolishes pharmacological preconditioning-induced cardioprotection due to impairment of the ROS-mediated AMPK pathway, a consequence of increased basal myocardial oxidative stress. Exercise training can prevent the attenuation of anesthetic cardioprotection in obesity by a mechanism including reduced basal oxidative stress and normalized ROS-mediated AMPK pathway [82].

In preclinical models of obesity, cardioprotection was also reported with several currently used antidiabetic drugs. Vildagliptin was found to be protective against IRI in obese-insulin resistant rats by improving mitochondrial function, oxidative stress and apoptosis in the ischemic myocardium [83]. The sodium-glucose cotransporter-2 (SGLT2) inhibitor dapagliflozin was also found to exert cardioprotection in high-fat diet-induced obese/insulin-resistant rats by decreasing the cleaved caspase 3 as well as mitochondrial anti-dynamin related protein-1, suggesting a role of dapagliflozin in the control of mitochondrial fission [84]. Empagliflozin reduced body weight, infarct size and improved redox regulation by decreasing inducible NOS (iNOS) expression and subsequently lipid peroxidation in mice fed a Western diet [85].

Adiponectin has been reported to play a protective role in the development of obesity-linked disorders. It has been shown that adiponectin protects against IRI in a pig model through its ability to suppress inflammation, apoptosis and oxidative stress [86]. Treatment with AC261066, a synthetic selective agonist for the retinoic acid β_2-receptor exerted protective effects in obese (high fat diet-fed) wild-type mice when their hearts were subjected to ischemia/reperfusion *ex vivo*. This cardioprotection was associated with decreased formation of ROS and toxic aldehydes [87]. Melatonin, a potent free radical scavenger and antioxidant reduced infarct size in a rat model of diet-induced obesity and prevented the metabolic abnormalities induced by diet-induced obesity [88]. MitoTEMPO, a mitochondria-targeted ROS scavenger, prevented cardiac

fibrosis and oxidative stress and ameliorated weight gain in a high fat diet rodent model [89]. Similar protective effects were observed with mitoQ, a synthetic mitochondrial antioxidant [90-92].

In summary, although it is difficult to separate the effects originating only from obesity from the effects induced by other comorbidities strongly associated with obesity, such as hyperlipidemia and diabetes, redox signaling triggers changes in cardiac function in obese hearts. Lowering oxidative stress to prevent metabolic disorders related to obesity constitutes to be an interesting therapeutic target. However, further studies are needed to clearly understand ROS generation, typology, and distribution in obesity.

3. Hyperlipidemia

According to WHO data, the global prevalence of hyperlipidemia (hypercholesterolemia) could be up to 40% [93]. Hyperlipidemia increases the risk of MI more than 8-fold (odds ratio 8.39) [94]. Low density lipoprotein (LDL) cholesterol is ranked eighth among the leading risk factors for disability-adjusted life years (years lived with severe illness) based on the global burden of disease data of the year 2019 [13]. Multiple experimental studies have shown that hyperlipidemia enhances infarct size and favors cardiolipotoxicity. Oxidative stress and NO play an important role in LDL accumulation in the vascular wall [95]. Hypercholesterolemia facilitates the reaction between ROS and NO inducing the generation of reactive nitrogen species (RNS) such as dinitrogen trioxide (N_2O_3) and peroxynitrite [96]. Nitrosation of protein thiols by peroxynitrite may also exert detrimental effects on protein synthesis contributing to the promotion of ROS and inflammation [97]. Both native LDL and oxidized LDL (oxLDL) stimulate superoxide/peroxynitrite production and uncouple eNOS [98] thereby reducing endothelial NO production by inhibiting eNOS activity [99]. Furthermore, hypercholesterolemia upregulates caveolin and promotes eNOS interaction with caveolin [100] and decreases the association of eNOS with heat shock protein (HSP) 90 [101] resulting in a further inhibition of eNOS activity. Finally, oxLDL decreases eNOS activity either by inhibiting phosphorylation of eNOS at serine 1177 [102] or by increased proteasomal degradation of eNOS [103]. Consistent with

experimental evidence, reduced bioavailability of NO has been demonstrated in hypercholesterolemic patients [97] and these patients also displayed impaired endothelial function (measured by venous occlusion plethysmography) [104]. Apart from the direct effects of lipids on ROS/NO formation, disturbed flow pattern with the development of atherosclerosis reduces endothelial NO production [105] and enhances ROS production in endothelial cells and in vascular smooth muscle cells (VSMC) [106]. Oxidative stress and ROS play opposite roles in the regulation of adhesion molecule expression and endothelial–leukocyte interaction. Endothelial NO inhibits cytokine-induced nuclear factor-κB (NFkB) activation and upregulation of vascular cell and intercellular adhesion molecules [107, 108], whereas inhibition of NO production increases leukocyte adherence [109]. On the contrary, ROS are implicated in upregulation of adhesion molecules induced by cytokines [107].

OxLDL exhibits a wide array of proatherogenic properties and many of these effects are mediated by oxidized phospholipids within the LDL molecules. Lipid peroxidation can occur through enzymatic mechanisms (e.g., by ROS derived from NOX, uncoupled eNOS) [110], myeloperoxidases, lipoxygenases, cyclooxygenases, and cytochrome P450). In some cases, ROS formation is based on the original enzyme activity, whereas in other cases ROS originate from undesired side reactions. The lipid peroxidation products, such as malondialdehyde, 4-hydroxynonenal etc. are highly reactive and can lead to the generation of structural neoepitopes termed oxidation-specific epitopes (OSEs) [111], which play an important role in the development of atherosclerosis. OxLDL leads to upregulation of proprotein convertase subtilisin/kexin type 9 (PCSK9) expression and release from extrahepatic tissues thereby contributing to an increase in the overall circulating PCSK9 concentration, which then impacts on LDL levels, but also aggravates atherosclerosis development *per se* and impairs cardiac function [112]-[113].

In addition to the direct effects of LDL on endothelial ROS production, hypercholesterolemia may indirectly enhance oxidative stress by potentiating the effects of angiotensin II via upregulation of angiotensin II type 1 receptor [114]. ROS are also produced as byproducts of mitochondrial respiration and can become pathologically elevated during metabolic perturbations such as those seen in hyperlipidemia [115]. OxLDL inhibits the normal function of mitochondria and thus

promotes mitochondrial ROS generation which is in turn involved in LDL oxidation creating a vicious cycle. Additionally, ROS may inhibit specific mitochondrial enzymes affecting cellular antioxidant and energetic capacities [116].

3.1.Hyperlipidemia and redox signaling in myocardial infarction

Hypercholesterolemia increased myocardial necrosis by 45% compared to normal animals and this contributed to increased oxidative stress in the ischemic myocardium such as protein oxidation, lipid peroxidation, and tyrosine nitration during IRI in the setting of hypercholesterolemia [117, 118]. Tyrosine nitration was also increased in Watanabe heritable hyperlipidemic rabbits [119]. Accumulating evidence indicates that the major enzymatic sources of ROS in the cardiovascular system are NOX, uncoupled eNOS, mitochondria and XO [120]. NOX and XO have been proposed to be the major sources of superoxide anion in the coronary artery of hypercholesterolemic patients with CAD [121] as well as cholesterol-fed rabbits [122]. NOX-derived oxidative stress has been shown to be a major mediator of atherosclerosis [123], since LDL oxidation can be induced by NOX-derived ROS [124]. As already mentioned above, obesity and hypercholesterolemia had additive effects on NOX2 activation (measured by sNox2-dp) [36] and higher sNox2-dp as well as oxLDL levels were even observed in in hypercholesterolemic children [125]. However, the different NOX isoforms seem to have different roles in development and progression of atherosclerosis. NOX1 and NOX2 are required for the development of atherosclerosis [126]. Deletion of *Nox1* in apoprotein (Apo)E knockout (KO) mice reduced aortic superoxide production, macrophage infiltration and lesion formation [127]. In contrast, several studies have shown a protective role of NOX4 in atherosclerosis [128]. Global *Nox4* knockout or induced deletion of *Nox4* increased atherosclerosis in *ApoE*-KO mice. The results of the above-mentioned study demonstrated that H_2O_2 production was reduced, however, increased inflammation, macrophage accumulation and fibrosis were observed in the aortae of *Nox4*/*ApoE* double KO mice. These data suggest that NOX4-derived H_2O_2 might mediate beneficial effects in atherosclerosis via inhibition of inflammation, which is contrary to the deleterious effects of ROS produced by NOX1 and NOX2 [129]. NOX5 is localized in both endothelial and VSMCs and it has been found in the coronary arteries from patients with CAD undergoing cardiac transplantation [130]. Moreover, NOX5 increases the proliferation of VSMCs [131], but so far, there is no

direct evidence available on the role of NOX5 in atherogenesis from animal models because rodents do not express NOX5.

Uncoupling of eNOS is likely to be a subsequent event secondary to oxidative stress mediated by NOXs and XO because of oxidation-induced tetrahydrobiopterin (BH4) deficiency [132]. Besides BH4 deficiency, L-arginine deficiency also represents an underlying cause of eNOS uncoupling in hypercholesterolemia. The latter has been supported by studies in *ApoE*-KO mice and in hyperlipidemic rabbits where upregulation of arginase expression and activity caused a decrease in L-arginine levels thereby affecting substrate availability for eNOS [133, 134]. ROS derived from uncoupled eNOS has been detected in LDL-treated endothelial cells, in hypercholesterolemic *ApoE*-KO mice and in hypercholesterolemic patients as well [135]. ROS derived from NOXs and uncoupled eNOS are also involved in the generation of OSEs. OSEs, including oxidized phospholipids and malondialdehyde-modified amino groups, have been documented on the surface of apoptotic cells and oxLDL molecules [136]. Peroxidation of phospholipids moieties promotes a change in the conformation of the apoB-100 molecule leading to enhanced nonreceptor-mediated capture of oxLDL by vascular cells [136]. Oxidized phospholipids induce the expression of chemoattractants and trigger monocyte binding to endothelial cells via toll-like receptor 4 [110]. Therefore, OSE sensing by endothelial cells is a key response in the development of atherosclerosis [111].

In addition to the role of ROS in hyperlipidemia, the effects of antioxidant defense systems are significant. The expression and activity of antioxidants and antioxidant enzymes (especially reduced glutathione, SOD and CAT) in the vascular system is reduced in hypercholesterolemia [97]. The effects of SOD on atherogenesis are dose-dependent. Moderate SOD1 upregulation reduces ROS burden, whereas SOD1 overexpression generates high amount of hydrogen peroxide, which can lead to the formation of hydroxyl radicals thereby exacerbating oxidative stress [137]. SOD2 is one of the first line defense enzymes against superoxide production of the mitochondrial electron transport chain (ETC). SOD2 deficiency leads to mitochondrial dysfunction and accelerated atherosclerosis in *ApoE*-KO mice [138]. SOD3 is abundantly expressed in the vascular wall and its role in atherogenesis is still unclear. Genetic deletion of SOD3 in *ApoE*-KO mice leads to a slight reduction in

atherosclerosis after one-month atherogenic diet, whereas no effect is observed after three months [139].

Glutathione peroxidase (GPx)-1 deficiency increases LDL oxidation, foam cell formation, and macrophage proliferation [140]. The protective role of GPx1 against atherogenesis has been shown in experimental studies where deficiency of GPx1 enhanced atherosclerosis in ApoE-KO mice [141, 142]. GPx4 reduces the level of hydrogen peroxide and other lipid hydroperoxides, including oxidized phospholipids and cholesterol hydroperoxides, and likely explaining why GPx4 overexpression reduces atherosclerosis in *ApoE*-KO mice [143].

The paraoxonase family proteins (Pon1, Pon2, and Pon3) reduce oxidative stress, decrease lipid peroxidation, and diminish atherosclerosis. Pon1 is primarily synthesized by the liver and associates with high density lipoprotein (HDL) particles. HDL-associated Pon1 inhibits the formation of oxidized phospholipids and therefore LDL oxidation [132]. Pon2 is expressed in the vascular wall and in intracellular structures, such as the membranes of the endoplasmic reticulum or mitochondria and can translocate to the plasma membrane in response to oxidative stress where it suppresses lipid peroxidation [144]. Pon2 prevents LDL peroxidation, reduces oxidative stress in vascular cells, and protects against atherosclerosis in mouse models [145]. Pon2 knockout mice display increased ROS formation and endothelial dysfunction as well as higher tissue factor levels and a procoagulant phenotype [146]. Pon3 is found both in serum and cells and prevents LDL oxidation like Pon1 [147]. Pon2/3 antioxidant effects result from the prevention of mitochondrial superoxide formation through an interaction with coenzyme Q10 (ubiquinone) [148]. Pon3 expression is reduced in vascular cells of atherosclerotic patients [149].

In contrast to the regulated production of NO by neuronal NOS and eNOS, iNOS may generate large amounts of NO over long periods of time and iNOS induction in the vasculature facilitates the generation of peroxynitrite [150], a key proatherosclerotic oxidant [151]. Importantly, the expression of iNOS in human atherosclerotic plaques is associated with nitrotyrosine staining, a marker of peroxynitrite formation [150, 152]. XO also plays a critical role in cholesterol crystal-induced ROS formation and subsequent inflammatory cytokine release by

macrophages. XO inhibition reduces vascular ROS levels, leading to improvement in endothelial function, and suppressing plaque formation in *ApoE*-KO mice [153].

ROS and RNS production, which may continue for hours after the beginning of reperfusion, play an important role in the genesis of reperfusion injury and in the recruitment of inflammatory cells [154]. Supplementary to increased ROS/RNS production, ischemia/reperfusion also reduces the levels of antioxidant enzymes such as glutathione peroxidase, and SOD [155], which are also influenced by the presence of hypercholesterolemia as mentioned above. Therefore, in the presence of hypercholesterolemia and atherosclerosis ROS/RNS production is unbalanced by cell defenses, inducing deleterious effects in a large number of pathways involved in cell cycle and survival pathways.

3.2 Pharmacological redox modulation in hyperlipidemia and cardioprotection

The increase in ROS generation induced by hypercholesterolemia may interfere with endogenous cardioprotective mechanisms such as cardiac preconditioning and postconditioning and may have a detrimental role in determining the severity of IRI [97]. Therefore, there is an urgent need to better understand the biology and the damage caused by ischemia/reperfusion and redox stress in hyperlipidemia before considering an appropriate treatment [156].

The attenuation of nitro-oxidative stress in hyperlipidemic animals has been proposed as a cardioprotective mechanism of statins in the setting of myocardial IRI. Three-week simvastatin treatment reduced infarct size and reversed the loss of postconditioning in hypercholesterolemic rabbits subjected to ischemia/reperfusion by attenuation of nitro-oxidative stress in the ischemic myocardium [157]. Short-term administration of pravastatin reduced infarction in cholesterol-fed rabbits independently of any lipid lowering effect, potentially through eNOS activation and attenuation of nitro-oxidative stress [158]. The reduction in infarct size by a natural constituent of olives and olive oil, oleuropein, was achieved by attenuation of reperfusion injury and reduced oxidative stress in hyperlipidemic rabbits [159].

Many studies have revealed that HSP70 is induced during myocardial ischemia/reperfusion and contributes to cardioprotection by suppression of ROS

generation, inhibition of cell apoptosis, attenuation of calcium overload; HSP70 is involved in the cardioprotection obtained by preconditioning and postconditioning [160]. HSP70 is upregulated in cardiomyocytes during IRI [161] and this may be attributed at least in part, to excessive oxidative stress [162], since the accumulated ROS may enhance the activity of heat shock factor 1 and facilitate its translocation into the nucleus, which contributes to the induction of HSP70 in ischemia/reperfusion [162]. Several studies have suggested that hyperlipidemia can impair the cardioprotective effects of HSP70 against IRI. Indeed, HSP70 downregulation was observed in cholesterol-fed rats subjected to myocardial ischemia/reperfusion [163], potentially due to activation of glycogen synthase kinase (GSK)3β [164]-[165] as well as accumulation of cholesterol in the membrane of cardiomyocytes, which might prevent accumulation of HSP70 during IRI [163].

The hypoxia-inducible factors (HIFs) and downstream genes are important factors in the protection of tissues from IRI. Redox signaling during IRI contributes to protective or adaptive responses and HIF-1α is one of the first response elements to IRI at the molecular level [166], and plays a pivotal role in the endogenous protective mechanism against ischemia [167]. HIF-1α expression was maintained at a very low level in hyperlipidemic rats and HIF activation using pharmacological prolyl hydroxylase inhibitors results in a level of cardioprotection similar to that obtained with ischemic postconditioning [168].

Nrf2 regulates antioxidant gene expression in vascular cells after exposure to modified LDL [169] and oxidized phospholipids *in vivo* [170]. Nrf2 deficiency in a more human-like hypercholesterolemia LDL receptor (LDLR)-KO/ApoB100/100 female mouse model, promoted plaque inflammation and oxidative stress leading to increased plaque instability, which is considered as a risk factor of MI in humans [169]. *Crocus sativus L*. aqueous extract induced cardioprotection in *ApoE*-KO mice undergoing myocardial IRI through activation of Nrf2 and its downstream targets SOD2 and heme oxygenase 1, with the subsequent regulation of nitro-oxidative stress indicating that the activation of Nrf-2 might be a central mechanism of the cardioprotective effect of *Crocus sativus L* [171].

In summary, hypercholesterolemia results in increased myocardial oxidative stress, mainly from NOXs, uncoupled eNOS, mitochondria, XO and

downregulation of antioxidant defense systems all of which play important role in IRI and may account for increased susceptibility of the myocardium to infarction. Increased LDL and oxLDL stimulate the production of ROS and reduce NO bioavailability predisposing the endothelial cells of large arteries to an inflammatory phenotype. Inflammation is associated with further increased ROS production that may overcome cellular defense mechanisms leading to atherogenesis, and eventually to loss of contractile function and vascular dysfunction [172]. As a result the infarct size is aggravated in a model of high fat diet and the protective effects of post-conditioning are lost (Figure 2) [173]. Statins, and pharmacological agents that modulate NO bioavailability, possess antioxidant properties and interfere with antioxidant defense systems may provide beneficial effect in the myocardium in hypercholesterolemic conditions.

4. Diabetes

According to WHO data, the global prevalence of diabetes mellitus (DM) in 2014 was estimated to be 9% [12]. DM increases the risk of MI almost 2-fold (odds ratio 1.89) [94]. High fasting blood glucose ranks third among the leading risk factors for disability-adjusted life years (years lived with severe illness) based on the global burden of disease data for 2019 [13]. Approximately 60% of preclinical studies examining type 2 diabetes mellitus (T2DM) in *in vivo* models of regional ischemia/reperfusion, demonstrated increased infarct size with T2DM when compared to non-diabetic controls; 20% of these studies showed that T2DM was without effect on infarct size [174]. However, in these preclinical *in vivo* models the T2DM animals were almost all untreated for the presence of diabetes, causing large differences in plasma glucose levels between diabetic and control animals (e.g. blood glucose values of 450-550 mg/dl in ZDF rats). This contrasts with T2DM in humans, where known diabetes is almost always treated by antidiabetic drugs or insulin to normalize plasma glucose levels. Therefore, preclinical studies possibly overestimate the effects of T2DM on infarct size by allowing these differences in glucose levels.

Indeed, when only the isolated hearts of T2DM animals were studied, which is commonly performed using similar perfusate glucose levels between groups, the

proportion of studies reporting a detrimental or neutral effect of T2DM on infarct size was equal [174]. Thus, it seems that elevated plasma glucose levels are a main determinant of infarct size. This is also in agreement with clinical studies examining infarct size during e.g. by-pass surgery or percutaneous coronary interventions (PCI) for diabetic and non-diabetic patients. Myocardial infarct size strongly correlated with plasma glucose levels and less so with T2DM, with even larger infarct size reported for non-diabetic than for diabetic patients presenting with similar glucose levels [175]. Thus, whereas it is clear that DM does in general increase CVD by 40-250% in DM patients receiving standard of care [176, 177], e.g. for incidence of cardiovascular death, HF or MI, the DM effects on sensitivity towards an ischemic event are less pronounced and are not always observed; this is also in accordance with the rather moderate odds ratio of MI associated with diabetes mentioned above.

4.1 Diabetes and redox signaling in myocardial infarction

Increased ischemic sensitivity of the heart is present with DM and can be ascribed to elevated plasma glucose and fatty acid levels and disturbed insulin, and/or to numerous molecular changes within the diabetic heart. Dysregulated redox signaling emerges prominently as one of the important T2DM-induced molecular changes and is mostly reflected by increased oxidative stress [178, 179]. Although increased reductive stress can also be detrimental to cardiac function [180], the diabetic heart commonly displays a depressed reductive stress response, as reflected by a diminished Nrf2-related gene response (e.g. depressed antioxidant enzyme complexes) [181]. The reduced reductive stress response will contribute to the net increase of oxidative stress within the diabetic heart. The cardiac oxidative stress is largely a result of metabolic overload by elevated plasma glucose and fatty acid levels. Both acute and chronic plasma glucose and fatty acid elevations cause oxidative stress in tissues and organs [182, 183] contributing to the increased ischemic sensitivity of diabetic heart [174]. There are many different cellular ROS sources in the heart that have been shown to be activated in the diabetic state [179, 184]. In addition, hyperglycemia is associated with a low-grade inflammatory phenotype, partly triggered by advanced glycation end-product (AGE)/receptor of AGE (RAGE) signaling [185, 186].

The three major sources in the cytosolic compartment are NOX2, uncoupled eNOS and XO in diabetic animals [187]. Other reports have proven that each of these

cytosolic ROS components may be activated with diabetes and can contribute to ischemia-reperfusion [188-192]. Genetic Nox2 deficiency prevented the major diabetic complications in streptozotocin (STZ)-treated mice [193] and insulin resistance-triggered endothelial cell dysfunction largely relies on NOX2 activity [194]. NOX1-derived ROS contribute to immune cell activation and vascular infiltration in diabetic ApoE-KO mice [195]. In contrast, NOX-4-derived H_2O_2 seems to be protective in diabetic mice [196].

The major sources in the mitochondrial compartment entail the ETC, MAO and $p66^{Shc}$ [179]. In a seminal report it was demonstrated that high glucose resulted in increased ETC-produced ROS in endothelial cells through increases in the mitochondrial membrane potential, affecting four different pathological biochemical pathways contributing to hyperglycemia-associated oxidative stress and damage [197, 198]. In these studies, it was suggested that high glucose resulted in increases of mitochondrial potential through increased delivery of oxidation-prone substrates and reducing factors (NADH, NADPH) to the ETC. Additionally, it is also possible that part of the increased mitochondrial potential is due to hyperglycemia-induced dislodgement of hexokinase II (HKII) binding to mitochondria [199, 200]. Decreasing the amount of mitochondria-bound HKII is known to increase ROS production in the heart [200-202], and diabetic hearts have been reported to have less HKII bound to mitochondria [203, 204]. Less HKII bound to mitochondria was also recently suggested as a possible explanation for increased oxidative stress with aging [205], providing at least one explanation for why sensitivity to an ischemic insult may be particularly exaggerated in the aging diabetic patients. The cytosolic adaptor protein $p66^{Shc}$ can translocate to the mitochondrial matrix upon high glucose-induced PKC activation. Once in the mitochondrial matrix the protein catalyzes electrons going from cytochrome C directly to oxygen, thereby contributing to H_2O_2 production [206]. Finally, MAOs at the outer mitochondrial membrane breakdown catecholamines and neurotransmitters with concomitant generation of H_2O_2 [207]. Both $p66^{Shc}$ and MAO related ROS production can contribute to increased cardiac ischemic sensitivity [42]. Genetic deficiency of mitochondrial aldehyde dehydrogenase resulted in increased immunohistochemical staining of cardiac 4-hydroxynonenal and diastolic dysfunction in diabetic mice [208].

Although many different cellular sources of ROS exist in the diabetic heart, it is important to recognize that these sources are not independent entities, because of the now well-accepted ROS-induced ROS production [209, 210], which is also well documented in the setting of diabetes [186]. Thus, although ROS production could start with one source, this can quickly result in the activation of other ROS sources, making it difficult to discern the primary cause of ROS production. ROS can contribute to cardiac infarct development by facilitating the opening of the mPTP during early reperfusion, resulting in mitochondrial dysfunction and activating necrotic pathways [211]. In addition, mitochondrial dysfunction and ROS generation will activate the innate immune receptor nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing NLRP 3, an immune receptor whose presence is already increased in diabetes, thereby contributing to cardiac infarct development through pyroptosis [212]. Finally, ROS can also induce the endoplasmic reticulum stress response, thereby contributing to infarct size through necroptosis [53, 213].

4.2 Pharmacological redox modulation in diabetes and cardioprotection

As discussed above, an excess of ROS induced by hyperglycemia contributes to the enhanced basal oxidative stress and is likely to aggravate myocardial IRI in diabetic patients. As such, therapeutic interventions to decrease oxidative stress could, in principle, protect against hyperglycemia-induced myocardial tissue damage. However, although increasing evidence favors protection by antioxidants and ROS scavengers, the potential of reducing oxidative stress to treat the diabetic heart is still controversial and equivocal in human studies. Antioxidants such as ascorbic acid and N-acetylcysteine prevent NOS uncoupling in the diabetic heart resulting in increased bioavailability of NO and increased tolerance to IRI in diabetic rat heart [214].

Diabetic heart mitochondria demonstrate an enhanced susceptibility to injury, mediated by redox-dependent shifts in mPTP opening [215]**.** In this context, diabetic mice treated with a mitochondria-targeted antioxidant (MitoTEMPO) displayed preserved heart rates and better survival after MI by suppression of calmodulin-dependent protein kinase-II (CAMK-II) oxidation [216] and mitochondrial ROS/RNS generation, apoptosis and myocardial hypertrophy [217]. The latter observations were also confirmed *ex vivo* in cultured cardiomyocytes subjected to hyperglycemia. Compounds other than direct antioxidants, that attenuate mPTP opening, such as a

newly developed cyclophilin D inhibitor (NIM811), were reported to reduce infarct size when administrated at reperfusion in STZ diabetic rats [218].Pharmacological inhibition of histone deacetylase 6, which confers redox regulation and suppresses cellular stress responses, showed highly beneficial effects in STZ-induced and ischemia/reperfusion-subjected diabetic hearts, potentially based on modulation of acetylation of peroxiredoxin 1 (Prdx1) and thereby decreasing ROS levels [219]. As another mitochondria-targeted approach, inhibition of MAO attenuated diabetic cardiomyopathy [53, 179].

Stabilization of HIF-1α has been reported to promote tolerance against acute myocardial IRI by decreasing mitochondrial oxidative stress and inhibiting mPTP opening [220], while the HIF-1α signaling pathway is compromised in the diabetic setting [221]. When diabetic rats were treated with N-acetylcysteine or the XO inhibitor allopurinol, HIF-1α/heme oxygenase-1-dependent signaling was stabilized and consequently myocardial IRI was attenuated [222]. Further studies have revealed that cobalt (II) chloride ($CoCl_2$) can activate the impaired HIF-1α pathway under diabetic conditions [223]. $CoCl_2$ or deferoxamime-activated HIF-1α signaling pathway restored the sevoflurane postconditioning-dependent myocardial protection in diabetic rats by improving myocardial mitochondrial respiratory function and mitophagy and reducing ROS generation [224-226].

Phosphodiesterase-5 (PDE5) inhibitors have been described to protect the heart against IRI through several mechanisms involved in increased expression of NOS, activation of protein kinase G (PKG)-dependent hydrogen sulfide (H_2S) generation, and phosphorylation of GSK-3β – which modulates mPTP directly [227]. PDE5 inhibition improves endothelial function and promotes antioxidant activity in the diabetic heart through increasing NO bioavailability [228]. In this context, tadalafil therapy attenuates oxidative stress and improves mitochondrial integrity while reduces myocardial infarct size following IRI in db/db mice [229].

Melatonin, a cellular antioxidant and direct ROS scavenger, exerts protection against myocardial IRI in T2DM rats by limiting reperfusion-induced ROS formation and endoplasmic reticulum stress in a SIRT1-dependent manner [230]. In acute hyperglycemia, melatonin rescued the thioredoxin (Trx) system in the heart by reducing Trx-interacting protein expression via neurogenic locus notch homolog protein

(Notch)1/ enhancer of split 1 (Hes1)/ Akt signaling [230, 231]. Furthermore, melatonin prevented myocardial IRI in STZ-induced diabetic rats by normalizing mitochondrial function and oxidative stress as well as stimulation of mitochondrial biogenesis via AMPK-PGC1α-SIRT3 signaling [232].

Resveratrol has been shown to have pleiotropic and beneficial effects on cardiovascular complications in DM, including amelioration of mitochondrial function and oxidative stress as well as amelioration of endothelial function mainly through mechanisms involving NO and SIRT pathways [233-236]. In addition, pterostilbene, a naturally-occurring dimethylated analogue of resveratrol with antidiabetic effects, significantly reduced post-ischemic cardiac infarct size, oxidative stress, and apoptosis in diabetic rats. Pterostilbene enhanced the viability of cardiomyocytes exposed to hypoxia-reoxygenation under high glucose conditions and decreased ROS formation [237]. Other bioflavonoids (e.g. quercetin, rutin or benzenetriol), also displayed cardioprotective effects in IRI in diabetic rats, which partially rely on the attenuation of oxidative stress and improvement of antioxidant reserves [238, 239]. On the other hand, quercetin was not effective in preventing myocardial IRI in ZDF rats implying that other confounding factors may abolish the cardioprotective effect [240].

Other polyphenolic compounds such as luteolin, butin, and berberine may inhibit oxidative stress and protect against IRI in diabetic mice via eNOS/ Kelch-like ECH-associated protein (Keap1)/Nrf2 or AMPK/Akt/GSK-3β/Nrf2 dependent pathways [241-244]. (-)-Epigallocatechin-3-gallate, a green tea polyphenol with potent antioxidant properties, decreased myocardial infarct size and apoptosis as well as oxidative stress via SIRT1-dependent pathways in STZ-diabetic rats with myocardial IRI [245]. Furthermore, attenuation of myocardial IRI in diabetic rats was observed by the dietary flavonoid kaempferol by suppression of AGE-RAGE/mitogen activated protein kinase (MAPK)-dependent inflammation and oxidative stress [246].

Increasing evidence documents the beneficial effects of SLGT2 inhibitors in the heart, directly or indirectly, in animal and human studies, including decreasing oxidative stress and preventing IRI [247, 248]. Long term, but not short term, SGLT2 inhibition by empagliflozin, attenuated myocardial IRI *in vivo* in diabetic and non-diabetic mice through regulation of oxidative stress [85, 249]. Treatment with empagliflozin significantly attenuated the DM-induced increase in acute mortality after

MI in a model of T2DM through preservation of myocardial antioxidant defense and normalization of the size and number of mitochondria [247, 250, 251]. Studies on the effects of a diverse range of antioxidants on cardiac effects in cardiometabolic comorbidities are presented in Table 1.

In conclusion, the diabetic comorbidity is associated in general with exacerbated ROS generation within the heart, originating from both cytosolic and mitochondrial sources, and most often driven by metabolic overload of glucose and fatty acids as well as an inflammatory phenotype. Increased oxidative stress diminishes the heart's resistance against ischemic episodes or increases the sensitivity of the heart to ischemia, which, at least in preclinical studies, can be prevented by antioxidant strategies. As a result of this impaired redox balance, infarct size is aggravated in a model of diabetes and further exacerbated by genetic heme oxygenase-1 deficiency (Figure 2) [252]. Strategies to combat this oxidative stress therefore seem warranted.

5. Hypertension/hypertrophy

According to WHO data, the global prevalence of hypertension was estimated to be approximately 30% in the adult population [253]. Hypertension increases the risk of MI almost 3-fold (odds ratio 3.11) [94] and ranks first among the leading risk factors for disability-adjusted life years (years lived with severe illness) based on the global burden of disease data of the year 2019 [13]. The term "hypertensive heart disease" can be applied broadly to describe the composite result of the morphological, metabolic, microvascular and electrophysiological perturbations that predispose to greater CVD risk in patients with hypertension. A key feature of hypertensive heart disease is concentric left ventricular hypertrophy (LVH) [254]. Increased left ventricular muscle mass initially helps to pump more efficiently against an increased ventricular afterload. Estimates vary but more than 20% of hypertensive patients may develop echocardiographic evidence of LVH [255-257] and it is well established that hypertensive patients with LVH have a worse prognosis than those without detectable LVH. While hypertension is a major risk factor for the development of IHD, hypertensive LVH presents an additive risk for all forms of cardiac rhythm

disturbances, sudden cardiac death, HF and, most pertinent in the context of the current review, atherothrombotic events including MI [258-260].

5.1 Hypertension/LVH and redox signaling in myocardial infarction

Widespread disturbance of cellular redox balance throughout the circulatory system is recognized as a general feature of arterial hypertension, whether of primary or secondary etiology. In experimental models of hypertension or other forms of pressure overload, LVH is accompanied by many biochemical, metabolic and signaling disturbances that have been associated with cardiomyocyte hypertrophy, altered myofibrillar contractility, interstitial fibrosis and gradual progression to decompensation and HF**.** Many studies show that increased ROS-generating capacity, reduced endogenous anti-oxidant defense and impaired NO generation are general features of hypertrophic myocardium and are related to altered sensitivity of hypertrophied tissue to stressful stimuli such as ischemia-reperfusion [261-264].

The major sources of ROS in cardiomyocytes and the key antioxidant systems have been reviewed extensively before [265, 266]. Redox signaling is a critical factor in physiological myocyte hypertrophy in post-natal growth and in response to stressful stimuli. In arterial hypertension, the progression from a state of adaptive cardiac hypertrophy to a maladaptive state, when myocyte contractility is impaired and HF develops, is clearly associated with oxidative stress. The nature and causes of the imbalance between ROS generation and antioxidant defense mechanisms in hypertension are unclear although they are likely to be complex, multifactorial and dependent on the etiology of hypertension in humans or the nature of the experimental model in *in vivo* and *in vitro* models.

Many of the kinase cascades and their target proteins that regulate transcription, protein synthesis and myocyte growth, for example members of the MAPK family extracellular signal-regulated kinases (ERK)1/2, Akt, GSK3β and the nuclear factor of activated T-cells (NFAT) family of transcription factors, are ROS-activated or redox-sensitive [267-270]. In evolving or compensated hypertrophy, ROS may be from mitochondrial or non-mitochondrial sources. The major neurohormonal mediators of myocyte hypertrophy in hypertension, namely catecholamines and angiotensin II, stimulate hypertrophy *in vivo* or *in vitro* through mitochondrial ROS generation via the ETC complexes [271-273]. MAO-associated ROS generation may also contribute beyond ROS generated by the ETC complexes. MAO-A and MAO-B activities were

shown to be enhanced in cardiomyocytes from spontaneously hypertensive rats at a stage before detectable hypertrophy was established [274-276] [277]. However, cytosolic (non-mitochondrial) ROS-generating enzymes also appear to play important roles in physiological myocyte hypertrophy. These include XO [266]. In Dahl salt-sensitive rats, high salt diet increased myocardial XO activity, was accompanied by increases in blood pressure, LV mass index and interstitial fibrosis during the initial 8-week period of hypertension and LVH development. Febuxostat, a selective XO inhibitor attenuated these increases as well as markers of oxidative stress, suggesting that in this model of hypertension, XO-derived ROS are mediators of cardiomyocyte hypertrophy and interstitial fibrosis [278].

Evolving experimental evidence suggests that other non-mitochondrial sources of ROS may be relevant to both physiological cardiac hypertrophy and pathological decompensation leading to HF. Most prominent are the NOX isoforms of non-phagocytic origin of which NOX2 and NOX4 have received most attention [279-282]. Calcium/calmodulin-dependent NOX5 may also be implicated [283]. The extent to which these various pathways of ROS production are co-regulated or exhibit cross-talk is unclear. However, it is of interest that selective XO inhibition in the Dahl salt-sensitive rat also reduced total NOX activity [278] and the angiotensin II type 1 receptor antagonist, candesartan, decreased both XO and NOX activities in parallel [284].

Progression of LVH from an adapted (compensated) state to decompensation and HF appears to be associated with multiple biochemical and metabolic alterations that shift redox balance towards a state of oxidant stress. Although the functional decline is often difficult to define clinically and even more difficult to model experimentally, many studies show that enhanced oxidant stress is a feature of the progression. Alterations in substrate metabolism [285] and the ETC complexes [286], increased expression and activity of MAO [275, 287-290], upregulation of XO [261] and increased activity of NOX isoforms [291] have been implicated in mediating excessive ROS production associated with LVH progression and decompensation.

There is also evidence that many endogenous antioxidant systems are depleted or become inactivated during the progression of LVH, either as a cause or a consequence of decompensation. For example, reduced total (cytosolic and mitochondrial) SOD activity [261] is a feature even in the compensated state and accompanied by reduction in the ratio of reduced glutathione (GSH)/oxidized glutathione (GSSG) [292] in the transition to HF. Trx1 inhibits cardiac hypertrophy

through a number of redox-controlled downstream mechanisms [293]. Depletion or inhibition of Trx increases hypertrophy and may predispose to decompensation. A growing body of evidence suggests that the gaseous thiol H_2S, generated through regulated enzymatic pathways in myocardium and the coronary vasculature, may also represent an important antioxidant in myocardium although the mechanisms are as yet unclear. While direct chemical interaction and scavenging of ROS would seem to be a simple mechanism, evidence is emerging of more complex redox regulation by H_2S, especially in the mitochondria (reviewed in [294]). Recent evidence indicates that deletion of the most abundant H_2S-generating enzyme in the heart, 3-mercaptopyruvate sulfurtransferase (3-MST), had no effects on blood pressure or LV mass in young animals but was associated with hypertension and LVH in aged mice [295]. There is limited evidence of mechanisms by which H_2S might modify physiological and pathological processes in hypertrophy. SIRT3 is a mitochondrial histone deacetylase controlling protein deacetylation and thereby influences substrate metabolism and mitochondrial redox status. In human LV tissue, SIRT3 expression correlated inversely with the severity of pathological changes [296]. In experimental LVH, elevation of H_2S availability through exogenous administration increased the expression of SIRT-3, improved several measures of mitochondrial function and attenuated the hypertrophic response to pressure overload in a SIRT3-dependent manner [297].

Enhanced oxidative stress through increased ROS generation and/or depletion of intracellular antioxidant systems may predispose the hypertrophied myocardium to altered responses to acute ischemia/reperfusion and modify the response to protective interventions, notably preconditioning and postconditioning treatments. Responses to ischemia/reperfusion in experimental LVH have been comprehensively reviewed elsewhere [11, 156]. Briefly, many experimental studies confirm that the severity of arrhythmias during both coronary occlusion and reperfusion is increased in compensated LVH, mirroring extensive clinical observations of enhanced susceptibility to malignant arrhythmias and sudden death in patients with LVH. There is also experimental evidence that myocardial stunning (delayed recovery of contractile function during reperfusion following ischemia) is exaggerated in LVH [261, 263, 298]. Augmented irreversible tissue injury, measured as infarct size, has been observed in short-term experimental models of myocardial infarction in hypertensive LVH [299-301] although not consistently [302, 303]. However, it is conceivable that long-term responses to MI could be modified in LVH due to the combination of decreased

microvascular density, interstitial/perivascular fibrosis and persistent oxidant stress. Although experimental evidence is lacking, one could predict an exaggerated post-infarct inflammatory response in the hypertrophied heart leading to less favorable tissue remodeling and worse outcome [304].

5.2 Pharmacological redox modulation in hypertension/hypertrophy and cardioprotection

Protection of the hypertrophied myocardium from the consequences of ischemia/reperfusion has arguably received less attention than it deserves. Long-term treatment with antihypertensive drugs can lead to regression of LVH. Although blood pressure lowering and control of LV afterload is clearly an important goal, some antihypertensive drug classes are associated with better LVH regression and their effects on LV mass go beyond blood pressure control. For example, angiotensin converting enzyme inhibitors, β-adrenoceptor antagonists and L-type calcium channel blockers induce LVH regression which is not observed with thiazide diuretics or older vasodilators such as hydralazine and minoxidil. However, it remains unclear if LVH regression induced by antihypertensive drug therapy is truly associated with reduced risk of major events and improved prognosis [305, 306]. Given this uncertainty, cardioprotection of the hypertrophied myocardium against ischemia-reperfusion injury remains an important therapeutic goal.

Several studies suggest that the endogenous cardioprotective mechanism, ischemic preconditioning, is applicable and effective in young animals with experimental LVH, at least during the early stage of hemodynamic compensation [302, 303, 307-311]. However, in long-standing or progressive LVH, even without evidence of decompensation, preconditioning protection (ischemic or pharmacological) may be attenuated or require a higher intensity preconditioning stimulus to be effective compared to age-matched control animals [312, 313]. Observations of postconditioning in hypertrophied myocardium are limited but the bulk of evidence to date suggests that the postconditioning mechanism is abrogated even in young animals with short-term hypertension [314-317].

Excessive ROS accumulation, particularly from mitochondrial sources, is known to trigger mPTP opening during early reperfusion [318] and it has been

suggested that the greater susceptibility of hypertrophied myocardium to IRI is, at least in part, related to enhanced opening of mPTP [301, 319]. There is some evidence that oxidative stress and the impairment of mitochondrial homeostasis and redox signaling mechanisms that is seen in advanced or decompensated LVH may be related to attenuation of the preconditioning response. For example, isoflurane preconditioning increased SOD2 activity in normotensive rats and limited infarct size but these responses were lost in hypertensive animals with established LVH [299]. Fantinelli and colleagues [320] have demonstrated a change in ischemic preconditioning threshold required to confer protection in hypertrophied hearts but protection was associated with preservation of GSH (an indicator of reduced oxidant stress) and decreased cytosolic accumulation of SOD2 (a surrogate indicator of mPTP opening).

Although there is clear evidence that oxidant stress is a mediator of pathological hypertrophy development/decompensation and of enhanced IRI in LVH models, the potential of exogenous antioxidants as clinical cardioprotective agents has so far met with limited success. Key issues, common to many experimental ischemia-reperfusion studies, have been the right antioxidant, in the appropriate biological compartment (extracellular/cytosolic/mitochondrial), at the right concentration, at the right time. The experimental literature is extensive and extends over several decades. It includes antioxidant enzymes (CAT, SOD); inhibitors of ROS-generating enzymes (e.g. allopurinol); phytochemical ROS-scavenging agents such as purified derivatives or galenical plant extracts containing polyphenolic secondary metabolites (e.g. flavonoids such as quercetin, curcuminoids, anthocyanins and stilbenoids like resveratrol); vitamins, notably ascorbate/vitamin C and tocopherol derivatives/vitamin E; and synthetic agents such as N-acetylcysteine and 4-hydoxy-TEMPO (Tempol). Some of these agents have been applied as tools for investigation of the role of oxidant stress both in the mediation of experimental hypertrophy and ischemia-reperfusion injury (**see Table 2**). It is important to note that action may not be specific and the difficulties of dose standardization, particularly in the case of the complex phytochemical preparations.

Despite clear evidence of oxidative stress in the pathophysiology of hypertensive LVH and ischemia/reperfusion injury, and promising beneficial effects in some laboratory models, no antioxidants so far have been established in large randomized control trials to exert benefit in hypertension, either through attenuation of hypertrophy progression towards decompensation/HF, or cardioprotection against

ischemia/reperfusion injury (see [321] for extensive review). Smaller clinical studies that have investigated allopurinol as adjunct to standard treatment for hypertension or heart failure have shown marginal benefit or even a detrimental effect [322, 323].

The reasons for this divergence between experimental and clinical experience are likely to be wide-ranging. Reasons may include the vast number of biological targets for antioxidant action some of which may be essential redox pathways controlling normal homeostasis; the huge diversity of chemical structure and mechanisms of action of antioxidants; lack of specificity of antioxidant compounds; and the complexities of multiple-morbidity and co-existing drug treatments (some of which may have inherent antioxidant activity [324, 325]. These difficulties render the demonstration of antioxidant benefits in human hypertension a challenging and high-risk endeavor.

In conclusion, redox signaling is a critical molecular mechanism controlling cardiomyocyte hypertrophy in pressure overload conditions like hypertension. Although LVH is initially an essential adaptive phenomenon that maintains cardiac output in the face of increased afterload, chronic pressure overload and neurohormonal influences contribute to increasing oxidative stress, characterized by excessive ROS production and reduced antioxidant capacity. These factors predispose the hypertrophied myocardium to exaggerated IRI and development of HF. Under experimental conditions, *in vivo* and *in vitro*, a wide variety of antioxidants have been shown to modify the hypertrophic response to pressure overload or pro-hypertrophic neurohormonal stimuli and mitigate against the deterioration to HF. However, clinical application of antioxidant approaches for hypertensive heart disease has so far been limited in scope and requires further exploration as a possible approach to management of this insidious condition.

6. Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)

NAFLD accounts for an appreciable part of chronic liver disease with a prevalence of ~30% of the US population [326]. Approximately 10-15% of the patients with NAFLD develop NASH , which is characterized by hepatic apoptosis, inflammation, steatosis, and fibrosis, with a substantially higher risk of cirrhosis and primary liver cancer [327]. Of note, there is a clear association of cardiovascular risk and mortality with the severity

of NASH [328], as supported by increased carotid intima-media thickness as well as aggravated coronary calcification and endothelial dysfunction in patients with NASH [328, 329]. NASH increases the risk of MI by 50% (odds ratio 1.5) [94] and fatty liver disease also contributes significantly to the global burden of disease in terms of disability-adjusted life years [330]. Previous reports provided indirect proof for a role of oxidative stress in hepatic endothelial dysfunction [331], which was also supported by improved hepatic endothelial function upon infusion of high dose vitamin C in patients with liver cirrhosis [332]. NAFLD is connected with DM, which represents another metabolic disease with a clear association with oxidative stress and higher cardiovascular risk [333, 334], thereby supporting the notion of liver disease as a cardiovascular comorbidity [335].

So far, there are only a limited number of studies that have investigated the correlation of liver damage progression with oxidative stress or cardiovascular risk. Studies that explored the benefit of combined pharmacological targeting of liver and cardiovascular inflammation are rare. Whereas macrophages, freshly recruited or resident ones, may represent a common pathophysiological feature, their detailed role in NASH as well as their pharmacological modulation remain insufficiently studied [336, 337]. In line with this notion, hepatic levels of TNF-α, interleukin (IL)-6, IL-1β, and cyclooxygenase-2 were found to be increased in NASH animal models [338-340]. As a consequence, hepatic ROS levels are higher in NASH and have been proposed for therapeutic targeting [341]. Therefore, inflammation provides a clear link between NAFLD/NASH and CVD since the progression of atherosclerosis in humans [342, 343] and arterial hypertension in animals [344, 345] is largely dependent on the recruitment and activation of immune cells. The inflammation-triggered oxidative stress impairs endothelial function and represents a prognostic marker for higher cardiovascular risk [346, 347] and, *vice versa*, oxidative stress can activate inflammatory pathways by different mechanisms leading to a vicious cycle [348, 349].

In conclusion, NASH represents an inflammatory liver disease with important features of atherosclerosis [350]. Macrophages and dendritic cells derived from blood monocytes as well as liver resident macrophages/Kupffer cells drive local immune responses in NASH [351] leading to higher levels of hepatic and cardiovascular ROS [341, 352]. In analogy to NASH, these cells also play an essential role for the progression of atherosclerosis [342, 343] and arterial hypertension [344, 353].

Therefore, CVD may significantly contribute to overall mortality in patients with NAFLD/NASH [329].

6.1 NAFLD/NASH and Redox Signaling in Myocardial infarction

Oxidative stress has adverse effects on endothelial function and CVD prognosis [346]. Oxidative stress plays a central role for NASH and NAFLD disease progression (including cardiovascular complications) [354, 355] and NOX-derived ROS represent key players in liver fibrosis [356]. Patients with NASH have higher levels of 8-isoprostanes and sNox2-dp correlating with the histological grading of steatosis as well as liver inflammation, ballooning and fibrosis [357]. Patients with NAFLD displayed higher sNox2-dp and 8-isoprostane levels that correlated with higher steatosis and portal inflammation [358] or with markers of infection [359]. NOX1 isoform was found to be upregulated in livers of NASH patients [360]. As shown by animal studies, genetic Nox1 or Nox2 deficiency attenuated the major biochemical and functional markers of NASH in high fat diet fed mice [360, 361]. A cell culture study demonstrated that advanced glycation end products may play a role for inflammatory activation of hepatic stellate cells by a NOX2-dependent pathway [362].

Apart from NOX isoforms, mitochondrial ROS formation has been identified as a major source of oxidative stress in the setting of NAFLD/NASH, which is a consequence of altered mitochondrial morphology and function as well as inhibition of the ETC [363-365]. Enhanced $p66^{shc}$ signaling, increased opening probability of the mPTP and higher levels of mitochondrial damage-associated molecular patterns (DAMPs) were reported for rodent models of NASH [366-368] that may explain the increased mitochondrial ROS formation. XO inhibition could efficiently prevent the major pathophysiological changes in rodent models of NASH [369, 370]. Finally, neuroinflammatory processes through the liver-brain-axis may come into play, again involving ROS formation (e.g. via NOX2) [371], which may affect neuronal stress hormone signaling and thereby affect cardiovascular function [372]. Of note, the above-mentioned ROS sources can activate each other in a crosstalk fashion and are recognized mediators of ischemia/reperfusion damage during myocardial infarction [373, 374].

Endothelial function measured by FMD, a prognostic parameter was reduced and carotid artery intima-media thickness was increased, indicating higher CVD risk in patients with NAFLD or NASH [375, 376]. Importantly, sNox2-dp and isoprostane levels in patients with NASH also correlated with peripheral endothelial dysfunction measured by FMD, all of which was corrected by administration of polyphenol-rich dark chocolate [377]. These data were in line with observations in a NASH model (methionine/choline-deficient diet) linking liver steatosis, inflammation, fibrosis and oxidative stress with an adverse vascular phenotype characterized by endothelial dysfunction, ROS formation from mitochondria, NOX1 and NOX2 as well as vascular inflammation in peripheral vessels [352]. Taken together, these data support and explain the higher risk of MI associated with NASH [94] and the higher CVD risk of patients with NAFLD [378, 379].

6.2 Pharmacological redox modulation in NAFLD/NASH and cardioprotection

Therapy with vitamin E and PPARγ agonists (e.g. pioglitazone) was recommended as combination therapy for NASH patients and confers potent antioxidant and anti-inflammatory protection; this provides further support for oxidative stress as a central pathophysiological mechanism in NASH [335]. These lines of evidence are supported by meta-analysis showing that vitamin E supplementation improves major disease parameters in NAFLD patients, endorsing the oxidative stress concept in fatty liver disease [380]. The synthetic ROS scavenger mito-TEMPO prevented NAFLD associated liver inflammation and steatosis [381] and the related compound mitoQ will most likely show similar beneficial effects [382]. The natural antioxidant flavonoid silibinin improved adverse effects of NASH on the liver and heart in a mouse model (methionine/choline-deficient diet) [383]. Similarly, resveratrol ameliorated all adverse features of NAFLD [384]. Treatment of NASH mice with nanoformulated SOD1 prevented the NASH phenotype [385]. Pharmacological activation of retinoic acid-related orphan receptor α lead to induction of SOD2 and GPx1 genes in association with an improved NASH phenotype in mice [386]. *Vice versa,* genetic deletion of SOD1 and the senescence marker protein-30 was associated with oxidative stress and hepatic steatosis [387].

Animal studies demonstrated a beneficial effect of incretin-based therapies (glucagon-like peptide-1 [GLP-1] mimetics and dipeptidyl peptidase-4 [DPP-4] inhibitors) on the vascular system, including inhibition of atherosclerosis, myocardial and kidney fibrosis [388-391]. A limited number of studies using NAFLD and NASH models demonstrated anti-inflammatory and antioxidant effects [392-394], although these studies focused mainly on aspects of hepatocyte damage and steatosis. Also, synergistic effects of GLP-1 administration on liver inflammation and systemic atherosclerosis were reported [395]. Effects of DPP-4 inhibitor (gliptin) therapy on NAFLD/NASH associated oxidative and inflammatory complications in the liver and vascular tissue were demonstrated using a NASH mouse model (methionine/choline-deficient diet) [352]. Gliptins increased GLP-1 levels and thereby suppressed NOX and mitochondria-derived ROS formation and markers of inflammation in the aorta. This may be explained by GLP-1-dependent inhibition of PKC and NFκB-mediated NOX activation and upregulation [396, 397]. Alternatively, higher GLP-1 levels may contribute to AMPK activation that controls macrophage polarization and antioxidant defense [350, 391]. The indirect antioxidant effects of incretin-based therapies are further supported by reports of reduced oxidative stress markers in models of DM [396-399], atherosclerosis [388, 400], sepsis [389, 391], cardiac IRI [401] and chronic MI.

Another novel antidiabetic drug class, SGLT2 inhibitors, are currently under consideration for the therapy of NAFLD/NASH [402]. Empagliflozin improved markers of liver fibrosis and steatosis in NAFLD patients with and without T2DM [403, 404]. The drug also ameliorates the phenotype of NASH (fibrosis and steatosis) in mice [405]. Importantly, empagliflozin was shown to possess highly beneficial cardioprotective effects by decreasing the cardiovascular mortality in larger scale studies in T2DM patients [406], which was mechanistically supported by potent antioxidant and anti-inflammatory effects of the drug in rodent models of type 1 and type 2 DM [334, 407]. These mechanistic considerations on the cardio-metabolic-renal benefits of SGLT2 inhibition have been reviewed in detail [408].

In conclusion, NAFLD and NASH are associated with a higher burden of oxidative stress within the liver and heart, based on activation of cytosolic and mitochondrial sources. NAFLD and NASH share similarities in their pathomechanisms with DM and the metabolic syndrome, including dysregulated lipid metabolism and an inflammatory phenotype (in part also mild

hyperglycemia) as well as progression of atherosclerosis. These adverse features of NAFLD and NASH explain the aggravated susceptibility to ischemia/reperfusion injury of the heart and higher risk of MI for patients with NAFLD and NASH. As oxidative stress plays a central role in NAFLD and NASH pathophysiology and disease progression as well as associated ischemic heart disease, several antioxidant treatment regimens were reported to display highly beneficial therapeutic effects in preclinical models of or patients with NAFLD and NASH.

7. Conclusions/Future Perspectives

In order to provide an impression of the increase in CVD risk by the different comorbidities discussed above, we summarize the odds ratios for the association of each of them with MI using data from a large scale population-based national study (55,099,280 patients) [94]. Hyperlipidemia showed the strongest association with MI with an odds ratio of 8.39 (95% CI: 8.21-8.58), followed by hypertension with an odds ratio of 3.11 (95% CI: 3.05-3.17). DM and NASH showed a comparable odds ratio of 1.89 (95%CI: 1.86-1.91) and 1.5 [95% CI: 1.40-1.62], respectively. Association of other risk factors with MI were smoking with an odds ratio of 2.83 (95% CI: 2.79-2.87), age above 65 years with an odds ratio of 1.47 (95% CI: 1.45-1.49) and male gender with an odds ratio of 1.53 (95% CI: 1.51-1.55).

The nature, source, location and rate of production of ROS generated in myocardium under physiological or pathological conditions, together with the availability of cellular antioxidant defense systems, will determine the balance between redox signaling (physiological) and oxidative stress (pathological). The primary major sources of ROS in ischemia/reperfusion damage (e.g. during MI) are the mitochondria and NOXs, whereas secondary sources are XO and uncoupled NOS [374]. The contribution of NOXs was supported by protective effects of the inhibitor apocynin [409], which also displayed protection in all discussed comorbidities. Mitochondrial ROS play a dual role and can be detrimental but also protective as blockade of the mitochondrial ATP-sensitive potassium channel by glibenclamide or 5-hydroxydecanoate increased infarct size and prevented the protective effects of

ischemic preconditioning [410, 411]. Also the inhibition of PKC can induce adverse or protective effects by suppression of preconditioning [412], whereas the PKC inhibitors chelerythrine or calphostin C conferred protection against most of the discussed comorbidities at the preclinical level or in isolated blood cells and platelets of patients.

Previously, the concept of redox crosstalk between different sources of ROS was proposed [413-416], which may help to explain the impact of the above described comorbidity factors on MI or cardiovascular death (**Figure 3**). Based on this concept, comorbidities such as arterial hypertension, DM, hypercholesterolemia or NAFLD/NASH would activate primary ROS sources such as NOX (e.g. via the renin-angiotensin-aldosterone or AGE). These ROS from primary sources may increase ischemia/reperfusion damage by aggravating mitochondrial ROS formation in a bonfire fashion, which will ultimately lead to potentiation of mitochondrial dysfunction (impaired ATP-based energy supply), mitochondrial DNA damage, cell death by apoptosis and necrosis. The amplification of mitochondrial ROS release will lead to damage of vascular signaling and activation of secondary ROS sources such as uncoupled eNOS. Aggravated inflammation by ROS-triggered pathways (e.g. redox activation of the NLRP3 inflammasome or the central hub of inflammation, HMGB1) as well as the increase in circulating levels of DAMPs may further contribute to comorbidity-induced ischemia/reperfusion injury [417].

Oxidative stress is an attractive target for novel therapies, as it represents the common pathway through which different CVD comorbidities exert their deleterious cardiovascular effects. Although sources such as NOX are common for all the comorbidities, other redox signaling alterations may be specific for each comorbidity. Therefore, there is an urgent need to better understand the biology of such comorbidities and their consequences on the redox system as well as subsequent events such as ischemia/reperfusion injury. More mechanistic studies are necessary to characterize the sequences of events and to potentially recognize components that may specifically be pharmacologically targeted by available drugs or by novel molecules. **Figure 3** presents novel/unexplored (mostly preclinical) redox therapeutic approaches to interfere with these comorbidity-induced adverse redox signaling pathways.

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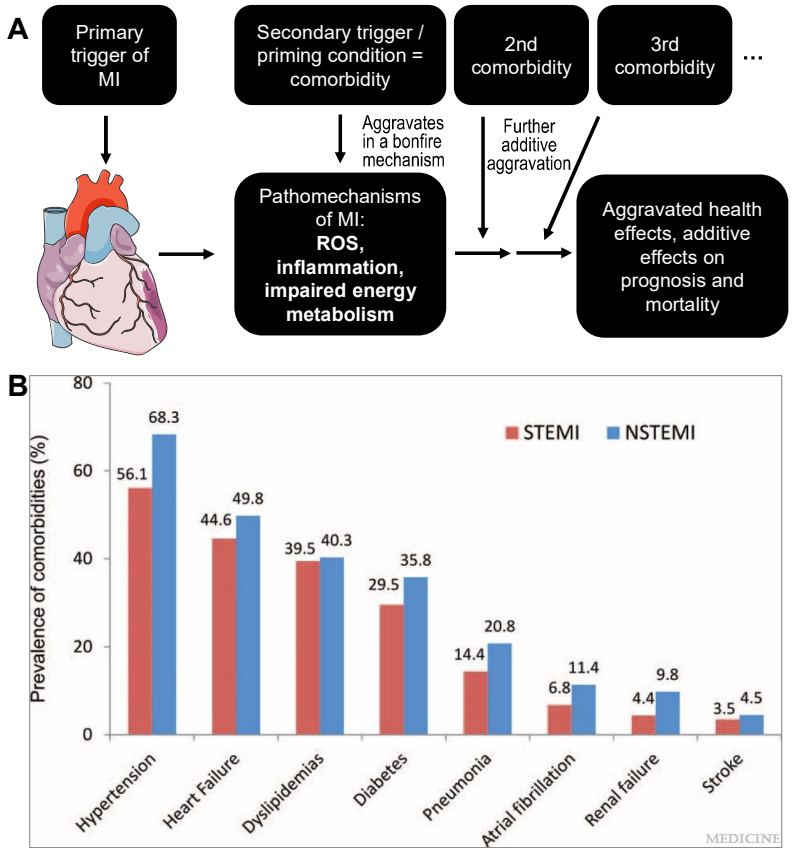

Figure 1. Proposed concept of comorbidities in myocardial infarction (MI) with oxidative stress and inflammation as central pathomechanisms. (A) Comorbidities aggravate adverse health outcomes of MI. **(B)** Overall burden of the major comorbidities in ST-segment elevation and non-ST-segment elevation MI (STEMI and NSTEMI) by a population-based study in Beijing (77,943 patients). Adapted from [418] with permission. Copyright © 2016, Wolters Kluwer Health.

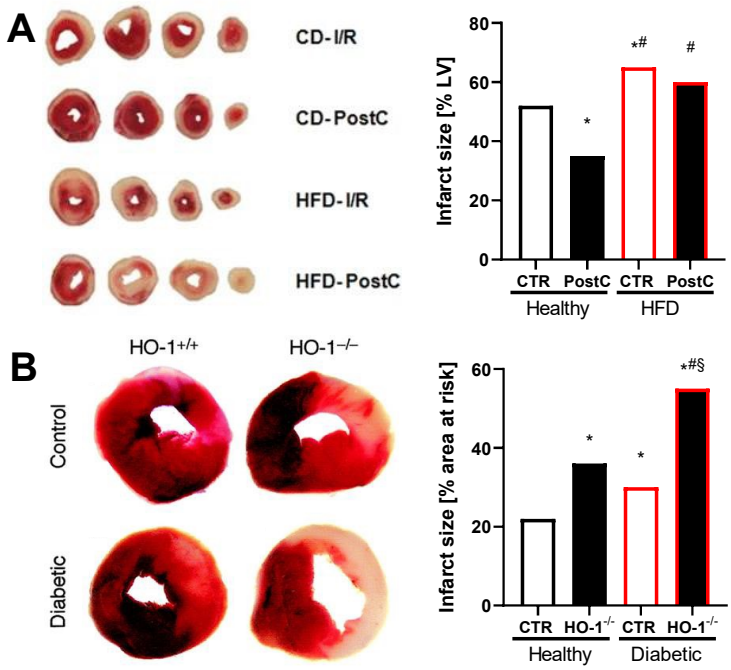

Figure 2. Experimental myocardial infarction in models of diabetes and hyperlipidemia (high fat diet). (A) All groups underwent induction of MI (I/R). Infarct size was aggravated by hyperlipidemia in high fat diet fed mice. The cardioprotective effects of post-conditioning (PostC) were abolished in the high fat diet group. The white color in the stainings shows infarcted, necrotic tissue. Reused from [173] with permission. Copyright © 2018, Springer Science Business Media, LLC, part of Springer Nature. **(B)** All groups underwent induction of MI. Infarct size was aggravated by diabetes in STZ-treated mice. Genetic heme oxygenase-1 deficiency further exacerbated the ischemic heart damage. The white color in the stainings shows infarcted, necrotic tissue. Reused from [252] with permission. Copyright © 2005, American Diabetes Association.

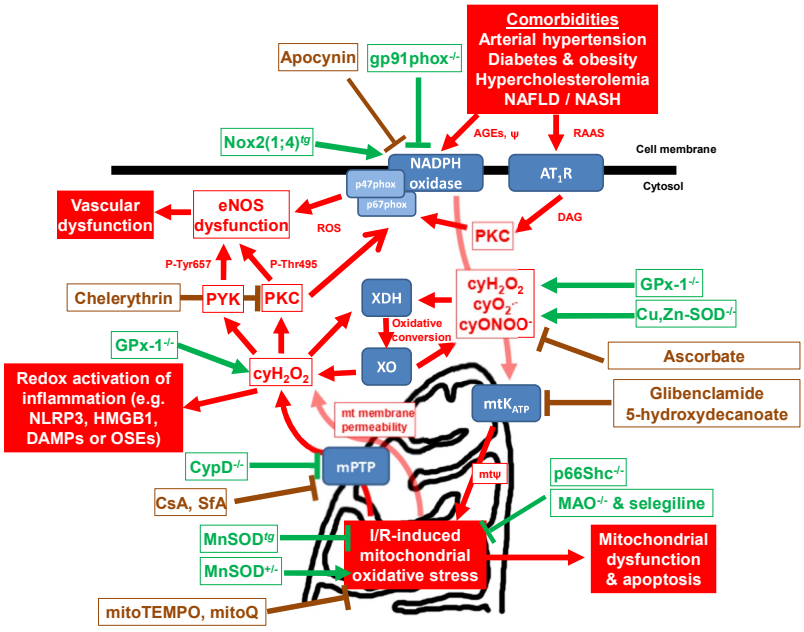

Figure 3. Proposed mechanism of the redox crosstalk between different ROS sources explaining the aggravation of ischemia/reperfusion damage by comorbidity factors. The green and brown boxes represent novel/unexplored genetic or pharmacological redox approaches to interfere with the vicious cycle between comorbidities and IRI (as observed during MI). **Abbreviations:** AT_1R, angiotensin-II receptor (type 1); cy, cytosolic; CsA, cyclosporine A; CypD, cyclophilin D; DAG, diacylglycerol; gp91phox, NOX2; MnSOD, manganese superoxide dismutase (SOD2); mtKATP, mitochondrial ATP-sensitive potassium channel; p47phox and p67phox, regulatory cytosolic subunits of NOX2; PYK, protein tyrosine kinase; RAAS, renin-angiotensin-aldosterone system; SfA, sangliferin A; XDH, xanthine dehydrogenase; Ψ, membrane potential; mtΨ, mitochondrial membrane potential. Summarized and updated from [413, 415, 416]. **Note to reviewers: This figure will be drawn by a graphical artist during revision of the MS.**

Table 1. Studies on the effects of a diverse range of antioxidants on cardiac effects in cardiometabolic comorbidities

Study	Antioxidant	Dose and administration	Experimental *in vivo* model	Major reported outcomes/effects	Mechanistic insights
Sivasinprasasn, S (2017) [83]	Vildagliptin	3 mg/kg daily, via intragastric gavage for 12 weeks	Ovariectomized rats received high-fat diet (HFO) for 12 weeks. In vivo cardiac IRI, 30-min ischemia and 120-min reperfusion	Reduction in the infarct size	Reduction of oxidative stress and apoptosis in the ischemic myocardium
Tanajak, P (2018) [84]	Dapagliflozin	1 mg/kg/day for 28 days	High-fat (HF) diet-induced obese insulin-resistant rats. In vivo cardiac IRI, 30-min ischemia and 120-min reperfusion	Reduction of infarct size, left ventricular (LV) function improvement	Markedly decreased mitochondrial fission and cardiac oxidative stress
Andreadou I (2017) [85]	Empagliflozin	10 mg/kg daily by gavage for 6 weeks	Mice fed with western diet for 14 weeks. In vivo cardiac IRI, 30-min ischemia and 120-min reperfusion	Improvement of left ventricular fractional shortening; reduction of infarct size	Improvement of redox regulation by decreasing iNOS expression and subsequently decreased of lipid peroxidation

Kondo K (2010) [86]	Adiponectin	Recombinant adiponectin protein was given as a bolus intracoronary injection during ischemia	Left anterior descending coronary artery was occluded in pigs for 45 minutes and then reperfused for 24 hours	Reduction in myocardial infarct size and improvement of left ventricular function in pigs after IRI	Suppression of inflammation, apoptosis, and oxidative stress
Marino A (2018) [87]	AC261066, a synthetic selective agonist for the retinoic acid β_2-receptor	Drinking water containing 3.0 mg AC261066/100 ml in 0.1% dimethylsulfoxide/H_2O for 6 weeks	Obese (HFD-fed) wild-type mice IRI in ex Vivo Mouse Hearts	Attenuation of infarct size, and alleviation of reperfusion arrhythmias.	Decreased formation of oxygen radicals and toxic aldehydes
Nduhirabandi, F (2011) [88]	Melatonin	4 mg/kg/day was administered in the drinking water for 16 weeks	A rat model of diet-induced obesity IRI in ex Vivo Rat Hearts	Reduction of infarct size and increased percentage recovery of functional performance of diet-induced obesity hearts.	Increased activation of Akt, ERK42/44 and reduced p38 MAPK activation
Iliodromitis EK (2010) [157]	Simvastatin	3 mg/kg, orally for 3 weeks	Cholesterol fed rabbits received for 6 weeks a diet enriched with 2 g of cholesterol.	Reduction of infarct size	Attenuation of oxidative and nitrosative stress

			IRI in vivo 30 min ischemia and 180 min reperfusion		
Andreadou I (2012) [158]	Pravastatin	3 mg/kg orally for 3 days	Cholesterol fed rabbits received for 6 weeks a diet enriched with 2 g of cholesterol. IRI in vivo 30 min ischemia and 180 min reperfusion	Reduction of infarct size	Activation of eNOS and attenuation of nitro-oxidative stress
Andreadou I (2007) [159]	Oleuropein	20 mg/kg daily, orally for 6 weeks and for 3 weeks	Cholesterol fed rabbits received for 6 weeks a diet enriched with 2 g of cholesterol. IRI in vivo 30 min ischemia and 180 min reperfusion	Reduction of infarct size	Protection against oxidative damage during ischemia-reperfusion, reduction of the protein carbonyl content and enhancement of SOD activity
Yadav, H.N (2012) [165]	GSK-3β inhibitors, SB 216763 and indirubin-3 monoxime (IND)	SB, 0.6 mg/kg, i.p., IND, 0.4 mg/kg, i.p., administered 24 h before the isolation of heart	Rat by feeding high-fat diet for 6 weeks IRI in Ex Vivo Rat Hearts	Decrease of myocardial infarct size	HSP acts on pathway of GSK-3β and plays a significant role in cardioprotection
Sloan (2012) [218]	NIM811-(cyclosporin A analogue)	5 μM at the onset of reperfusion	STZ-induced diabetic rats	Reduction in infarct size	Inhibition of mPTP

			IRI in Ex Vivo Rat Hearts		
Leng (2018) [219]	Tubastatin A (HDAC6 inhibitor)	10 mg/kg, i.p., for 7days	STZ-induced diabetic rats In vivo IRI; 45min ischemia and 180 min reperfusion	Improved cardiac function; reduced infarct size and release of LDH and CK-MB	Attenuation of ROS generation, lipid peroxidation and apoptosis; increased acetylated-Prdx1 levels
Koka (2013) [229]	Tadalafil (PDE5 inhibitor)	1mg/kg/day, i.p., for 28days	Type 2 diabetes (db/db mice) Ex vivo global IRI	Reduction in infarct size	Attenuation of ROS generation and myocardial lipid peroxidation; attenuation of NADPH oxidase activity and expression of subunits pRac1 and $gp91^{phox}$
Yu (2017) [232]	Melatonin	10 mg/kg orally for 5 days and i.p once before reperfusion	STZ-induced diabetic rats In vivo IRI; 30min ischemia and 180 min reperfusion	Improved cardiac function; reduced infarct size; reduced apoptosis	Reduced mitochondrial oxidative stress and enhanced biogenesis; activated AMPK/PGC-1α-SIRT3 signaling and increased expression of SOD2, NRF1 and TFAM
Yu (2016) [231]	Melatonin	10 mg/kg/d i.p. for 5 days	Acute hyperglycemia (500	Improved cardiac function; reduced	Reduced oxidative stress; activated Notch1

			g/L HG, 4 ml/kg/h, i.v.) In vivo IRI; 30min ischemia/4h-72h reperfusion	infarct size; reduced apoptosis	signaling by increasing Trx activity while decreasing Txnip
Yu (2015) [230]	Melatonin	20 mg/kg/day orally	T2D (HFD-STZ) rat model In vivo IRI; 30min ischemia/4h-72h reperfusion	Improved cardiac function; reduced infarct size; reduced apoptosis	Attenuation of oxidative stress and ER stress via activation of SIRT1 signaling
Yu (2018) [419]	Melatonin	10 mg/kg/d i.p. for 5 days	STZ-induced diabetic rats In vivo IRI; 30min ischemia/4h reperfusion	Improved cardiac function; reduced infarct size; reduced apoptosis	Activation of cGMP-PKGIα / Nrf-2-HO-1 signaling
Mao (2013) [222]	Antioxidants (NAC and Allopurinol)	Combination of NAC (1.5 g/kg/day) and ALP (100 mg/kg/day) for 4 weeks	STZ-induced diabetic rats In vivo IRI; 30min ischemia/ 2h reperfsuion	Improved cardiac function; reduced infarct size and release of CK-MB	Enhanced GSH/GSSG; Increased expression of HO-1 and HIF-1α

Nayak (2019) [239]	Phloroglucinol (benzenetriol)	100 mg/kg/day or 200mg/kg/day administered orally for 28 days	STZ-induced diabetic rats Ex vivo IRI; 15 min ischemia/30 min reperfsuion	Improved hemodynamic parameters before I/R; reduced infarct size and release of CK-MB	Increased GSH levels; decreased lipid peroxidation
Xiao (2019) [243]	Luteolin (polyphenol)	100 mg/kg/day, i.g., for 2 weeks	STZ-induced diabetic rats Ex vivo global IRI, 30 min ischemia/120min reperfusion	Improved cardiac function and myocardial viability	Decreased oxidative stress and lipid peroxidation; enhanced eNOS/Keap1/Nrf2 signaling and upregulation of antioxidant enzymes
Yang (2015) [244]	Luteolin (polyphenol)	100 mg/kg/day, i.g fot 2 weeks	STZ-induced diabetic rats Ex vivo global IRI, 30 min ischemia/120 min reperfusion	Improved cardiac function and decreased LDH release	Upregulation of eNOS and MnSOD; inhibition of mPTP
Duan (2017) [242]	Butin (plant flavonoid)	10, 20 and 40 mg/kg i.g for 15 days	STZ-induced diabetic mice In vivo IRI, 20 min ischemia/6h reperfusion	Improved cardiac functional recovery; reduced infarct size; decreased apoptosis	Upregulation of Nrf2 and HO-1 via activation of AMPK/Akt/GSK3β signaling pathway

Suchal (2017) [246]	Kaempferol (plant flavonoid)	20 mg/kg; i.p. daily for 28 days	STZ-induced diabetic rats In vivo IRI, 45 min ischemia/60min reperfusion	Improved hemodynamic parameters and cardiac function; decreased apoptosis	Inhibition of the MAPK and AGE-RAGE pathways; attenuation of oxidative stress and inflammation
Thirunavukkarasu (2007) [236]	Resveratrol	2.5mg/kg orally for 2 weeks	STZ-induced diabetic rats Ex vivo IRI, 30 min ischemia/2h reperfusion	Improved cardiac functional recovery; reduction in infarct size and apoptosis	NO mediated induction of Trx-1, HO-1 and VEGF; activation of Mn-SOD
Fourny (2019) [234]	Resveratrol	1 mg/kg/day orally for 8 weeks	Type 2 diabetic female Goto-Kakizaki rats Ex vivo IRI	Improved cardiac function	Improved mitochondrial function; increased expression of eNOS/ SIRT1
Wu (2017) [245]	Epigallocatechin-3-gallate (EGCG)	100mg/kg/day i.p. for 14 days	STZ-induced diabetic rats In vivo IRI; 30 min ischemia /2h reperfusion	Improvement of cardiac functional recovery; reduction of I/R-induced myocardial infarct size	Decreased oxidative stress and fibrosis; increased expression of SIRT1and MnSOD

The selection in this table is restricted to studies on ischemia/reperfusion injury (IRI) in metabolic comorbidities where antioxidants were administered exogenously. Studies were excluded if full-text was not readily available or if experimental details and/or data were incompletely reported.

Abbreviations used in this Table: AGE, advanced glycation end-products; AMPK, AMP-activated protein kinase; eNOS, endothelial nitric oxide synthase; ERK,42/44 extracellular (signal) regulated kinase; GSK-3β, glycogen synthase kinase-3β; HO-1, heme oxygenase-1; HSP, heat shock protein; Keap1, Kelch-like ECH-associated protein1; LDH, lactate-dehydrogenase; MAPK, mitogen-activated protein kinase; MnSOD manganese-dependent superoxide dismutase; Nrf2, nuclear factor erythroid 2-related factor; RAGE, receptor of advanced glycation end-products (AGE); SIRT1, sirtuin1; STZ, streptozotocin; Trx-1, thioredoxin-1; VEGF, *Vascular endothelial growth factor*.

Table 2. Recent studies of a diverse range of antioxidants in pressure-overload hypertrophy models *in vivo*

Study	Antioxidant	Dose and administration	Experimental *in vivo* model	Major reported outcomes/effects	Mechanistic insights
Matsuoka H (2019) [420]	Molecular Hydrogen (H_2)	2% H_2 in air for 6 weeks	Dahl salt-sensitive rat	Slight attenuation of hypertension development; reduced LV mass index; reduced myocyte cross sectional area	H_2 scavenges $^{\cdot}OH$ and $ONOO^-$. No specific molecular mechanism identified
Fan Z (2018) [421]	Molecular Hydrogen (H_2)	>0.6 mM H_2 in saline by i.p. injection daily for 6 weeks	Transverse abdominal aortic constriction, rat	Dose-dependent attenuation of LV mass index; reduced collagen fraction; reduced LV natriuretic peptide expression	Effects associated with reduced LV protein level of JAK and STAT3 and phospho-STAT3
Xu X (2020) [422]	Pyrolloquinoline quinone	0.4, 2 or 10 mg/kg daily by gavage for 6 weeks	Transverse abdominal aortic constriction, rat	Prevention of cardiac hypertrophy; preservation of EF by echocardiography; reduced collagen fraction	Reduced ROS production and preservation of mitochondrial membrane potential in isolated cardiac myocytes treated with Ang II
Liao HH (2019) [423]	Myricetin (plant polyphenol)	200 mg/kg daily by gavage for 6 weeks	Thoracic aortic constriction, mouse	Attenuation of LV mass index; preservation of EF and other echocardiographic indices.	Effects associated with reduced activation of TAK1, p38 MAPK and JNK1/2

Zhang Y (2019) [424]	Isorhyncophylline (plant tetracyclic oxindole alkaloid)	0.2% in feed for 6 weeks	Thoracic aortic constriction, mouse	Attenuation of LV mass index; reduced LV echocardiographic dimensions; reduced LV natriuretic peptide expression; reduced collagen fraction	Increased activity of SOD and catalase. *In vitro*, antihypertrophic effects of isorhyncophylline are Nrf2 dependent.
Liu C (2019) [425]	Zingerone (plant methoxyphenol)	10 or 20 mg/kg daily by gastric gavage for 25 days	Thoracic aortic constriction, mouse	Attenuation of cardiac index; reduced LV natriuretic peptide expression; reduced collagen fraction; improved echocardiographic indices	*In vitro*, suppression of phenylephrine induced cardiac myocyte hypertrophy and reduced ROS generation, abolished by Nrf2 knockdown. Enhanced eNOS activity and NO generation
Xu M (2019) [426]	Oridonin (plant diterpenoid flavonoid)	40 mg/kg daily by gavage	Thoracic aortic constriction, mouse	Attenuation of cardiac hypertrophy; reduced natriuretic peptide expression; preserved EF; improved echocardiographic indices; reduced collagen fraction; enhanced autophagy markers	*In vitro*, suppression of Ang II-induced myocyte hypertrophy; autophagy effects of oridonin P21-dependent
Ba L (2019) [427]	Allicin (plant organosulfur	5, 10 or 20 mg/kg daily by i.p.	Abdominal aortic constriction, rat	Attenuation of cardiac hypertrophy at 10 or 20 mg/kg; reduced myocyte cross	*In vitro*, suppression of Ang II-induced myocyte hypertrophy; inhibition of

	compound [thiosulfinate])	injection for 4 weeks		sectional area; reduced natriuretic peptide expression; reduced expression of autophagy markers	autophagy was via activation of PI3k/Akt/mTOR and MAPK/mTOR pathways
Bradic J (2019) [428]	*Galium verum* (L) extract (containing flavonoids)	Dried 1:5 methanolic extract in drinking water, ~500mg/kg daily for 4 weeks	Spontaneously hypertensive rat	Attenuation of hypertrophy; improved echocardiographic indices	Improved recovery of contractile function after 20 min global ischemia *ex vivo*; reduced plasma superoxide and lipid peroxides
Zeng J (2019) [429]	Lycopene (plant carotenoid terpene)	50 mg/kg daily by gavage for 1 week before and 4 weeks after surgery	Thoracic aortic constriction, mouse	Marked attenuation of LV hypertrophy; attenuation of echocardiographic changes; reduced LV ROS detection; increased SOD gene expression	*In vitro*, phenylephrine-induced myocyte hypertrophy attenuated; preservation of mitochondrial membrane potential and inhibition of mPTP opening
Liu Y (2018) [430]	Saikosaponin A (plant terpenoid)	5 mg/kg or 40 mg/kg daily by i.p injection for 4 weeks, starting 2 weeks after surgery	Aortic constriction, mouse (not stated if thoracic or abdominal)	No attenuation of LV hypertrophy; attenuation of natriuretic peptide expression; dose-dependent reduction of LV collagen fraction; attenuation of echocardiographic changes	Specific effect on fibrosis; *in vitro*, no attenuation of Ang II-induced myocyte hypertrophy; attenuation of TGF-beta1 stimulated cardiac fibroblast proliferation; inhibition of Smad signalling

				including improved ejection fraction	
Dong B (2018) [431]	Fisetin (plant flavonoid)	20 mg/kg daily by i.p. injection, from 1 week before to 4 weeks after surgery	Aortic constriction, mouse (not stated if thoracic or abdominal)	Attenuation of LV hypertrophy; improved ejection fraction and attenuation of other echocardiographic changes; attenuation of LV natriuretic peptide expression; reduced LV ROS production; increased LV expression of SOD1 and catalase mRNA	*In vitro*, attenuation of phenylephrine-induced myocyte hypertrophy; reduction in ERK1/2, p38 MAPK, JNK1/2 and mTOR phosphorylation *in vivo* and *in vitro*. No additive effect *in vitro* of N-acetylcysteine.
Chen K (2018) [432]	Quercetin (plant flavonoid)	5, 10 or 20 mg/kg daily by gavage for 8 weeks	Abdominal aortic constriction, rat	Prevention of cardiac hypertrophy; improved echocardiographic indices; inhibition/normalisation of proteasome activities; attenuation of interstitial fibrosis	Antihypertrophic action related to GSK-3 activation as a result of proteasome activation in vivo (and in Ang II -stimulated myocytes *in vitro*)
Meng G (2018) [297]	NaHS (H_2S donor)	50 umol/kg daily for 2 weeks	Thoracic aortic constriction, mouse	Attenuation of blood pressure increase and LV hypertrophy in wild type but not SIRT3 knockout mice; reduced	*In vitro*, attenuation of Ang II induced myocyte hypertrophy and natriuretic peptide expression plus improved

				myocardial ROS production in wild type but not SIRT3 knockout mice	mitochondrial function in SIRT-3 dependent manner
Zhang Q (2015) [433]	Polydatin (plant polyphenol)	50 mg/kg daily by gavage starting 7 days before Ang II treatment	Ang II infusion by minipump for 28 days, rat	Non-significant attenuation of blood pressure rises; attenuation of cardiac hypertrophy, myocyte cross-section area and collagen fraction;	Decreased cardiac NADPH oxidase activity and Nox 2 and Nox 4 expression; concentration-dependent antihypertrophic effect in cardiac myocytes *in vitro*
Dolinsky VW (2015) [434]	Resveratrol (plant polyphenol)	Orally in diet 4 g/kg, equivalent to 146 mg/kg daily (rat) or 320 mg/kg daily (mouse)	Spontaneously hypertensive rat, 5 weeks Ang II infusion by minipump for 14 days, mouse	Rat: attenuation of cardiac hypertrophy; increased phospho-AMPK, decreased phospho-P70S6K Mouse: attenuation of cardiac hypertrophy; increased phospho-AMPK, decreased phospho-Akt and phospho-P70S6K	*In vitro* activation of AMPK; inhibition of p70S6K and NFAT; no effect on SIRT1 expression. *In vivo*, no effects of resveratrol on physiological hypertrophy induced by exercise training (rat)

Articles specifically examining the effects of antioxidants on pressure overload hypertrophy and published in the date range 01 January 2015 to 07 November 2020 were retrieved from the PubMed database. The selection in this table is restricted to studies of pressure-overload models in vivo where antioxidants were administered exogenously. Studies were excluded if full-text was not readily available or if experimental details and/or data were incompletely reported.

Abbreviations used in this Table: AMPK, AMP-activated protein kinase; Ang II, angiotensin II; EF, LV ejection fraction; ERK, extracellular (signal) regulated kinase; GSK, glycogen synthase kinase; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen activated protein kinase; mPTP, mitochondrial permeability transition pore; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T-cells; Nox, NADPH oxidase; Nrf2, nuclear factor erythroid 2-related factor; SIRT, sirtuin; STAT, signal transducer and activator of transcription; TAK1, transforming growth factor beta-activated kinase; TGF, transforming growth factor.