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Review article

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

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Abbreviations

AGE	advanced glycation end-products
AMPK	AMP-activated protein kinase
Apo	apoprotein
BH4	tetrahydrobiopterin
BMI	body mass index
CAT	catalase
CR	caloric restriction
CVD	cardiovascular disease
DAMP	damage-associated molecular patterns
DM	diabetes mellitus
DPP	dipeptidyl protease
eNOS	endothelial nitric oxide synthase
ETC	electron transport chain
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
MMP	matrix metalloproteinase
mPTP	mitochondrial permeability transition pore
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NNT	nicotinamide nucleotide transhydrogenase
NO	nitric oxide
NOX	nicotinamide adenine dinucleotide phosphate (NADPH) oxidase
Nrf2	nuclear factor erythroid 2-related factor
NSTEMI	non-ST elevation myocardial infarction
OSE	oxidation specific epitopes
OxLDL	oxidised low-density lipoprotein (LDL)
PCSK9	proprotein convertase subtilisin/kexin type 9
PDE5	phosphodiesterase-5
PGC	peroxisome proliferator activated receptor-gamma (PPAR-γ) coactivator
PKC	protein kinase C
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
Trx	thioredoxin
XO	xanthine oxidase
ZDF	Zucker diabetic fatty (rat strain)

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 and myocardial dysfunction in the presence of the comorbidities. Thus, while early reperfusion represents the most favorable therapeutic strategy to prevent ischemia/reperfusion injury, redox therapeutic strategies may provide additive benefits, especially in patients with heart failure. While oxidative and nitrosative stress are harmful, controlled release of reactive oxygen species is however 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.

1. Introduction

Cardiovascular diseases (CVD), notably ischemic heart disease (IHD) and heart failure (HF) are the leading causes of disease burden and the primary causes of death worldwide [1]. Several chronic conditions (also termed comorbidities) increase the risk of CVD development. Their presence among patients with CVD is increasing due to reduced case fatality of IHD and prolonged life expectancy [2, 3]. The rising prevalence of diabetes mellitus (DM), obesity, non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), hypertension and hyperlipidemia drive the rising prevalence of CVD and is mitigating the benefits of effective cardiological interventions (cholesterol and blood pressure lowering, coronary interventions, etc.). Thus, these conditions constitute risk factors for the development of IHD and other CVD and they are common comorbidities in patients with established CVD that can affect clinical outcomes profoundly [4].

To provide an impression of the increase in CVD risk by the different comorbidities discussed below, 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) [5]. 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).

Obesity and DM synergistically cause myocardial dysfunction independent of coronary artery disease and hypertension since both conditions share similar pathophysiological mechanisms [6, 7]. 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 myocyte lipotoxicity, cardiac autonomic neuropathy, as well as increased inflammation and interstitial collagen deposition [8-10]. These pathological changes result in subclinical myocardial dysfunction (initially diastolic) and eventually the development of overt HF with preserved ejection fraction that may

progress to HF with reduced ejection fraction [11]. The numerous biochemical effects on the heart negatively affect the development of ischemia/reperfusion injury (IRI) and interfere with cardioprotective interventions, notably ischemic pre-and postconditioning. Ischemic preconditioning (where the heart is subjected to short non-lethal periods of ischemia and reperfusion, before the onset of sustained ischemia) and ischemic postconditioning (where the heart is subjected to short non-lethal episodes of ischemia and reperfusion, immediately after the sustained ischemic insult), are well described procedures that markedly enhance the ability of the heart to resist a prolonged ischemia/reperfusion period resulting in less arrhythmias, cell death and/or improved cardiac function [12]. In animal models, the role of redox signaling is of paramount importance for both cardioprotective strategies [13]. The mechanisms by which the remarkable cardioprotective effect of ischemic conditioning is attenuated or abolished in the presence of comorbidities are not fully understood [12]. 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 describe the effects of the major comorbidities on cardiac redox homeostasis, focusing on obesity, hyperlipidemia, DM, hypertension and NAFLD/NASH. We also consider the complex interactions between these conditions and possible therapeutic interventions to 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) [14]. 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 [15]. Obesity may have direct effects on the heart [16]. Obesity increases the risk of myocardial infarction (MI) by 20-40%. Framingham Heart Study data indicated that increased BMI correlates well with greater risk for developing HF both in men and women [11]. Human and animal studies show that the heart undergoes structural and functional changes in obesity [16],

namely increased left and right ventricular wall thickness, increased left atrium dimensions, fibrosis and accumulation of intracellular triglycerides [16]. Subclinical contractile alterations have been detected in obese patients, along with diastolic dysfunction. Similar results have been observed in experimental models of obesity, suggesting that obesity alone does not impair systolic function but affects diastolic relaxation [17, 18].

2.1. Obesity and redox signaling in myocardial infarction

Several [18-20] but not all [21,22] studies demonstrate greater susceptibility to IRI in experimental models of obesity and in patients. One possible reason for this discrepancy is that changes in hemodynamics may confound contractile defects *in vivo* [16]. In addition, obesity is associated with elevated circulating concentrations of insulin and fatty acids that might affect the extent of IRI [19, 20]. Indeed, one study found that obesity led to increased infarct size and reduced functional recovery after IRI *ex vivo*, but the presence of insulin and fatty acids in the buffer completely abolished these differences between obese and non-obese hearts [21]. Functional recovery of the heart after IRI is improved by increasing glucose oxidation during reperfusion [20]. An additional factor may be age, since aged obese hearts show reduced functional recovery when subjected to preconditioning [22].

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 [23]. Obesity is associated with alterations in mitochondrial function, number and turnover [24, 25]; thus, impairment in mitochondrial oxidative capacity observed in ob/ob mice inevitably results in increased superoxide formation [16]. Indeed, the mitochondrial respiratory chain (i.e. complexes I and III) is considered a relevant source of reactive oxygen species (ROS) in obese or diabetic hearts of mice [26]. An additional mechanism for mitochondrial ROS formation is $p66^{Shc}$ that, upon phosphorylation by protein kinase C (PKC), translocates to mitochondria to induce hydrogen peroxide (H_2O_2) formation [27]. $p66^{Shc}$ is critical for insulin signaling and glucose uptake and its phosphorylation

is increased in obesity and DM [28, 29]. Moreover, its deletion reduces oxidative stress in mice fed with high-fat diet [30].

Other intracellular enzymes contribute to altered 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 [31]. Importantly, $p66^{Shc}$ can also activate ras-related C3 botulinum toxin substrate 1 (rac1) and trigger NOX mediated ROS formation [31]. NOX activity is enhanced in obese animals and its inhibition prevents oxidative stress and cardiac dysfunction [32, 33].

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, amplified by NOX2 causing mitochondrial dysfunction and further oxidative stress in a vicious cycle [34]. 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 [33, 35]. Lipotoxicity via mitochondrial ROS further aggravates cardiac IRI [36, 37]. 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 [37]. MAO plays a major role in oxidative stress in diabetic cardiomyopathy [38] and could contribute to changes 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 [39]. 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 [40].

Expression and/or activity of many antioxidant enzymes is reduced in the heart and circulation of obese animals [33]. Moreover, mitochondrial peroxidases involved in ROS removal use NADPH provided mostly by nicotinamide nucleotide transhydrogenase (NNT) [41]. 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 [42]. In failing hearts, NNT activity can be reversed resulting in the depletion of mitochondrial antioxidant capacity and oxidative stress [43]. Whether alterations in NNT activity may be responsible for altered redox equilibrium in obese and ischemic hearts has not been investigated to date. As a general note of caution, the mouse strain C57BL/6J (B6J) displays a mutation of the Nnt gene leading to markedly lower NNT protein expression as compared with the control B6N strain, which may significantly influence the outcome of studies related to IRI and HF in these mice [44].

2.2. Pharmacological redox modulation in obesity and cardioprotection

Lifestyle intervention, caloric restriction (CR), exercise training and different pharmaceuticals and 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 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 [45] and decrease NOX activity [46]. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased the expression of heme oxygenase-1 (HO-1) by a mechanism dependent on nuclear factor erythroid 2-related factor 2 (Nrf2) [47]. Moreover, PUFA supplementation in humans resulted in increased expression of antioxidants such as catalase (CAT), HO-2, glutathione transferases (GST) and glutathione reductase (GR) and in the downregulation of antioxidant genes such as glutathione peroxidases (GPx) [48]. Polyphenols increased nitric oxide (NO) bioavailability by inducing eNOS activity, while reducing NOX1 in obese animals [49]. 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 [50, 51].

Several primary and secondary interventional studies have reported the benefit of CR, indicating its efficacy in improving the antioxidant response in obese individuals [52]. Notably, CR-mediated protection relies on the decrease of oxidative stress markers via sirtuins (SIRT), NAD^+-dependent deacetylases [53, 54], FOXO [55] and PGC-1α-mediated mitochondrial bioenergetics [56]. CR can induce cardioprotection in

obese animals via antioxidant adaptive genes associated with increased adiponectin expression and AMPK activation [57], as well as via SIRT1 and PGC-1α [58]. Polyphenols and exercise training were reported to induce stress response genes and mitochondrial biogenesis via AMPK and SIRT mediated reduction of FOXO activity [59], and exercise training restored anesthetic cardioprotection in obesity through reduced basal oxidative stress and normalized ROS-mediated AMPK pathway [60].

Of note, prebiotics, probiotics, and their optimal synergistic combination termed synbiotics were found to induce cardioprotection by counteracting mitochondrial dysfunction via the improvement of the electromechanical proton gradient in obese animals [61].

In obesity models, several antidiabetic drugs induce cardioprotection: Vildagliptin was found to be protective against IRI in obese-insulin resistant rats by improved cardiac mitochondrial function, reduced oxidative stress and reduced apoptosis [62]. The sodium-glucose cotransporter-2 (SGLT2) inhibitor dapagliflozin exerted cardioprotection in high-fat diet-induced obese/insulin-resistant rats by decreasing cleaved caspase 3 as well as mitochondrial anti-dynamin related protein-1, suggesting a mechanism involving control of mitochondrial fission [63]. Empagliflozin reduced body weight, attenuated infarct size and improved redox regulation by decreasing inducible NOS (iNOS) expression and subsequently lipid peroxidation in mice fed a Western diet [64].

Adiponectin has been reported to play a protective role in the development of obesity-linked disorders. Adiponectin protects against IRI in a pig model through its ability to suppress inflammation, apoptosis, and oxidative stress [65]. Treatment with AC261066, a retinoic acid β_2- receptor selective agonist, protected hearts from obese mice subjected to IRI *ex vivo*. This cardioprotection was associated with decreased ROS and toxic aldehyde formation [66]. Melatonin, a potent free radical scavenger and antioxidant reduced infarct size in a rat model of diet-induced obesity and prevented the metabolic abnormalities [67]. MitoTEMPO, a mitochondria-targeted ROS scavenger, prevented cardiac fibrosis and oxidative stress and ameliorated weight gain in a high fat diet model [68]. Similar protective effects were observed with mitoQ, a synthetic mitochondrial antioxidant [69, 70].

In summary, it is difficult to differentiate the effects of obesity from the effects induced by associated comorbidities (hyperlipidemia and DM). However, altered redox signaling triggers changes in cardiac function in obese hearts (Figure 2). Decreasing oxidative stress to prevent metabolic disorders related to obesity is an interesting therapeutic target but 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% [71]. 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 [15].

Multiple experimental studies have shown that hyperlipidemia enhances infarct size and favors cardiolipotoxicity. Oxidative and nitrosative stress play an important role in LDL accumulation in the vascular wall [72]. Hypercholesterolemia facilitates the reaction between superoxide and NO, generating reactive nitrogen species (RNS) such as dinitrogen trioxide (N_2O_3) and peroxynitrite [73]. Thiol nitrosation by peroxynitrite may also exert detrimental effects on protein synthesis contributing to the promotion of ROS and inflammation [74]. Both native LDL and oxidized LDL (oxLDL) stimulate superoxide/peroxynitrite production and uncouple eNOS [75] thereby reducing endothelial NO production [76]. Furthermore, hyperlipidemia upregulates caveolin and promotes eNOS interaction with caveolin [77] and decreases eNOS association with heat shock protein (HSP) 90 [78] resulting in further inhibition of eNOS activity. Finally, oxLDL decreases eNOS activity either by inhibiting serine 1177 phosphorylation of eNOS [79] or by increased proteasomal eNOS degradation [80]. Consistent with experimental evidence, reduced NO bioavailability was observed in hypercholesterolemic patients [74] who also display endothelial dysfunction [81]. Oxidative stress and NO play opposing roles in the regulation of adhesion molecule expression and endothelial–leukocyte interaction. Endothelial NO inhibits cytokine-induced nuclear factor-κB (NFkB) activation, downregulates vascular cell and

intercellular adhesion molecules, and decreases leukocyte adherence [82], whereas ROS are implicated in cytokine-induced upregulation of adhesion molecules [83].

OxLDL exhibits proatherogenic properties mediated by oxidized phospholipids within the LDL molecules. Lipid peroxidation can occur through enzymatic mechanisms (e.g. by ROS derived from NOX and uncoupled eNOS [84], myeloperoxidases, lipoxygenases, cyclooxygenases, and cytochrome P450). ROS formation may be through direct enzyme activity but may also originate from side reactions. Highly reactive lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal can lead to the generation of oxidation-specific epitopes (OSEs) [85]. OxLDL upregulates proprotein convertase subtilisin/kexin type 9 (PCSK9) expression and release from extrahepatic tissues and cardiomyocytes, increasing the overall circulating PCSK9 concentration, which then impacts on LDL levels, but also impairs cardiac function [86, 87].

In addition to the direct effects of LDL on endothelial ROS production, hyperlipidemia indirectly enhances oxidative stress by potentiating the effects of angiotensin II via upregulation of angiotensin II type 1 receptor [88]. As seen in Section 2, ROS are produced as byproducts of mitochondrial respiration and production is elevated during metabolic perturbation, including hyperlipidemia [89]. OxLDL inhibits the normal function of mitochondria and thus promotes mitochondrial ROS generation, which in turn oxidize LDL, creating a vicious cycle. Additionally, ROS may inhibit specific mitochondrial enzymes affecting cellular antioxidant and energetic capacities [90].

3.1.Hyperlipidemia and redox signaling in myocardial infarction

Hyperlipidemia increased infarct size by 45%, associated with increased protein oxidation, lipid peroxidation, and tyrosine nitration during IRI [91, 92]. Tyrosine nitration was also increased in Watanabe heritable hyperlipidemic rabbits [93]. NOX and XO were observed as the major sources of superoxide anion in the coronary artery of hypercholesterolemic patients with IHD [94] and in cholesterol-fed rabbits [95]. Obesity and hypercholesterolemia have additive effects on NOX2 activation (measured by sNox2-dp) [96]. Higher sNox2-dp and oxLDL levels were observed in hypercholesterolemic children [97].

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 [98]. Besides BH4 deficiency, L-arginine deficiency also represents an underlying cause of eNOS uncoupling in hyperlipidemia. This is supported by studies in hyperlipidemic rabbits where upregulation of arginase expression and activity caused a decrease in L-arginine as substrate for eNOS [99]. ROS derived from uncoupled eNOS have been detected in LDL-treated endothelial cells, in hypercholesterolemic *ApoE*-KO mice and in hypercholesterolemic patients [100]. ROS derived from NOXs and uncoupled eNOS are involved in the generation of OSEs including oxidized phospholipids and malondialdehyde-modified amino groups, which have been documented on the surface of apoptotic cells and oxLDL molecules [101].

Perturbations in antioxidant defense systems are significant in hyperlipidemia. The expression and activity of antioxidants (especially reduced glutathione, SOD and CAT) in the vascular system are reduced in hypercholesterolemia [74]. Glutathione peroxidase (GPx)-1 deficiency increases LDL oxidation, foam cell formation, and macrophage proliferation [102]. 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 [103].

In IRI, ROS and RNS production may continue for hours after reperfusion and play an important role in the genesis of reperfusion injury and inflammatory cell recruitment [13]. IRI also reduces the levels of antioxidant enzymes such as glutathione peroxidase and SOD [104], already impaired by hyperlipidemia as mentioned above. Therefore, in the presence of hyperlipidemia ROS/RNS production is unbalanced by cell defenses, inducing deleterious effects on pathways involved in cell cycle and survival pathways.

3.2 Pharmacological redox modulation in hyperlipidemia and cardioprotection

Increased ROS generation induced by hyperlipidemia may interfere with endogenous cardioprotective mechanisms such as cardiac preconditioning and postconditioning and may have a detrimental role in determining the severity of IRI [74, 105].

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 IRI by attenuation of myocardial nitro-oxidative stress [106]. 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 [107]. The reduction in infarct size by a constituent of olives and olive oil, oleuropein, was achieved by attenuation of reperfusion injury and reduced oxidative stress in hyperlipidemic rabbits [108] and by red palm oil in hypercholesterolemic rats [109]. Moreover, inhibition of the peroxynitrite-matrix metalloproteinase (MMP) signaling axis by MMPs inhibitors has been shown to confer cardioprotection even in the presence of hyperlipidemia [110, 111]**.**

HSP70 is induced during myocardial IRI and contributes to preconditioning and postconditioning cardioprotection through suppression of ROS generation, inhibition of cell apoptosis and attenuation of calcium overload [112]. HSP70 is upregulated in cardiomyocytes during IRI [113] attributable, at least in part, to oxidative stress, since ROS can activate heat shock factor 1 which contributes to HSP70 induction [114]. Several studies have suggested that hyperlipidemia impairs the cardioprotective effects of HSP70 against IRI. Indeed, HSP70 downregulation was observed in cholesterol-fed rats subjected to myocardial IRI [115], potentially due to activation of glycogen synthase kinase (GSK)3β [116] as well as accumulation of cholesterol in the membrane of cardiomyocytes, which might prevent HSP70 accumulation during IRI [115].

The hypoxia-inducible factors (HIFs) and downstream genes are important factors in the protection of tissues from IRI. HIF-1α is one of the first response elements to IRI [117], and plays a pivotal role in the endogenous protective mechanism against ischemia [118]. HIF-1α expression was maintained at a very low level in hyperlipidemic rats and HIF activation using prolyl hydroxylase inhibitors resulted in a level of cardioprotection similar to that obtained with ischemic postconditioning [119].

Nrf2 regulates antioxidant gene expression in vascular cells after exposure to modified LDL [120] and oxidized phospholipids *in vivo* [121]. *Crocus sativus L.*

aqueous extract induced cardioprotection in *ApoE*-KO mice undergoing myocardial IRI through activation of Nrf2 and its downstream targets SOD2 and HO-1, with the subsequent regulation of nitro-oxidative stress in myocardium [122].

In summary, hyperlipidemia results in increased myocardial oxidative stress, through increased ROS production and downregulation of antioxidant defense systems which accounts for increased susceptibility to IRI (Figure 2). LDL and oxLDL predispose endothelial cells to inflammation with further ROS production leading to loss of cardiac contractile function and vascular dysfunction [123]. As a result, the infarct size is aggravated in a model of high fat diet and the protective effects of post-conditioning are lost [124]. Statins and pharmacological agents that modulate NO bioavailability, possess antioxidant properties or enhance antioxidant defense systems, or inhibits downstream targets of ROS/RNS signaling like e.g. MMP inhibitors may provide beneficial effects in the hyperlipidemic myocardium.

4. Diabetes

According to WHO data, the global prevalence of diabetes mellitus (DM) in 2014 was estimated to be 9% [14]. 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 [15].

Approximately 60% of studies examining type 2 diabetes mellitus (T2DM) in *in vivo* models of regional IRI, demonstrated increased infarct size with T2DM when compared to non-diabetic controls [125]. However, in these models the T2DM animals were almost all untreated for DM, 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 DM 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.

Studies on isolated hearts from diabetic animals show that elevated plasma glucose levels are a main determinant of infarct size [125]. Also, in 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 [126]. Thus, whereas it is clear that DM does in general increase CVD by 40-250% in DM patients receiving standard of care [127], effects on susceptibility to IRI are less pronounced or not observed, consistent with the moderate odds ratio of MI associated with DM.

4.1 Diabetes and redox signaling in myocardial infarction

Dysregulated redox signaling emerges as one of the prominent T2DM-induced molecular changes, reflected by increased oxidative stress [128]. Although increased reductive stress can also be detrimental to cardiac function [129], the diabetic heart commonly displays a depressed reductive stress response, as reflected by a diminished Nrf2-related gene response [130]. The reduced reductive stress response will contribute to the net increase of oxidative stress within the diabetic heart. Cardiac oxidative stress is largely a result of metabolic overload by elevated plasma glucose and fatty acid levels. Acute and chronic elevations of plasma glucose and fatty acid cause oxidative stress [131] contributing to increased ischemic sensitivity of diabetic heart [125]. Hyperglycemia is associated with a low-grade inflammatory phenotype, partly triggered by advanced glycation end-product (AGE)/receptor of AGE (RAGE) signaling [132].

In diabetic animals, the three major cytosolic sources of ROS are NOX2, uncoupled eNOS and XO [133]. These components may be activated by DM and can contribute to IRI [134-136]. Genetic Nox2 deficiency prevented the major diabetic complications in streptozotocin (STZ)-diabetic mice [137] and insulin resistance-triggered endothelial cell dysfunction largely relies on NOX2 activity [138]. NOX1-derived ROS contribute to immune cell activation and vascular infiltration in diabetic *ApoE*-KO mice [139]. In contrast, NOX-4-derived H_2O_2 seems to be protective in diabetic mice [140].

The major mitochondrial sources of ROS in the diabetic heart are the ETC, MAO and $p66^{Shc}$ [128]. High glucose increased ETC-produced ROS in endothelial cells

through increases in the mitochondrial membrane potential [141]. Increased mitochondrial potential may be due to hyperglycemia-induced reduction of hexokinase II (HKII) binding to mitochondria [142, 143]. Decreasing the amount of mitochondria-bound HKII increases ROS production in the heart [143, 144], and diabetic hearts have been reported to have less HKII bound to mitochondria [145]. Less mitochondrial HKII binding is suggested as a possible explanation for increased oxidative stress with aging [146], providing at least one explanation for why sensitivity to IRI may be exaggerated in aging diabetic patients. Genetic deficiency of mitochondrial aldehyde dehydrogenase resulted in increased immunohistochemical staining of cardiac 4-hydroxynonenal and diastolic dysfunction in diabetic mice [147].

4.2 Pharmacological redox modulation in diabetes and cardioprotection

Antioxidants such as ascorbic acid and N-acetylcysteine prevent NOS uncoupling in the diabetic rat heart resulting in increased bioavailability of NO and increased tolerance to IRI [148]. Diabetic heart mitochondria demonstrate an enhanced susceptibility to injury, mediated by redox-dependent shifts in mPTP opening [149]. In this context, diabetic mice treated with MitoTEMPO displayed preserved heart rates and better survival after MI by suppression of calmodulin-dependent protein kinase-II (CAMK-II) oxidation [150] and mitochondrial ROS/RNS generation [151]. Compounds that attenuate mPTP opening, such as NIM811, a cyclophilin D inhibitor, were reported to reduce infarct size when administered at reperfusion to STZ diabetic rats [152]. Pharmacological inhibition of histone deacetylase 6, which confers redox regulation and suppresses cellular stress responses, showed benefit in STZ diabetic hearts subjected to IRI, potentially through modulation of peroxiredoxin 1 (Prdx1) acetylation, thereby decreasing ROS levels [153]. As another mitochondria-targeted approach, inhibition of MAO attenuated diabetic cardiomyopathy [38, 128].

Stabilization of HIF-1α promotes tolerance to myocardial IRI by decreasing mitochondrial oxidative stress and inhibiting mPTP opening [154], but the HIF-1α signaling pathway is compromised in DM [155]. When diabetic rats were treated with N-acetylcysteine or the XO inhibitor allopurinol, HIF-1α/HO-1-dependent signaling was stabilized and myocardial IRI was attenuated [156]. Further studies have revealed that cobalt (II) chloride ($CoCl_2$) can activate the impaired HIF-1α pathway under diabetic conditions [157]. $CoCl_2$ or deferoxamine-activated HIF-1α signaling restored

sevoflurane postconditioning protection in diabetic rats by improving myocardial mitochondrial respiratory function and mitophagy and reducing ROS generation [158, 159].

Phosphodiesterase-5 (PDE5) inhibitors have been shown 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 [160]. PDE5 inhibition improves endothelial function and promotes antioxidant activity in the diabetic heart through increasing NO bioavailability [161]. In this context, tadalafil therapy attenuates oxidative stress and improves mitochondrial integrity while reducing myocardial infarct size in db/db mice [162].

Melatonin exerts protection against myocardial IRI in diabetic rats by limiting reperfusion-induced ROS formation and endoplasmic reticulum stress in a SIRT1-dependent manner [163]. 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 [164]. Furthermore, melatonin reduced myocardial IRI in STZ diabetic rats by normalizing mitochondrial function and oxidative stress as well as stimulation of mitochondrial biogenesis via AMPK-PGC1α-SIRT3 signaling [165].

Pterostilbene, a naturally occurring dimethylated analogue of resveratrol with antidiabetic effects, significantly reduced infarct size, oxidative stress, and apoptosis in diabetic rats. [166]. Other bioflavonoids (e.g. quercetin, rutin or benzenetriol), also displayed cardioprotective effects against IRI in diabetic rats, which partially rely on the attenuation of oxidative stress and improvement of antioxidant reserves [167, 168]. 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 [169, 170]. (-)-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 [171]. Furthermore, attenuation of myocardial IRI in diabetic rats was observed by kaempferol by suppression of AGE-

RAGE/mitogen activated protein kinase (MAPK)-dependent inflammation and oxidative stress [172].

Increasing evidence documents beneficial effects of SLGT2 inhibitors in the heart, directly or indirectly. Benefits include decreasing oxidative stress and preventing IRI [173, 174]. 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 [64, 175]. Treatment with empagliflozin significantly attenuated the increase in acute mortality after MI in a model of T2DM through preservation of myocardial antioxidant defense and normalization of mitochondrial size and number [173, 176].

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

In conclusion, DM is associated 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 (Figure 2). Increased oxidative stress diminishes the diabetic heart's resistance to IRI or increases the sensitivity of the heart to ischemia, which, at least in preclinical studies, can be prevented by antioxidant strategies. As a result infarct size is aggravated in a model of diabetes and further exacerbated by genetic heme oxygenase-1 deficiency [177]. Strategies to combat oxidative stress in patients with DM therefore seem warranted.

5. Hypertension/hypertrophy

According to WHO data, the global prevalence of systemic arterial hypertension was estimated to be approximately 30% in the adult population [178]. Hypertension 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 [15]. A key feature of hypertensive heart disease is concentric left ventricular hypertrophy (LVH) [179]. Estimates vary but more than 20% of hypertensive patients may develop echocardiographic evidence of LVH [180, 181] 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 [182, 183].

5.1 Hypertension/LVH and redox signaling in myocardial infarction

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 IRI [184-186]. The progression from adaptive cardiac hypertrophy to a maladaptive state, when myocyte contractility is impaired and HF develops, is clearly associated with increasing oxidative stress. The nature and causes of the imbalance between ROS generation and antioxidant defense mechanisms in hypertension are unclear although they are multifactorial and dependent on the etiology of hypertension in humans or the nature of the experimental model.

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 [187-189]. 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 [190, 191]. MAO-associated ROS generation may also contribute further. 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 [192, 193]. However, non-mitochondrial ROS-generating enzymes also appear to play important roles in physiological myocyte hypertrophy. These include XO [194]. 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.

Other non-mitochondrial sources of ROS may be relevant to both physiological cardiac hypertrophy and pathological decompensation leading to HF. NOX2 and NOX4

have received most attention [195, 196] although calcium//calmodulin-dependent NOX5 may also be implicated [197]. 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 [198] and the angiotensin II type 1 receptor antagonist, candesartan, decreased both XO and NOX activities in parallel [199].

Progression of LVH to decompensation and HF appears to be associated with multiple biochemical and metabolic alterations that shift redox balance towards a state of oxidative stress. Although the functional decline is often difficult to define clinically or model experimentally, many studies show that enhanced oxidative stress is a feature of the progression. Alterations in substrate metabolism [200] and the ETC complexes [201], increased expression and activity of MAO [193, 202-204], upregulation of XO [184] and increased activity of NOX isoforms [205] have been implicated in mediating excessive ROS production associated with LVH progression and decompensation in animal models.

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 [184] is a feature even in the compensated state and accompanied by reduction in the ratio of reduced glutathione (GSH)/oxidized glutathione (GSSG) [206] in the transition to HF. Trx1 inhibits cardiac hypertrophy through a number of redox-controlled downstream mechanisms [207]. Depletion or inhibition of Trx increases hypertrophy and may predispose to decompensation. Evidence suggests that 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 scavenging of ROS is plausible, there is evidence of more complex redox regulation by H_2S, especially in the mitochondria (reviewed in [208]). 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 [209]. There is limited evidence of mechanisms by which H_2S might modify physiological and

pathological processes in hypertrophy. SIRT3 influences substrate metabolism and mitochondrial redox status. In human LV tissue, SIRT3 expression correlated inversely with the severity of pathological changes [210]. In experimental LVH, exogenous H_2S 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 [211].

Enhanced oxidative stress through increased ROS generation and/or depletion of intracellular antioxidant systems may sensitize the hypertrophied myocardium to IRI and modify the response to protective interventions, notably preconditioning and postconditioning treatments. IRI responses in experimental LVH have been comprehensively reviewed elsewhere [12, 105]. Augmented irreversible tissue injury, measured as infarct size, has been observed in short-term experimental models of myocardial infarction in hypertensive LVH [212, 213] although not consistently [214, 215]. 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 leading to exaggerated post-infarct inflammatory response, less favorable tissue remodeling and worse outcome [216].

Several studies suggest that ischemic preconditioning is applicable and effective in young animals with experimental LVH, at least during the early stage of hemodynamic compensation [214, 215, 217, 218]. 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 [219, 220]. 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 [221-223].

Excessive ROS accumulation, particularly from mitochondrial sources, is known to trigger mPTP opening during early reperfusion [224] and the greater susceptibility of hypertrophied myocardium to IRI may, at least in part, be related to enhanced opening of mPTP [213, 225]. There is some evidence that oxidative stress

and the impairment of mitochondrial homeostasis and redox signaling mechanisms 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 [226]. An increase in ischemic preconditioning threshold required to confer protection was observed in hypertrophied hearts but protection was associated with preservation of GSH and decreased cytosolic accumulation of SOD2 (a surrogate indicator of mPTP opening) [227].

5.2 Pharmacological redox modulation in hypertension/hypertrophy

There is clear evidence from many hundreds of experimental studies with antioxidant compounds that oxidative stress is a mediator of pathological hypertrophy development/decompensation and of enhanced IRI in LVH models. The list includes exogenous antioxidant enzymes (CAT, SOD); inhibitors of ROS-generating enzymes (e.g. the XO inhibitor allopurinol); phytochemical ROS-scavenging agents such as purified derivatives or galenical plant extracts containing polyphenolic secondary metabolites (e.g. flavonoids; curcuminoids; anthocyanins; and stilbenoids); 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 oxidative stress both in the mediation of experimental hypertrophy and IRI (see summary in **suppl. Table S1**). The generally consistent picture in relatively short-term animal pressure overload models is that antioxidant treatment attenuates hypertrophy development, mitigates the histological changes associated with hypertrophy and delays or prevents the decline in cardiac function consistent with HF development.

However, despite clear evidence of oxidative stress in the pathophysiology of hypertensive LVH, progression to HF and increased susceptibility to IRI, and promising beneficial effects in 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 IRI (see [228] 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 [229]. Thus, the potential of exogenous antioxidants as cardioprotective agents in hypertrophied myocardium has so far met with limited success in therapeutic translation. Key issues have been the right antioxidant, in the appropriate biological compartment (extracellular/cytosolic/mitochondrial), at the right concentration, at the right time.

In conclusion, redox signaling is a critical molecular mechanism controlling cardiomyocyte hypertrophy in pressure overload conditions (Figure 2). 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 altered myocardial metabolism and 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, which to date have not translated to the clinical setting.

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 [230]. 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 [231]. Of note, there is a clear association of cardiovascular risk and mortality with the severity of NASH [232], as supported by increased carotid intima-media thickness as well as aggravated coronary calcification and endothelial dysfunction in patients with NASH [232, 233]. Fatty liver disease also contributes significantly to the global burden of disease in terms of disability-adjusted life years [234]. Previous reports provided indirect proof for a role of oxidative stress in hepatic endothelial dysfunction [235], which was also supported by improved hepatic endothelial function upon infusion of high dose vitamin C in patients with liver cirrhosis [236]. NAFLD is connected with

DM (see Section 4), which is clearly associated with oxidative stress and higher cardiovascular risk in mice [237], thereby supporting the notion of liver disease as a cardiovascular comorbidity in patients [238].

NASH represents an inflammatory liver disease with important features of atherosclerosis as shown in mice [239]. Macrophages and dendritic cells derived from blood monocytes as well as liver resident macrophages/Kupffer cells drive local immune responses in NASH [240] leading to higher levels of hepatic and cardiovascular ROS in animals and men [241, 242]. In analogy to NASH, these cells also play an essential role for the progression of atherosclerosis in patients [243, 244] and arterial hypertension in mice [245, 246]. Therefore, CVD may significantly contribute to overall mortality in patients with NAFLD/NASH [233].

6.1 NAFLD/NASH and redox signaling in myocardial infarction

Oxidative stress plays a central role in NASH and NAFLD disease progression (including cardiovascular complications) [247, 248] and NOX-derived ROS represent key players in liver fibrosis [249]. 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 [250]. Patients with NAFLD displayed higher oxidative stress burden (increased sNox2-dp and 8-isoprostane levels) that correlated with higher steatosis and portal inflammation [251] or with markers of infection [252], all of which are also accepted indicators of an increased CVD risk [253].

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 in the hepatocyte [254, 255] but also cardiac and vascular tissue of mice [242]. Enhanced $p66^{shc}$ signaling, increased opening probability of the mPTP and higher levels of mitochondrial damage-associated molecular patterns (DAMPs) were reported as a pathomechanism of liver damage in rodent models of NASH as well as patients [256, 257] that may explain the increased mitochondrial ROS formation. Similar mitochondrial abnormalities were also reported for cardiac tissue in models of fibrotic liver disease [258]. XO inhibition prevented the major pathophysiological changes in

rodent models of NASH [259] and XO inhibitors were also cardioprotective in animals and patients with NAFLD/NASH or other metabolic disease [260]. Finally, neuroinflammatory processes through the liver-brain axis may come into play in mice with NASH, again involving ROS formation (e.g. via NOX2) [261], which may affect neuronal stress hormone signaling and thereby affect cardiovascular function as shown for animals and humans [262]. Of note, the above-mentioned ROS sources can activate each other in a crosstalk fashion and are recognized mediators of IRI and heart failure [263, 264].

Endothelial function was reduced and carotid artery intima-media thickness was increased, indicating higher CVD risk in patients with NAFLD or NASH [265, 266]. Importantly, sNox2-dp and isoprostane levels in patients with NASH also correlated with peripheral endothelial dysfunction measured by flow-mediated dilatation (FMD); these were corrected by administration of polyphenol-rich dark chocolate [267]. 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, mitochondrial ROS formation, NOX1 and NOX2 as well as vascular inflammation in peripheral vessels [242]. Taken together, these data support and explain the higher risk of MI associated with NASH [5] and the higher CVD risk of patients with NAFLD [268, 269].

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, supporting oxidative stress as a central pathophysiological mechanism in NASH [238]. 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 [270]. The flavonoid silibinin improved adverse effects of NASH on the liver and heart in a mouse model (methionine/choline-deficient diet) [271]. Similarly, resveratrol ameliorated all adverse features of NAFLD in mice [272] and also prevented endothelial dysfunction and cardiac oxidative stress in atherosclerotic mice [273].

In NAFLD and NASH models cardioprotective, anti-inflammatory and antioxidant effects have been shown for incretin-based therapies (glucagon-like peptide-1 [GLP-1] mimetics and dipeptidyl peptidase-4 [DPP-4] inhibitors) by animal studies [274-276]. Although these preclinical studies focused mainly on aspects of hepatocyte damage and steatosis they also revealed synergistic effects of GLP-1 administration on liver inflammation and systemic atherosclerosis [277]. Effects of DPP-4 inhibitor (gliptin) therapy on NAFLD/NASH associated oxidative and inflammatory complications in the liver and cardiovascular tissue were demonstrated using a NASH mouse model (methionine/choline-deficient diet) [242]. 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 in cultured human aortic endothelial cells [278]. Alternatively, higher GLP-1 levels may contribute to AMPK activation that controls macrophage polarization and antioxidant defense in animals and humans [239, 279]. The indirect antioxidant effects of incretin-based therapies are further supported by reports of reduced oxidative stress markers in models of atherosclerosis [280, 281], sepsis [279, 282] and cardiac IRI [283].

Antidiabetic SGLT2 inhibitors are currently under consideration for the therapy of NAFLD/NASH [284]. Empagliflozin improved markers of liver fibrosis and steatosis in NAFLD patients with and without T2DM [285, 286]. The drug also ameliorates the phenotype of NASH (fibrosis and steatosis) in mice [287]. Importantly, empagliflozin was shown to possess cardioprotective effects by decreasing the cardiovascular mortality in larger scale studies in T2DM patients [288], which was mechanistically supported by potent antioxidant and anti-inflammatory effects of the drug in rodent models of type 1 and type 2 DM [289, 290]. These mechanistic considerations on the cardio-metabolic-renal benefits of SGLT2 inhibition have been reviewed in detail [291].

In conclusion, NAFLD and NASH are associated with a higher burden of oxidative stress within the liver and heart derived from cytosolic and mitochondrial sources (Figure 2). NAFLD and NASH share similarities in their pathomechanisms with DM and the metabolic syndrome, including dysregulated lipid metabolism, mild hyperglycemia, an inflammatory phenotype and progression of atherosclerosis. These adverse features of NAFLD and NASH

explain the aggravated susceptibility to myocardial IRI 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 IHD, antioxidant treatment regimens display beneficial cardioprotective effects in preclinical models or in patients with NAFLD and NASH.

7. Conclusions/Mechanistic Implications and Future Perspectives

The primary major sources of ROS in IRI (e.g. during MI) are the mitochondria and NOXs, whereas secondary sources are XO and uncoupled NOS [264]. The contribution of NOXs was supported by protective effects of the inhibitor apocynin [292], which also displayed protection in the comorbidities we have discussed. Mitochondrial ROS play a dual role; ROS are undoubtedly detrimental in chronic cardiometabolic disorders but regulated mitochondrial redox signaling may play a critical role in cardioprotective pathways (e.g. blockade of the mitochondrial ATP-sensitive potassium channel glibenclamide or 5-hydroxydecanoate increased infarct size and prevented the protective effects of ischemic preconditioning) [293, 294]. Also the inhibition of PKC can induce adverse or protective effects by suppression of preconditioning [295], 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.

The concept of redox crosstalk between different sources of ROS has been proposed [296-299], and may help to explain the impact of the various comorbidities on MI or cardiovascular death (**Figure 3**). Based on this concept, comorbidities such as arterial hypertension, DM, hyperlipidemia 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 IRI 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 IRI [300]. Also altered endoplasmic reticulum (ER) function and accumulation of misfolded proteins is a common hallmark of most cardiovascular comorbidities such as DM, obesity and NAFLD/NASH [301-303]. Cardiac hypoxia and hypertrophy are linked to the induction of protein misfolding and ER stress leading to HF. ER-mediated ROS formation and apoptotic signaling are central pathomechanisms counterbalanced by protective processes such as the unfolded protein response (UPR) [301]. Mammalian target of rapamycin (mTOR) signaling also plays an important role for ER stress as well as protective pathways [302], a pathway that largely regulates autophagy and protein quality control in cardiometabolic diseases [303].

Oxidative stress is an attractive target for novel therapies, as it represents the common pathway through which different CVD comorbidities and risk factors exert their deleterious cardiovascular effects **(Figure 2)**. 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 IRI. More mechanistic studies are necessary to characterize the sequences of events and to identify components that could be specifically 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. In designing further studies, particularly with antioxidants, we should be mindful of some caveats whch require careful consideration since there are discrepancies between outcomes in laboratory and clinical studies. The reasons for divergences 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; the complexities of multiple-morbidity where several cardiometabolic conditions are present simultaneously; and co-existing drug treatments (some of which may have inherent antioxidant activity) [304, 305]. These difficulties render the demonstration of

improved outcomes from antioxidant treatment in cardiovascular comorbidities a challenging endeavor.

Finally, the concept of oxidative stress as a primary target for attenuation of acute IRI, explored for four decades, has waned considerably in appeal; it is now well-established that prompt reperfusion [e.g. by primary percutaneous coronary intervention (PCI)] is the only way to save myocardial tissue in patients with MI [306]. However, therapeutic approaches that target redox signaling or oxidative stress may hold greater promise to prevent the chronic processes underlying HF development [307], as there is good evidence for an important role of oxidative stress in the pathophysiology of HF of various etiologies [308-311]. NOXs represent important sources of ROS in HF and contribute to cardiomyocyte hypertrophy, atrial fibrillation, interstitial fibrosis, and post-MI remodelling by modulation of matrix metalloproteinase activity (an important drug target for both acute cardioprotection and HF) [111, 312] and finally to myocyte death [205]. This topic is extensively covered by another review article in the same Special Issue [264]. Importantly, we should always keep in mind that cell culture and animal models have limitations and cannot fully reflect the clinical situation as outlined previously [313, 314].

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References

[1] Diseases, G. B. D.; Injuries, C. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* **396:**1204-1222; 2020.
[2] Diederichs, C.; Berger, K.; Bartels, D. B. The measurement of multiple chronic diseases--a systematic review on existing multimorbidity indices. *J Gerontol A Biol Sci Med Sci* **66:**301-311; 2011.
[3] Tran, J.; Norton, R.; Conrad, N.; Rahimian, F.; Canoy, D.; Nazarzadeh, M.; Rahimi, K. Patterns and temporal trends of comorbidity among adult patients with incident cardiovascular disease in the UK between 2000 and 2014: A population-based cohort study. *PLoS Med* **15:**e1002513; 2018.
[4] Bozkurt, B.; Aguilar, D.; Deswal, A.; Dunbar, S. B.; Francis, G. S.; Horwich, T.; Jessup, M.; Kosiborod, M.; Pritchett, A. M.; Ramasubbu, K.; Rosendorff, C.; Yancy, C.; American Heart Association Heart, F.; Transplantation Committee of the Council on Clinical, C.; Council on Cardiovascular, S.; Anesthesia; Council on, C.; Stroke, N.; Council on, H.; Council on, Q.; Outcomes, R. Contributory Risk and Management of Comorbidities of Hypertension, Obesity, Diabetes Mellitus, Hyperlipidemia, and Metabolic Syndrome in Chronic Heart Failure: A Scientific Statement From the American Heart Association. *Circulation* **134:**e535-e578; 2016.
[5] Ghoneim, S.; Dhorepatil, A.; Shah, A. R.; Ram, G.; Ahmad, S.; Kim, C.; Asaad, I. Non-alcoholic steatohepatitis and the risk of myocardial infarction: A population-based national study. *World J Hepatol* **12:**378-388; 2020.
[6] Obokata, M.; Reddy, Y. N. V.; Pislaru, S. V.; Melenovsky, V.; Borlaug, B. A. Evidence Supporting the Existence of a Distinct Obese Phenotype of Heart Failure With Preserved Ejection Fraction. *Circulation* **136:**6-19; 2017.
[7] Pechanova, O.; Varga, Z. V.; Cebova, M.; Giricz, Z.; Pacher, P.; Ferdinandy, P. Cardiac NO signalling in the metabolic syndrome. *Br J Pharmacol* **172:**1415-1433; 2015.
[8] Koncsos, G.; Varga, Z. V.; Baranyai, T.; Boengler, K.; Rohrbach, S.; Li, L.; Schluter, K. D.; Schreckenberg, R.; Radovits, T.; Olah, A.; Matyas, C.; Lux, A.; Al-Khrasani, M.; Komlodi, T.; Bukosza, N.; Mathe, D.; Deres, L.; Bartekova, M.; Rajtik, T.; Adameova, A.; Szigeti, K.; Hamar, P.; Helyes, Z.; Tretter, L.; Pacher, P.; Merkely, B.; Giricz, Z.; Schulz, R.; Ferdinandy, P. Diastolic dysfunction in prediabetic male rats: Role of mitochondrial oxidative stress. *Am J Physiol Heart Circ Physiol* **311:**H927-H943; 2016.
[9] Varga, Z. V.; Giricz, Z.; Liaudet, L.; Hasko, G.; Ferdinandy, P.; Pacher, P. Interplay of oxidative, nitrosative/nitrative stress, inflammation, cell death and autophagy in diabetic cardiomyopathy. *Biochim Biophys Acta* **1852:**232-242; 2015.
[10] Varga, Z. V.; Kupai, K.; Szucs, G.; Gaspar, R.; Paloczi, J.; Farago, N.; Zvara, A.; Puskas, L. G.; Razga, Z.; Tiszlavicz, L.; Bencsik, P.; Gorbe, A.; Csonka, C.; Ferdinandy, P.; Csont, T. MicroRNA-25-dependent up-regulation of NADPH oxidase 4 (NOX4) mediates hypercholesterolemia-induced oxidative/nitrative stress and subsequent dysfunction in the heart. *J Mol Cell Cardiol* **62:**111-121; 2013.
[11] Kenchaiah, S.; Evans, J. C.; Levy, D.; Wilson, P. W.; Benjamin, E. J.; Larson, M. G.; Kannel, W. B.; Vasan, R. S. Obesity and the risk of heart failure. *N Engl J Med* **347:**305-313; 2002.
[12] Ferdinandy, P.; Hausenloy, D. J.; Heusch, G.; Baxter, G. F.; Schulz, R. Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion

injury and cardioprotection by preconditioning, postconditioning, and remote conditioning. *Pharmacol Rev* **66:**1142-1174; 2014.
[13] Pagliaro, P.; Penna, C. Redox signalling and cardioprotection: translatability and mechanism. *Br J Pharmacol* **172:**1974-1995; 2015.
[14] WHO GLOBAL STATUS REPORT on noncommunicable diseases 2014. *https://www.who.int/nmh/publications/ncd-status-report-2014/en/*.
[15] Collaborators, G. B. D. R. F. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* **396:**1223-1249; 2020.
[16] Abel, E. D.; Litwin, S. E.; Sweeney, G. Cardiac remodeling in obesity. *Physiol Rev* **88:**389-419; 2008.
[17] Christoffersen, C.; Bollano, E.; Lindegaard, M. L.; Bartels, E. D.; Goetze, J. P.; Andersen, C. B.; Nielsen, L. B. Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology* **144:**3483-3490; 2003.
[18] Verreth, W.; De Keyzer, D.; Pelat, M.; Verhamme, P.; Ganame, J.; Bielicki, J. K.; Mertens, A.; Quarck, R.; Benhabiles, N.; Marguerie, G.; Mackness, B.; Mackness, M.; Ninio, E.; Herregods, M. C.; Balligand, J. L.; Holvoet, P. Weight-loss-associated induction of peroxisome proliferator-activated receptor-alpha and peroxisome proliferator-activated receptor-gamma correlate with reduced atherosclerosis and improved cardiovascular function in obese insulin-resistant mice. *Circulation* **110:**3259-3269; 2004.
[19] Jonassen, A. K.; Sack, M. N.; Mjos, O. D.; Yellon, D. M. Myocardial protection by insulin at reperfusion requires early administration and is mediated via Akt and p70s6 kinase cell-survival signaling. *Circ Res* **89:**1191-1198; 2001.
[20] Lopaschuk, G. D.; Spafford, M. A.; Davies, N. J.; Wall, S. R. Glucose and palmitate oxidation in isolated working rat hearts reperfused after a period of transient global ischemia. *Circ Res* **66:**546-553; 1990.
[21] du Toit, E. F.; Smith, W.; Muller, C.; Strijdom, H.; Stouthammer, B.; Woodiwiss, A. J.; Norton, G. R.; Lochner, A. Myocardial susceptibility to ischemic-reperfusion injury in a prediabetic model of dietary-induced obesity. *Am J Physiol Heart Circ Physiol* **294:**H2336-2343; 2008.
[22] Webster, I.; Salie, R.; Marais, E.; Fan, W. J.; Maarman, G.; Huisamen, B.; Lochner, A. Myocardial susceptibility to ischaemia/reperfusion in obesity: a re-evaluation of the effects of age. *BMC Physiol* **17:**3; 2017.
[23] Gramlich, Y.; Daiber, A.; Buschmann, K.; Oelze, M.; Vahl, C. F.; Munzel, T.; Hink, U. Oxidative Stress in Cardiac Tissue of Patients Undergoing Coronary Artery Bypass Graft Surgery: The Effects of Overweight and Obesity. *Oxid Med Cell Longev* **2018:**6598326; 2018.
[24] Niemann, B.; Chen, Y.; Teschner, M.; Li, L.; Silber, R. E.; Rohrbach, S. Obesity induces signs of premature cardiac aging in younger patients: the role of mitochondria. *J Am Coll Cardiol* **57:**577-585; 2011.
[25] Duncan, J. G.; Fong, J. L.; Medeiros, D. M.; Finck, B. N.; Kelly, D. P. Insulin-resistant heart exhibits a mitochondrial biogenic response driven by the peroxisome proliferator-activated receptor-alpha/PGC-1alpha gene regulatory pathway. *Circulation* **115:**909-917; 2007.
[26] Boudina, S.; Sena, S.; O'Neill, B. T.; Tathireddy, P.; Young, M. E.; Abel, E. D. Reduced mitochondrial oxidative capacity and increased mitochondrial uncoupling impair myocardial energetics in obesity. *Circulation* **112:**2686-2695; 2005.
[27] Giorgio, M.; Migliaccio, E.; Orsini, F.; Paolucci, D.; Moroni, M.; Contursi, C.; Pelliccia, G.; Luzi, L.; Minucci, S.; Marcaccio, M.; Pinton, P.; Rizzuto, R.; Bernardi,

P.; Paolucci, F.; Pelicci, P. G. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell* **122:**221-233; 2005.
[28] Natalicchio, A.; De Stefano, F.; Perrini, S.; Laviola, L.; Cignarelli, A.; Caccioppoli, C.; Quagliara, A.; Melchiorre, M.; Leonardini, A.; Conserva, A.; Giorgino, F. Involvement of the p66Shc protein in glucose transport regulation in skeletal muscle myoblasts. *Am J Physiol Endocrinol Metab* **296:**E228-237; 2009.
[29] Tomilov, A. A.; Ramsey, J. J.; Hagopian, K.; Giorgio, M.; Kim, K. M.; Lam, A.; Migliaccio, E.; Lloyd, K. C.; Berniakovich, I.; Prolla, T. A.; Pelicci, P.; Cortopassi, G. A. The Shc locus regulates insulin signaling and adiposity in mammals. *Aging Cell* **10:**55-65; 2011.
[30] Napoli, C.; Martin-Padura, I.; de Nigris, F.; Giorgio, M.; Mansueto, G.; Somma, P.; Condorelli, M.; Sica, G.; De Rosa, G.; Pelicci, P. Deletion of the p66Shc longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet. *Proc Natl Acad Sci U S A* **100:**2112-2116; 2003.
[31] De Marchi, E.; Baldassari, F.; Bononi, A.; Wieckowski, M. R.; Pinton, P. Oxidative stress in cardiovascular diseases and obesity: role of p66Shc and protein kinase C. *Oxid Med Cell Longev* **2013:**564961; 2013.
[32] Serpillon, S.; Floyd, B. C.; Gupte, R. S.; George, S.; Kozicky, M.; Neito, V.; Recchia, F.; Stanley, W.; Wolin, M. S.; Gupte, S. A. Superoxide production by NAD(P)H oxidase and mitochondria is increased in genetically obese and hyperglycemic rat heart and aorta before the development of cardiac dysfunction. The role of glucose-6-phosphate dehydrogenase-derived NADPH. *Am J Physiol Heart Circ Physiol* **297:**H153-162; 2009.
[33] Niemann, B.; Rohrbach, S.; Miller, M. R.; Newby, D. E.; Fuster, V.; Kovacic, J. C. Oxidative Stress and Cardiovascular Risk: Obesity, Diabetes, Smoking, and Pollution: Part 3 of a 3-Part Series. *J Am Coll Cardiol* **70:**230-251; 2017.
[34] Joseph, L. C.; Barca, E.; Subramanyam, P.; Komrowski, M.; Pajvani, U.; Colecraft, H. M.; Hirano, M.; Morrow, J. P. Inhibition of NAPDH Oxidase 2 (NOX2) Prevents Oxidative Stress and Mitochondrial Abnormalities Caused by Saturated Fat in Cardiomyocytes. *PLoS One* **11:**e0145750; 2016.
[35] D'Souza, K.; Nzirorera, C.; Kienesberger, P. C. Lipid metabolism and signaling in cardiac lipotoxicity. *Biochim Biophys Acta* **1861:**1513-1524; 2016.
[36] Dewald, O.; Sharma, S.; Adrogue, J.; Salazar, R.; Duerr, G. D.; Crapo, J. D.; Entman, M. L.; Taegtmeyer, H. Downregulation of peroxisome proliferator-activated receptor-alpha gene expression in a mouse model of ischemic cardiomyopathy is dependent on reactive oxygen species and prevents lipotoxicity. *Circulation* **112:**407-415; 2005.
[37] Pchejetski, D.; Kunduzova, O.; Dayon, A.; Calise, D.; Seguelas, M. H.; Leducq, N.; Seif, I.; Parini, A.; Cuvillier, O. Oxidative stress-dependent sphingosine kinase-1 inhibition mediates monoamine oxidase A-associated cardiac cell apoptosis. *Circ Res* **100:**41-49; 2007.
[38] Deshwal, S.; Forkink, M.; Hu, C. H.; Buonincontri, G.; Antonucci, S.; Di Sante, M.; Murphy, M. P.; Paolocci, N.; Mochly-Rosen, D.; Krieg, T.; Di Lisa, F.; Kaludercic, N. Monoamine oxidase-dependent endoplasmic reticulum-mitochondria dysfunction and mast cell degranulation lead to adverse cardiac remodeling in diabetes. *Cell Death Differ* **25:**1671-1685; 2018.
[39] Nagy, C. T.; Koncsos, G.; Varga, Z. V.; Baranyai, T.; Tuza, S.; Kassai, F.; Ernyey, A. J.; Gyertyan, I.; Kiraly, K.; Olah, A.; Radovits, T.; Merkely, B.; Bukosza,

N.; Szenasi, G.; Hamar, P.; Mathe, D.; Szigeti, K.; Pelyhe, C.; Jelemensky, M.; Onodi, Z.; Helyes, Z.; Schulz, R.; Giricz, Z.; Ferdinandy, P. Selegiline reduces adiposity induced by high-fat, high-sucrose diet in male rats. *Br J Pharmacol* **175:**3713-3726; 2018.

[40] Jia, G.; Habibi, J.; Bostick, B. P.; Ma, L.; DeMarco, V. G.; Aroor, A. R.; Hayden, M. R.; Whaley-Connell, A. T.; Sowers, J. R. Uric acid promotes left ventricular diastolic dysfunction in mice fed a Western diet. *Hypertension* **65:**531-539; 2015.

[41] Kaludercic, N.; Di Lisa, F. The energetic cost of NNT-dependent ROS removal. *J Biol Chem* **295:**16217-16218; 2020.

[42] Smith, C. D.; Schmidt, C. A.; Lin, C. T.; Fisher-Wellman, K. H.; Neufer, P. D. Flux through mitochondrial redox circuits linked to nicotinamide nucleotide transhydrogenase generates counterbalance changes in energy expenditure. *J Biol Chem*; 2020.

[43] Nickel, A. G.; von Hardenberg, A.; Hohl, M.; Loffler, J. R.; Kohlhaas, M.; Becker, J.; Reil, J. C.; Kazakov, A.; Bonnekoh, J.; Stadelmaier, M.; Puhl, S. L.; Wagner, M.; Bogeski, I.; Cortassa, S.; Kappl, R.; Pasieka, B.; Lafontaine, M.; Lancaster, C. R.; Blacker, T. S.; Hall, A. R.; Duchen, M. R.; Kastner, L.; Lipp, P.; Zeller, T.; Muller, C.; Knopp, A.; Laufs, U.; Bohm, M.; Hoth, M.; Maack, C. Reversal of Mitochondrial Transhydrogenase Causes Oxidative Stress in Heart Failure. *Cell Metab* **22:**472-484; 2015.

[44] Ronchi, J. A.; Figueira, T. R.; Ravagnani, F. G.; Oliveira, H. C.; Vercesi, A. E.; Castilho, R. F. A spontaneous mutation in the nicotinamide nucleotide transhydrogenase gene of C57BL/6J mice results in mitochondrial redox abnormalities. *Free Radic Biol Med* **63:**446-456; 2013.

[45] Fan, C.; Zirpoli, H.; Qi, K. n-3 fatty acids modulate adipose tissue inflammation and oxidative stress. *Curr Opin Clin Nutr Metab Care* **16:**124-132; 2013.

[46] Han, C. Y.; Umemoto, T.; Omer, M.; Den Hartigh, L. J.; Chiba, T.; LeBoeuf, R.; Buller, C. L.; Sweet, I. R.; Pennathur, S.; Abel, E. D.; Chait, A. NADPH oxidase-derived reactive oxygen species increases expression of monocyte chemotactic factor genes in cultured adipocytes. *J Biol Chem* **287:**10379-10393; 2012.

[47] Kusunoki, C.; Yang, L.; Yoshizaki, T.; Nakagawa, F.; Ishikado, A.; Kondo, M.; Morino, K.; Sekine, O.; Ugi, S.; Nishio, Y.; Kashiwagi, A.; Maegawa, H. Omega-3 polyunsaturated fatty acid has an anti-oxidant effect via the Nrf-2/HO-1 pathway in 3T3-L1 adipocytes. *Biochem Biophys Res Commun* **430:**225-230; 2013.

[48] Schmidt, S.; Stahl, F.; Mutz, K. O.; Scheper, T.; Hahn, A.; Schuchardt, J. P. Transcriptome-based identification of antioxidative gene expression after fish oil supplementation in normo- and dyslipidemic men. *Nutr Metab (Lond)* **9:**45; 2012.

[49] Agouni, A.; Lagrue-Lak-Hal, A. H.; Mostefai, H. A.; Tesse, A.; Mulder, P.; Rouet, P.; Desmoulin, F.; Heymes, C.; Martinez, M. C.; Andriantsitohaina, R. Red wine polyphenols prevent metabolic and cardiovascular alterations associated with obesity in Zucker fatty rats (Fa/Fa). *PLoS One* **4:**e5557; 2009.

[50] Rivera, L.; Moron, R.; Zarzuelo, A.; Galisteo, M. Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochem Pharmacol* **77:**1053-1063; 2009.

[51] Andriantsitohaina, R.; Auger, C.; Chataigneau, T.; Etienne-Selloum, N.; Li, H.; Martinez, M. C.; Schini-Kerth, V. B.; Laher, I. Molecular mechanisms of the cardiovascular protective effects of polyphenols. *Br J Nutr* **108:**1532-1549; 2012.

[52] Most, J.; Tosti, V.; Redman, L. M.; Fontana, L. Calorie restriction in humans: An update. *Ageing Res Rev* **39:**36-45; 2017.

[53] Canto, C.; Auwerx, J. Targeting sirtuin 1 to improve metabolism: all you need is NAD(+)? *Pharmacol Rev* **64:**166-187; 2012.
[54] Brunet, A.; Sweeney, L. B.; Sturgill, J. F.; Chua, K. F.; Greer, P. L.; Lin, Y.; Tran, H.; Ross, S. E.; Mostoslavsky, R.; Cohen, H. Y.; Hu, L. S.; Cheng, H. L.; Jedrychowski, M. P.; Gygi, S. P.; Sinclair, D. A.; Alt, F. W.; Greenberg, M. E. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* **303:**2011-2015; 2004.
[55] Perrot, V.; Rechler, M. M. Characterization of insulin inhibition of transactivation by a C-terminal fragment of the forkhead transcription factor Foxo1 in rat hepatoma cells. *J Biol Chem* **278:**26111-26119; 2003.
[56] Gureev, A. P.; Shaforostova, E. A.; Popov, V. N. Regulation of Mitochondrial Biogenesis as a Way for Active Longevity: Interaction Between the Nrf2 and PGC-1alpha Signaling Pathways. *Front Genet* **10:**435; 2019.
[57] Shinmura, K.; Tamaki, K.; Saito, K.; Nakano, Y.; Tobe, T.; Bolli, R. Cardioprotective effects of short-term caloric restriction are mediated by adiponectin via activation of AMP-activated protein kinase. *Circulation* **116:**2809-2817; 2007.
[58] Waldman, M.; Cohen, K.; Yadin, D.; Nudelman, V.; Gorfil, D.; Laniado-Schwartzman, M.; Kornwoski, R.; Aravot, D.; Abraham, N. G.; Arad, M.; Hochhauser, E. Regulation of diabetic cardiomyopathy by caloric restriction is mediated by intracellular signaling pathways involving 'SIRT1 and PGC-1alpha'. *Cardiovasc Diabetol* **17:**111; 2018.
[59] Rodriguez-Bies, E.; Tung, B. T.; Navas, P.; Lopez-Lluch, G. Resveratrol primes the effects of physical activity in old mice. *Br J Nutr* **116:**979-988; 2016.
[60] Li, L.; Meng, F.; Li, N.; Zhang, L.; Wang, J.; Wang, H.; Li, D.; Zhang, X.; Dong, P.; Chen, Y. Exercise training prevents the attenuation of anesthetic pre-conditioning-mediated cardioprotection in diet-induced obese rats. *Acta Anaesthesiol Scand* **59:**85-97; 2015.
[61] Tunapong, W.; Apaijai, N.; Yasom, S.; Tanajak, P.; Wanchai, K.; Chunchai, T.; Kerdphoo, S.; Eaimworawuthikul, S.; Thiennimitr, P.; Pongchaidecha, A.; Lungkaphin, A.; Pratchayasakul, W.; Chattipakorn, S. C.; Chattipakorn, N. Chronic treatment with prebiotics, probiotics and synbiotics attenuated cardiac dysfunction by improving cardiac mitochondrial dysfunction in male obese insulin-resistant rats. *Eur J Nutr* **57:**2091-2104; 2018.
[62] Sivasinprasasn, S.; Tanajak, P.; Pongkan, W.; Pratchayasakul, W.; Chattipakorn, S. C.; Chattipakorn, N. DPP-4 Inhibitor and Estrogen Share Similar Efficacy Against Cardiac Ischemic-Reperfusion Injury in Obese-Insulin Resistant and Estrogen-Deprived Female Rats. *Sci Rep* **7:**44306; 2017.
[63] Tanajak, P.; Sa-Nguanmoo, P.; Sivasinprasasn, S.; Thummasorn, S.; Siri-Angkul, N.; Chattipakorn, S. C.; Chattipakorn, N. Cardioprotection of dapagliflozin and vildagliptin in rats with cardiac ischemia-reperfusion injury. *J Endocrinol* **236:**69-84; 2018.
[64] Andreadou, I.; Efentakis, P.; Balafas, E.; Togliatto, G.; Davos, C. H.; Varela, A.; Dimitriou, C. A.; Nikolaou, P. E.; Maratou, E.; Lambadiari, V.; Ikonomidis, I.; Kostomitsopoulos, N.; Brizzi, M. F.; Dimitriadis, G.; Iliodromitis, E. K. Empagliflozin Limits Myocardial Infarction in Vivo and Cell Death in Vitro: Role of STAT3, Mitochondria, and Redox Aspects. *Front Physiol* **8:**1077; 2017.
[65] Kondo, K.; Shibata, R.; Unno, K.; Shimano, M.; Ishii, M.; Kito, T.; Shintani, S.; Walsh, K.; Ouchi, N.; Murohara, T. Impact of a single intracoronary administration of adiponectin on myocardial ischemia/reperfusion injury in a pig model. *Circ Cardiovasc Interv* **3:**166-173; 2010.

[66] Marino, A.; Sakamoto, T.; Tang, X. H.; Gudas, L. J.; Levi, R. A Retinoic Acid beta2-Receptor Agonist Exerts Cardioprotective Effects. *J Pharmacol Exp Ther* **366:**314-321; 2018.
[67] Nduhirabandi, F.; Du Toit, E. F.; Blackhurst, D.; Marais, D.; Lochner, A. Chronic melatonin consumption prevents obesity-related metabolic abnormalities and protects the heart against myocardial ischemia and reperfusion injury in a prediabetic model of diet-induced obesity. *J Pineal Res* **50:**171-182; 2011.
[68] Gutierrez-Tenorio, J.; Marin-Royo, G.; Martinez-Martinez, E.; Martin, R.; Miana, M.; Lopez-Andres, N.; Jurado-Lopez, R.; Gallardo, I.; Luaces, M.; San Roman, J. A.; Gonzalez-Amor, M.; Salaices, M.; Nieto, M. L.; Cachofeiro, V. The role of oxidative stress in the crosstalk between leptin and mineralocorticoid receptor in the cardiac fibrosis associated with obesity. *Sci Rep* **7:**16802; 2017.
[69] Fink, B. D.; Herlein, J. A.; Guo, D. F.; Kulkarni, C.; Weidemann, B. J.; Yu, L.; Grobe, J. L.; Rahmouni, K.; Kerns, R. J.; Sivitz, W. I. A mitochondrial-targeted coenzyme q analog prevents weight gain and ameliorates hepatic dysfunction in high-fat-fed mice. *J Pharmacol Exp Ther* **351:**699-708; 2014.
[70] Feillet-Coudray, C.; Fouret, G.; Ebabe Elle, R.; Rieusset, J.; Bonafos, B.; Chabi, B.; Crouzier, D.; Zarkovic, K.; Zarkovic, N.; Ramos, J.; Badia, E.; Murphy, M. P.; Cristol, J. P.; Coudray, C. The mitochondrial-targeted antioxidant MitoQ ameliorates metabolic syndrome features in obesogenic diet-fed rats better than Apocynin or Allopurinol. *Free Radic Res* **48:**1232-1246; 2014.
[71] Global Health Observatory (GHO) data – Mean Cholesterol. *https://www.who.int/gho/ncd/risk_factors/cholesterol_mean_text/en/*.
[72] Cardona-Sanclemente, L. E.; Born, G. V. Effect of inhibition of nitric oxide synthesis on the uptake of LDL and fibrinogen by arterial walls and other organs of the rat. *Br J Pharmacol* **114:**1490-1494; 1995.
[73] Pirro, M.; Schillaci, G.; Mannarino, M. R.; Savarese, G.; Vaudo, G.; Siepi, D.; Paltriccia, R.; Mannarino, E. Effects of rosuvastatin on 3-nitrotyrosine and aortic stiffness in hypercholesterolemia. *Nutr Metab Cardiovasc Dis* **17:**436-441; 2007.
[74] Mollazadeh, H.; Carbone, F.; Montecucco, F.; Pirro, M.; Sahebkar, A. Oxidative burden in familial hypercholesterolemia. *J Cell Physiol* **233:**5716-5725; 2018.
[75] Pritchard, K. A., Jr.; Groszek, L.; Smalley, D. M.; Sessa, W. C.; Wu, M.; Villalon, P.; Wolin, M. S.; Stemerman, M. B. Native low-density lipoprotein increases endothelial cell nitric oxide synthase generation of superoxide anion. *Circ Res* **77:**510-518; 1995.
[76] Li, H.; Forstermann, U. Uncoupling of endothelial NO synthase in atherosclerosis and vascular disease. *Curr Opin Pharmacol* **13:**161-167; 2013.
[77] Feron, O.; Dessy, C.; Moniotte, S.; Desager, J. P.; Balligand, J. L. Hypercholesterolemia decreases nitric oxide production by promoting the interaction of caveolin and endothelial nitric oxide synthase. *J Clin Invest* **103:**897-905; 1999.
[78] Stepp, D. W.; Ou, J.; Ackerman, A. W.; Welak, S.; Klick, D.; Pritchard, K. A., Jr. Native LDL and minimally oxidized LDL differentially regulate superoxide anion in vascular endothelium in situ. *Am J Physiol Heart Circ Physiol* **283:**H750-759; 2002.
[79] Chavakis, E.; Dernbach, E.; Hermann, C.; Mondorf, U. F.; Zeiher, A. M.; Dimmeler, S. Oxidized LDL inhibits vascular endothelial growth factor-induced endothelial cell migration by an inhibitory effect on the Akt/endothelial nitric oxide synthase pathway. *Circulation* **103:**2102-2107; 2001.

[80] Steffen, Y.; Jung, T.; Klotz, L. O.; Schewe, T.; Grune, T.; Sies, H. Protein modification elicited by oxidized low-density lipoprotein (LDL) in endothelial cells: protection by (-)-epicatechin. *Free Radic Biol Med* **42:**955-970; 2007.
[81] Heitzer, T.; Yla-Herttuala, S.; Luoma, J.; Kurz, S.; Munzel, T.; Just, H.; Olschewski, M.; Drexler, H. Cigarette smoking potentiates endothelial dysfunction of forearm resistance vessels in patients with hypercholesterolemia. Role of oxidized LDL. *Circulation* **93:**1346-1353; 1996.
[82] Kubes, P.; Suzuki, M.; Granger, D. N. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci U S A* **88:**4651-4655; 1991.
[83] Khan, B. V.; Harrison, D. G.; Olbrych, M. T.; Alexander, R. W.; Medford, R. M. Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc Natl Acad Sci U S A* **93:**9114-9119; 1996.
[84] Bochkov, V. N.; Oskolkova, O. V.; Birukov, K. G.; Levonen, A. L.; Binder, C. J.; Stockl, J. Generation and biological activities of oxidized phospholipids. *Antioxid Redox Signal* **12:**1009-1059; 2010.
[85] Binder, C. J.; Papac-Milicevic, N.; Witztum, J. L. Innate sensing of oxidation-specific epitopes in health and disease. *Nat Rev Immunol* **16:**485-497; 2016.
[86] Schluter, K. D.; Wolf, A.; Weber, M.; Schreckenberg, R.; Schulz, R. Oxidized low-density lipoprotein (oxLDL) affects load-free cell shortening of cardiomyocytes in a proprotein convertase subtilisin/kexin 9 (PCSK9)-dependent way. *Basic Res Cardiol* **112:**63; 2017.
[87] Wolf, A.; Kutsche, H. S.; Schreckenberg, R.; Weber, M.; Li, L.; Rohrbach, S.; Schulz, R.; Schluter, K. D. Autocrine effects of PCSK9 on cardiomyocytes. *Basic Res Cardiol* **115:**65; 2020.
[88] Nickenig, G.; Baumer, A. T.; Temur, Y.; Kebben, D.; Jockenhovel, F.; Bohm, M. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation* **100:**2131-2134; 1999.
[89] Haendeler, J.; Eckers, A.; Lukosz, M.; Unfried, K.; Altschmied, J. Endothelial NADPH oxidase 2: when does it matter in atherosclerosis? *Cardiovasc Res* **94:**1-2; 2012.
[90] Ballinger, S. W.; Patterson, C.; Yan, C. N.; Doan, R.; Burow, D. L.; Young, C. G.; Yakes, F. M.; Van Houten, B.; Ballinger, C. A.; Freeman, B. A.; Runge, M. S. Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. *Circ Res* **86:**960-966; 2000.
[91] Osipov, R. M.; Bianchi, C.; Feng, J.; Clements, R. T.; Liu, Y.; Robich, M. P.; Glazer, H. P.; Sodha, N. R.; Sellke, F. W. Effect of hypercholesterolemia on myocardial necrosis and apoptosis in the setting of ischemia-reperfusion. *Circulation* **120:**S22-30; 2009.
[92] Andreadou, I.; Schulz, R.; Badimon, L.; Adameova, A.; Kleinbongard, P.; Lecour, S.; Nikolaou, P. E.; Falcao-Pires, I.; Vilahur, G.; Woudberg, N.; Heusch, G.; Ferdinandy, P. Hyperlipidaemia and cardioprotection: Animal models for translational studies. *Br J Pharmacol*; 2019.
[93] Warnholtz, A.; Mollnau, H.; Heitzer, T.; Kontush, A.; Moller-Bertram, T.; Lavall, D.; Giaid, A.; Beisiegel, U.; Marklund, S. L.; Walter, U.; Meinertz, T.; Munzel, T. Adverse effects of nitroglycerin treatment on endothelial function, vascular nitrotyrosine levels and cGMP-dependent protein kinase activity in hyperlipidemic Watanabe rabbits. *J Am Coll Cardiol* **40:**1356-1363; 2002.

[94] Guzik, T. J.; Sadowski, J.; Guzik, B.; Jopek, A.; Kapelak, B.; Przybylowski, P.; Wierzbicki, K.; Korbut, R.; Harrison, D. G.; Channon, K. M. Coronary artery superoxide production and nox isoform expression in human coronary artery disease. *Arterioscler Thromb Vasc Biol* **26:**333-339; 2006.
[95] Warnholtz, A.; Nickenig, G.; Schulz, E.; Macharzina, R.; Brasen, J. H.; Skatchkov, M.; Heitzer, T.; Stasch, J. P.; Griendling, K. K.; Harrison, D. G.; Bohm, M.; Meinertz, T.; Munzel, T. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin-angiotensin system. *Circulation* **99:**2027-2033; 1999.
[96] Loffredo, L.; Martino, F.; Carnevale, R.; Pignatelli, P.; Catasca, E.; Perri, L.; Calabrese, C. M.; Palumbo, M. M.; Baratta, F.; Del Ben, M.; Angelico, F.; Violi, F. Obesity and hypercholesterolemia are associated with NOX2 generated oxidative stress and arterial dysfunction. *J Pediatr* **161:**1004-1009; 2012.
[97] Loffredo, L.; Pignatelli, P.; Martino, F.; Carnevale, R.; Bartimoccia, S.; Catasca, E.; Colantoni, C.; Zanoni, C.; Perri, L.; Violi, F. Early increase of NOX2-derived oxidative stress in children: relationship with age. *Pediatr Res* **73:**788-793; 2013.
[98] Forstermann, U.; Xia, N.; Li, H. Roles of Vascular Oxidative Stress and Nitric Oxide in the Pathogenesis of Atherosclerosis. *Circ Res* **120:**713-735; 2017.
[99] Hayashi, T.; Esaki, T.; Sumi, D.; Mukherjee, T.; Iguchi, A.; Chaudhuri, G. Modulating role of estradiol on arginase II expression in hyperlipidemic rabbits as an atheroprotective mechanism. *Proc Natl Acad Sci U S A* **103:**10485-10490; 2006.
[100] Stroes, E.; Kastelein, J.; Cosentino, F.; Erkelens, W.; Wever, R.; Koomans, H.; Luscher, T.; Rabelink, T. Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. *J Clin Invest* **99:**41-46; 1997.
[101] Leopold, J. A.; Loscalzo, J. Oxidative risk for atherothrombotic cardiovascular disease. *Free Radic Biol Med* **47:**1673-1706; 2009.
[102] Cheng, F.; Torzewski, M.; Degreif, A.; Rossmann, H.; Canisius, A.; Lackner, K. J. Impact of glutathione peroxidase-1 deficiency on macrophage foam cell formation and proliferation: implications for atherogenesis. *PLoS One* **8:**e72063; 2013.
[103] Nomura, J.; Busso, N.; Ives, A.; Matsui, C.; Tsujimoto, S.; Shirakura, T.; Tamura, M.; Kobayashi, T.; So, A.; Yamanaka, Y. Xanthine oxidase inhibition by febuxostat attenuates experimental atherosclerosis in mice. *Sci Rep* **4:**4554; 2014.
[104] Vaage, J.; Antonelli, M.; Bufi, M.; Irtun, O.; DeBlasi, R. A.; Corbucci, G. G.; Gasparetto, A.; Semb, A. G. Exogenous reactive oxygen species deplete the isolated rat heart of antioxidants. *Free Radic Biol Med* **22:**85-92; 1997.
[105] Ferdinandy, P.; Schulz, R.; Baxter, G. F. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. *Pharmacol Rev* **59:**418-458; 2007.
[106] Iliodromitis, E. K.; Andreadou, I.; Prokovas, E.; Zoga, A.; Farmakis, D.; Fotopoulou, T.; Ioannidis, K.; Paraskevaidis, I. A.; Kremastinos, D. T. Simvastatin in contrast to postconditioning reduces infarct size in hyperlipidemic rabbits: possible role of oxidative/nitrosative stress attenuation. *Basic Res Cardiol* **105:**193-203; 2010.
[107] Andreadou, I.; Farmakis, D.; Prokovas, E.; Sigala, F.; Zoga, A.; Spyridaki, K.; Papalois, A.; Papapetropoulos, A.; Anastasiou-Nana, M.; Kremastinos, D. T.; Iliodromitis, E. K. Short-term statin administration in hypercholesterolaemic rabbits resistant to postconditioning: effects on infarct size, endothelial nitric oxide synthase, and nitro-oxidative stress. *Cardiovasc Res* **94:**501-509; 2012.
[108] Andreadou, I.; Iliodromitis, E. K.; Mikros, E.; Constantinou, M.; Agalias, A.; Magiatis, P.; Skaltsounis, A. L.; Kamber, E.; Tsantili-Kakoulidou, A.; Kremastinos, D.

T. The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits. *J Nutr* **136:**2213-2219; 2006.
[109] Szucs, G.; Bester, D. J.; Kupai, K.; Csont, T.; Csonka, C.; Esterhuyse, A. J.; Ferdinandy, P.; Van Rooyen, J. Dietary red palm oil supplementation decreases infarct size in cholesterol fed rats. *Lipids Health Dis* **10:**103; 2011.
[110] Giricz, Z.; Lalu, M. M.; Csonka, C.; Bencsik, P.; Schulz, R.; Ferdinandy, P. Hyperlipidemia attenuates the infarct size-limiting effect of ischemic preconditioning: role of matrix metalloproteinase-2 inhibition. *J Pharmacol Exp Ther* **316:**154-161; 2006.
[111] Dorman, G.; Cseh, S.; Hajdu, I.; Barna, L.; Konya, D.; Kupai, K.; Kovacs, L.; Ferdinandy, P. Matrix metalloproteinase inhibitors: a critical appraisal of design principles and proposed therapeutic utility. *Drugs* **70:**949-964; 2010.
[112] Song, Y. J.; Zhong, C. B.; Wang, X. B. Heat shock protein 70: A promising therapeutic target for myocardial ischemia-reperfusion injury. *J Cell Physiol* **234:**1190-1207; 2019.
[113] Pantos, C.; Mourouzis, I.; Dimopoulos, A.; Markakis, K.; Panagiotou, M.; Xinaris, C.; Tzeis, S.; Kokkinos, A. D.; Cokkinos, D. V. Enhanced tolerance of the rat myocardium to ischemia and reperfusion injury early after acute myocardial infarction. *Basic Res Cardiol* **102:**327-333; 2007.
[114] Costa, V. M.; Silva, R.; Ferreira, R.; Amado, F.; Carvalho, F.; de Lourdes Bastos, M.; Carvalho, R. A.; Carvalho, M.; Remiao, F. Adrenaline in pro-oxidant conditions elicits intracellular survival pathways in isolated rat cardiomyocytes. *Toxicology* **257:**70-79; 2009.
[115] Csont, T.; Balogh, G.; Csonka, C.; Boros, I.; Horvath, I.; Vigh, L.; Ferdinandy, P. Hyperlipidemia induced by high cholesterol diet inhibits heat shock response in rat hearts. *Biochem Biophys Res Commun* **290:**1535-1538; 2002.
[116] Yadav, H. N.; Singh, M.; Sharma, P. L. Pharmacological inhibition of GSK-3beta produces late phase of cardioprotection in hyperlipidemic rat: possible involvement of HSP 72. *Mol Cell Biochem* **369:**227-233; 2012.
[117] Shyu, K. G.; Lu, M. J.; Chang, H.; Sun, H. Y.; Wang, B. W.; Kuan, P. Carvedilol modulates the expression of hypoxia-inducible factor-1alpha and vascular endothelial growth factor in a rat model of volume-overload heart failure. *J Card Fail* **11:**152-159; 2005.
[118] Lee, S. H.; Wolf, P. L.; Escudero, R.; Deutsch, R.; Jamieson, S. W.; Thistlethwaite, P. A. Early expression of angiogenesis factors in acute myocardial ischemia and infarction. *N Engl J Med* **342:**626-633; 2000.
[119] Li, X.; Zhao, H.; Wu, Y.; Zhang, S.; Zhao, X.; Zhang, Y.; Wang, J.; Wang, J.; Liu, H. Up-regulation of hypoxia-inducible factor-1alpha enhanced the cardioprotective effects of ischemic postconditioning in hyperlipidemic rats. *Acta Biochim Biophys Sin (Shanghai)* **46:**112-118; 2014.
[120] Ruotsalainen, A. K.; Inkala, M.; Partanen, M. E.; Lappalainen, J. P.; Kansanen, E.; Makinen, P. I.; Heinonen, S. E.; Laitinen, H. M.; Heikkila, J.; Vatanen, T.; Horkko, S.; Yamamoto, M.; Yla-Herttuala, S.; Jauhiainen, M.; Levonen, A. L. The absence of macrophage Nrf2 promotes early atherogenesis. *Cardiovasc Res* **98:**107-115; 2013.
[121] Jyrkkanen, H. K.; Kansanen, E.; Inkala, M.; Kivela, A. M.; Hurttila, H.; Heinonen, S. E.; Goldsteins, G.; Jauhiainen, S.; Tiainen, S.; Makkonen, H.; Oskolkova, O.; Afonyushkin, T.; Koistinaho, J.; Yamamoto, M.; Bochkov, V. N.; Yla-Herttuala, S.; Levonen, A. L. Nrf2 regulates antioxidant gene expression evoked by oxidized phospholipids in endothelial cells and murine arteries in vivo. *Circ Res* **103:**e1-9; 2008.

[122] Efentakis, P.; Rizakou, A.; Christodoulou, E.; Chatzianastasiou, A.; Lopez, M. G.; Leon, R.; Balafas, E.; Kadoglou, N. P. E.; Tseti, I.; Skaltsa, H.; Kostomitsopoulos, N.; Iliodromitis, E. K.; Valsami, G.; Andreadou, I. Saffron (Crocus sativus) intake provides nutritional preconditioning against myocardial ischemia-reperfusion injury in Wild Type and ApoE((-/-)) mice: Involvement of Nrf2 activation. *Nutr Metab Cardiovasc Dis* **27:**919-929; 2017.
[123] Andreadou, I.; Iliodromitis, E. K.; Lazou, A.; Gorbe, A.; Giricz, Z.; Schulz, R.; Ferdinandy, P. Effect of hypercholesterolaemia on myocardial function, ischaemia-reperfusion injury and cardioprotection by preconditioning, postconditioning and remote conditioning. *Br J Pharmacol* **174:**1555-1569; 2017.
[124] Mazo, T.; V, D. A.; Zaobornyj, T.; Perez, V.; Gomez, A.; Berg, G.; Barchuk, M.; Ossani, G.; Martinefski, M.; Tripodi, V.; Lago, N.; Gelpi, R. J. High-fat diet abolishes the cardioprotective effects of ischemic postconditioning in murine models despite increased thioredoxin-1 levels. *Mol Cell Biochem* **452:**153-166; 2019.
[125] Penna, C.; Andreadou, I.; Aragno, M.; Beauloye, C.; Bertrand, L.; Lazou, A.; Falcao-Pires, I.; Bell, R.; Zuurbier, C. J.; Pagliaro, P.; Hausenloy, D. J. Effect of hyperglycaemia and diabetes on acute myocardial ischaemia-reperfusion injury and cardioprotection by ischaemic conditioning protocols. *Br J Pharmacol*; 2020.
[126] Eitel, I.; Hintze, S.; de Waha, S.; Fuernau, G.; Lurz, P.; Desch, S.; Schuler, G.; Thiele, H. Prognostic impact of hyperglycemia in nondiabetic and diabetic patients with ST-elevation myocardial infarction: insights from contrast-enhanced magnetic resonance imaging. *Circ Cardiovasc Imaging* **5:**708-718; 2012.
[127] Anker, S. D.; Butler, J.; Filippatos, G.; Khan, M. S.; Marx, N.; Lam, C. S. P.; Schnaidt, S.; Ofstad, A. P.; Brueckmann, M.; Jamal, W.; Bocchi, E.; Ponikowski, P.; Perrone, S. V.; Januzzi, J. L.; Verma, S.; Bohm, M.; Ferreira, J. P.; Pocock, S. J.; Zannad, F.; Packer, M.; Committees, E. M.-R. T.; Investigators. Effect of Empagliflozin on Cardiovascular and Renal Outcomes in Patients With Heart Failure by Baseline Diabetes Status - Results from the EMPEROR-Reduced Trial. *Circulation*; 2020.
[128] Kaludercic, N.; Di Lisa, F. Mitochondrial ROS Formation in the Pathogenesis of Diabetic Cardiomyopathy. *Front Cardiovasc Med* **7:**12; 2020.
[129] Shanmugam, G.; Wang, D.; Gounder, S. S.; Fernandes, J.; Litovsky, S. H.; Whitehead, K.; Radhakrishnan, R. K.; Franklin, S.; Hoidal, J. R.; Kensler, T. W.; Dell'Italia, L.; Darley-Usmar, V.; Abel, E. D.; Jones, D. P.; Ping, P.; Rajasekaran, N. S. Reductive Stress Causes Pathological Cardiac Remodeling and Diastolic Dysfunction. *Antioxid Redox Signal* **32:**1293-1312; 2020.
[130] Thorwald, M. A.; Godoy-Lugo, J. A.; Rodriguez, G. J.; Rodriguez, M. A.; Jamal, M.; Kinoshita, H.; Nakano, D.; Nishiyama, A.; Forman, H. J.; Ortiz, R. M. Nrf2-related gene expression is impaired during a glucose challenge in type II diabetic rat hearts. *Free Radic Biol Med* **130:**306-317; 2019.
[131] Marfella, R.; Quagliaro, L.; Nappo, F.; Ceriello, A.; Giugliano, D. Acute hyperglycemia induces an oxidative stress in healthy subjects. *J Clin Invest* **108:**635-636; 2001.
[132] Daiber, A.; Steven, S.; Vujacic-Mirski, K.; Kalinovic, S.; Oelze, M.; Di Lisa, F.; Munzel, T. Regulation of Vascular Function and Inflammation via Cross Talk of Reactive Oxygen and Nitrogen Species from Mitochondria or NADPH Oxidase-Implications for Diabetes Progression. *Int J Mol Sci* **21**; 2020.
[133] Wenzel, P.; Schulz, E.; Oelze, M.; Muller, J.; Schuhmacher, S.; Alhamdani, M. S.; Debrezion, J.; Hortmann, M.; Reifenberg, K.; Fleming, I.; Munzel, T.; Daiber, A.

AT1-receptor blockade by telmisartan upregulates GTP-cyclohydrolase I and protects eNOS in diabetic rats. *Free Radic Biol Med* **45:**619-626; 2008.
[134] Cadenas, S. ROS and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection. *Free Radic Biol Med* **117:**76-89; 2018.
[135] Desco, M. C.; Asensi, M.; Marquez, R.; Martinez-Valls, J.; Vento, M.; Pallardo, F. V.; Sastre, J.; Vina, J. Xanthine oxidase is involved in free radical production in type 1 diabetes: protection by allopurinol. *Diabetes* **51:**1118-1124; 2002.
[136] Dumitrescu, C.; Biondi, R.; Xia, Y.; Cardounel, A. J.; Druhan, L. J.; Ambrosio, G.; Zweier, J. L. Myocardial ischemia results in tetrahydrobiopterin (BH4) oxidation with impaired endothelial function ameliorated by BH4. *Proc Natl Acad Sci U S A* **104:**15081-15086; 2007.
[137] Xiang, F. L.; Lu, X.; Strutt, B.; Hill, D. J.; Feng, Q. NOX2 deficiency protects against streptozotocin-induced beta-cell destruction and development of diabetes in mice. *Diabetes* **59:**2603-2611; 2010.
[138] Sukumar, P.; Viswambharan, H.; Imrie, H.; Cubbon, R. M.; Yuldasheva, N.; Gage, M.; Galloway, S.; Skromna, A.; Kandavelu, P.; Santos, C. X.; Gatenby, V. K.; Smith, J.; Beech, D. J.; Wheatcroft, S. B.; Channon, K. M.; Shah, A. M.; Kearney, M. T. Nox2 NADPH oxidase has a critical role in insulin resistance-related endothelial cell dysfunction. *Diabetes* **62:**2130-2134; 2013.
[139] Di Marco, E.; Gray, S. P.; Chew, P.; Kennedy, K.; Cooper, M. E.; Schmidt, H. H.; Jandeleit-Dahm, K. A. Differential effects of NOX4 and NOX1 on immune cell-mediated inflammation in the aortic sinus of diabetic ApoE-/- mice. *Clin Sci (Lond)* **130:**1363-1374; 2016.
[140] Di Marco, E.; Gray, S. P.; Kennedy, K.; Szyndralewiez, C.; Lyle, A. N.; Lassegue, B.; Griendling, K. K.; Cooper, M. E.; Schmidt, H.; Jandeleit-Dahm, K. A. M. NOX4-derived reactive oxygen species limit fibrosis and inhibit proliferation of vascular smooth muscle cells in diabetic atherosclerosis. *Free Radic Biol Med* **97:**556-567; 2016.
[141] Giardino, I.; Edelstein, D.; Brownlee, M. BCL-2 expression or antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation endproducts in bovine endothelial cells. *J Clin Invest* **97:**1422-1428; 1996.
[142] da-Silva, W. S.; Gomez-Puyou, A.; de Gomez-Puyou, M. T.; Moreno-Sanchez, R.; De Felice, F. G.; de Meis, L.; Oliveira, M. F.; Galina, A. Mitochondrial bound hexokinase activity as a preventive antioxidant defense: steady-state ADP formation as a regulatory mechanism of membrane potential and reactive oxygen species generation in mitochondria. *J Biol Chem* **279:**39846-39855; 2004.
[143] Pasdois, P.; Parker, J. E.; Halestrap, A. P. Extent of mitochondrial hexokinase II dissociation during ischemia correlates with mitochondrial cytochrome c release, reactive oxygen species production, and infarct size on reperfusion. *J Am Heart Assoc* **2:**e005645; 2012.
[144] Nederlof, R.; Eerbeek, O.; Hollmann, M. W.; Southworth, R.; Zuurbier, C. J. Targeting hexokinase II to mitochondria to modulate energy metabolism and reduce ischaemia-reperfusion injury in heart. *Br J Pharmacol* **171:**2067-2079; 2014.
[145] Gurel, E.; Ustunova, S.; Kapucu, A.; Yilmazer, N.; Eerbeek, O.; Nederlof, R.; Hollmann, M. W.; Demirci-Tansel, C.; Zuurbier, C. J. Hexokinase cellular trafficking in ischemia-reperfusion and ischemic preconditioning is altered in type I diabetic heart. *Mol Biol Rep* **40:**4153-4160; 2013.
[146] Vyssokikh, M. Y.; Holtze, S.; Averina, O. A.; Lyamzaev, K. G.; Panteleeva, A. A.; Marey, M. V.; Zinovkin, R. A.; Severin, F. F.; Skulachev, M. V.; Fasel, N.; Hildebrandt, T. B.; Skulachev, V. P. Mild depolarization of the inner mitochondrial

membrane is a crucial component of an anti-aging program. *Proc Natl Acad Sci U S A* **117:**6491-6501; 2020.
[147] Wang, C.; Fan, F.; Cao, Q.; Shen, C.; Zhu, H.; Wang, P.; Zhao, X.; Sun, X.; Dong, Z.; Ma, X.; Liu, X.; Han, S.; Wu, C.; Zou, Y.; Hu, K.; Ge, J.; Sun, A. Mitochondrial aldehyde dehydrogenase 2 deficiency aggravates energy metabolism disturbance and diastolic dysfunction in diabetic mice. *J Mol Med (Berl)* **94:**1229-1240; 2016.
[148] Okazaki, T.; Otani, H.; Shimazu, T.; Yoshioka, K.; Fujita, M.; Iwasaka, T. Ascorbic acid and N-acetyl cysteine prevent uncoupling of nitric oxide synthase and increase tolerance to ischemia/reperfusion injury in diabetic rat heart. *Free Radic Res* **45:**1173-1183; 2011.
[149] Bugger, H.; Abel, E. D. Mitochondria in the diabetic heart. *Cardiovasc Res* **88:**229-240; 2010.
[150] Luo, M.; Guan, X.; Luczak, E. D.; Lang, D.; Kutschke, W.; Gao, Z.; Yang, J.; Glynn, P.; Sossalla, S.; Swaminathan, P. D.; Weiss, R. M.; Yang, B.; Rokita, A. G.; Maier, L. S.; Efimov, I. R.; Hund, T. J.; Anderson, M. E. Diabetes increases mortality after myocardial infarction by oxidizing CaMKII. *J Clin Invest* **123:**1262-1274; 2013.
[151] Ni, R.; Cao, T.; Xiong, S.; Ma, J.; Fan, G. C.; Lacefield, J. C.; Lu, Y.; Le Tissier, S.; Peng, T. Therapeutic inhibition of mitochondrial reactive oxygen species with mito-TEMPO reduces diabetic cardiomyopathy. *Free Radic Biol Med* **90:**12-23; 2016.
[152] Sloan, R. C.; Moukdar, F.; Frasier, C. R.; Patel, H. D.; Bostian, P. A.; Lust, R. M.; Brown, D. A. Mitochondrial permeability transition in the diabetic heart: contributions of thiol redox state and mitochondrial calcium to augmented reperfusion injury. *J Mol Cell Cardiol* **52:**1009-1018; 2012.
[153] Leng, Y.; Wu, Y.; Lei, S.; Zhou, B.; Qiu, Z.; Wang, K.; Xia, Z. Inhibition of HDAC6 Activity Alleviates Myocardial Ischemia/Reperfusion Injury in Diabetic Rats: Potential Role of Peroxiredoxin 1 Acetylation and Redox Regulation. *Oxid Med Cell Longev* **2018:**9494052; 2018.
[154] Ong, S. G.; Lee, W. H.; Theodorou, L.; Kodo, K.; Lim, S. Y.; Shukla, D. H.; Briston, T.; Kiriakidis, S.; Ashcroft, M.; Davidson, S. M.; Maxwell, P. H.; Yellon, D. M.; Hausenloy, D. J. HIF-1 reduces ischaemia-reperfusion injury in the heart by targeting the mitochondrial permeability transition pore. *Cardiovasc Res* **104:**24-36; 2014.
[155] Heather, L. C.; Clarke, K. Metabolism, hypoxia and the diabetic heart. *J Mol Cell Cardiol* **50:**598-605; 2011.
[156] Mao, X.; Wang, T.; Liu, Y.; Irwin, M. G.; Ou, J. S.; Liao, X. L.; Gao, X.; Xu, Y.; Ng, K. F.; Vanhoutte, P. M.; Xia, Z. N-acetylcysteine and allopurinol confer synergy in attenuating myocardial ischemia injury via restoring HIF-1alpha/HO-1 signaling in diabetic rats. *PLoS One* **8:**e68949; 2013.
[157] Koivunen, P.; Serpi, R.; Dimova, E. Y. Hypoxia-inducible factor prolyl 4-hydroxylase inhibition in cardiometabolic diseases. *Pharmacol Res* **114:**265-273; 2016.
[158] Wu, J.; Yang, L.; Xie, P.; Yu, J.; Yu, T.; Wang, H.; Maimaitili, Y.; Wang, J.; Ma, H.; Yang, Y.; Zheng, H. Cobalt Chloride Upregulates Impaired HIF-1alpha Expression to Restore Sevoflurane Post-conditioning-Dependent Myocardial Protection in Diabetic Rats. *Front Physiol* **8:**395; 2017.
[159] Yang, L.; Xie, P.; Wu, J.; Yu, J.; Li, X.; Ma, H.; Yu, T.; Wang, H.; Ye, J.; Wang, J.; Zheng, H. Deferoxamine Treatment Combined With Sevoflurane Postconditioning Attenuates Myocardial Ischemia-Reperfusion Injury by Restoring HIF-1/BNIP3-Mediated Mitochondrial Autophagy in GK Rats. *Front Pharmacol* **11:**6; 2020.

[160] Salloum, F. N.; Chau, V. Q.; Hoke, N. N.; Abbate, A.; Varma, A.; Ockaili, R. A.; Toldo, S.; Kukreja, R. C. Phosphodiesterase-5 inhibitor, tadalafil, protects against myocardial ischemia/reperfusion through protein-kinase g-dependent generation of hydrogen sulfide. *Circulation* **120:**S31-36; 2009.
[161] Das, A.; Durrant, D.; Salloum, F. N.; Xi, L.; Kukreja, R. C. PDE5 inhibitors as therapeutics for heart disease, diabetes and cancer. *Pharmacol Ther* **147:**12-21; 2015.
[162] Koka, S.; Das, A.; Salloum, F. N.; Kukreja, R. C. Phosphodiesterase-5 inhibitor tadalafil attenuates oxidative stress and protects against myocardial ischemia/reperfusion injury in type 2 diabetic mice. *Free Radic Biol Med* **60:**80-88; 2013.
[163] Yu, L.; Liang, H.; Dong, X.; Zhao, G.; Jin, Z.; Zhai, M.; Yang, Y.; Chen, W.; Liu, J.; Yi, W.; Yang, J.; Yi, D.; Duan, W.; Yu, S. Reduced silent information regulator 1 signaling exacerbates myocardial ischemia-reperfusion injury in type 2 diabetic rats and the protective effect of melatonin. *J Pineal Res* **59:**376-390; 2015.
[164] Yu, L.; Fan, C.; Li, Z.; Zhang, J.; Xue, X.; Xu, Y.; Zhao, G.; Yang, Y.; Wang, H. Melatonin rescues cardiac thioredoxin system during ischemia-reperfusion injury in acute hyperglycemic state by restoring Notch1/Hes1/Akt signaling in a membrane receptor-dependent manner. *J Pineal Res* **62**; 2017.
[165] Yu, L.; Gong, B.; Duan, W.; Fan, C.; Zhang, J.; Li, Z.; Xue, X.; Xu, Y.; Meng, D.; Li, B.; Zhang, M.; Bin, Z.; Jin, Z.; Yu, S.; Yang, Y.; Wang, H. Melatonin ameliorates myocardial ischemia/reperfusion injury in type 1 diabetic rats by preserving mitochondrial function: role of AMPK-PGC-1alpha-SIRT3 signaling. *Sci Rep* **7:**41337; 2017.
[166] Kosuru, R.; Cai, Y.; Kandula, V.; Yan, D.; Wang, C.; Zheng, H.; Li, Y.; Irwin, M. G.; Singh, S.; Xia, Z. AMPK Contributes to Cardioprotective Effects of Pterostilbene Against Myocardial Ischemia- Reperfusion Injury in Diabetic Rats by Suppressing Cardiac Oxidative Stress and Apoptosis. *Cell Physiol Biochem* **46:**1381-1397; 2018.
[167] Annapurna, A.; Reddy, C. S.; Akondi, R. B.; Rao, S. R. Cardioprotective actions of two bioflavonoids, quercetin and rutin, in experimental myocardial infarction in both normal and streptozotocin-induced type I diabetic rats. *J Pharm Pharmacol* **61:**1365-1374; 2009.
[168] Pranav Nayak, B.; Ganesha, K. R.; Minaz, N.; Razdan, R.; Goswami, S. K. Phloroglucinol, a nutraceutical for IR-induced cardiac damage in diabetic rats. *Animal Model Exp Med* **2:**210-216; 2019.
[169] Duan, J.; Guan, Y.; Mu, F.; Guo, C.; Zhang, E.; Yin, Y.; Wei, G.; Zhu, Y.; Cui, J.; Cao, J.; Weng, Y.; Wang, Y.; Xi, M.; Wen, A. Protective effect of butin against ischemia/reperfusion-induced myocardial injury in diabetic mice: involvement of the AMPK/GSK-3beta/Nrf2 signaling pathway. *Sci Rep* **7:**41491; 2017.
[170] Yang, J. T.; Qian, L. B.; Zhang, F. J.; Wang, J.; Ai, H.; Tang, L. H.; Wang, H. P. Cardioprotective effects of luteolin on ischemia/reperfusion injury in diabetic rats are modulated by eNOS and the mitochondrial permeability transition pathway. *J Cardiovasc Pharmacol* **65:**349-356; 2015.
[171] Wu, Y.; Xia, Z. Y.; Zhao, B.; Leng, Y.; Dou, J.; Meng, Q. T.; Lei, S. Q.; Chen, Z. Z.; Zhu, J. (-)-Epigallocatechin-3-gallate attenuates myocardial injury induced by ischemia/reperfusion in diabetic rats and in H9c2 cells under hyperglycemic conditions. *Int J Mol Med* **40:**389-399; 2017.
[172] Suchal, K.; Malik, S.; Khan, S. I.; Malhotra, R. K.; Goyal, S. N.; Bhatia, J.; Ojha, S.; Arya, D. S. Molecular Pathways Involved in the Amelioration of Myocardial Injury in Diabetic Rats by Kaempferol. *Int J Mol Sci* **18**; 2017.

[173] Andreadou, I.; Bell, R. M.; Botker, H. E.; Zuurbier, C. J. SGLT2 inhibitors reduce infarct size in reperfused ischemic heart and improve cardiac function during ischemic episodes in preclinical models. *Biochim Biophys Acta Mol Basis Dis* **1866:**165770; 2020.
[174] Lopaschuk, G. D.; Verma, S. Mechanisms of Cardiovascular Benefits of Sodium Glucose Co-Transporter 2 (SGLT2) Inhibitors: A State-of-the-Art Review. *JACC Basic Transl Sci* **5:**632-644; 2020.
[175] Nikolaou, P. E.; Efentakis, P.; Qourah, F. A.; Femmino, S.; Makridakis, M.; Kanaki, Z.; Varela, A.; Tsoumani, M.; Davos, C. H.; Dimitriou, C. A.; Tasouli, A.; Dimitriadis, G.; Kostomitsopoulos, N.; Zuurbier, C. J.; Vlahou, A.; Klinakis, A.; Brizzi, M. F.; Iliodromitis, E. K.; Andreadou, I. Chronic Empaglifozin treatment reduces myocardial infarct size in non-diabetic mice through STAT-3 mediated protection on microvascular endothelial cells and reduction of oxidative stress. *Antioxid Redox Signal*; 2020.
[176] Oshima, H.; Miki, T.; Kuno, A.; Mizuno, M.; Sato, T.; Tanno, M.; Yano, T.; Nakata, K.; Kimura, Y.; Abe, K.; Ohwada, W.; Miura, T. Empagliflozin, an SGLT2 Inhibitor, Reduced the Mortality Rate after Acute Myocardial Infarction with Modification of Cardiac Metabolomes and Antioxidants in Diabetic Rats. *J Pharmacol Exp Ther* **368:**524-534; 2019.
[177] Liu, X.; Wei, J.; Peng, D. H.; Layne, M. D.; Yet, S. F. Absence of heme oxygenase-1 exacerbates myocardial ischemia/reperfusion injury in diabetic mice. *Diabetes* **54:**778-784; 2005.
[178] Mills, K. T.; Bundy, J. D.; Kelly, T. N.; Reed, J. E.; Kearney, P. M.; Reynolds, K.; Chen, J.; He, J. Global Disparities of Hypertension Prevalence and Control: A Systematic Analysis of Population-Based Studies From 90 Countries. *Circulation* **134:**441-450; 2016.
[179] Yildiz, M.; Oktay, A. A.; Stewart, M. H.; Milani, R. V.; Ventura, H. O.; Lavie, C. J. Left ventricular hypertrophy and hypertension. *Prog Cardiovasc Dis* **63:**10-21; 2020.
[180] Savage, D. D.; Garrison, R. J.; Kannel, W. B.; Levy, D.; Anderson, S. J.; Stokes, J., 3rd; Feinleib, M.; Castelli, W. P. The spectrum of left ventricular hypertrophy in a general population sample: the Framingham Study. *Circulation* **75:**I26-33; 1987.
[181] Tuzcu, E. M.; Golz, S. J.; Lever, H. M.; Salcedo, E. E. Left ventricular hypertrophy in persons age 90 years and older. *Am J Cardiol* **63:**237-240; 1989.
[182] Massie, B. M.; Tubau, J. F.; Szlachcic, J.; O'Kelly, B. F. Hypertensive heart disease: the critical role of left ventricular hypertrophy. *J Cardiovasc Pharmacol* **13 Suppl 1:**S18-24; 1989.
[183] Prisant, L. M. Hypertensive heart disease. *J Clin Hypertens (Greenwich)* **7:**231-238; 2005.
[184] Batist, G.; Mersereau, W.; Malashenko, B. A.; Chiu, R. C. Response to ischemia-reperfusion injury in hypertrophic heart. Role of free-radical metabolic pathways. *Circulation* **80:**III10-13; 1989.
[185] Anderson, P. G.; Allard, M. F.; Thomas, G. D.; Bishop, S. P.; Digerness, S. B. Increased ischemic injury but decreased hypoxic injury in hypertrophied rat hearts. *Circ Res* **67:**948-959; 1990.
[186] Osbakken, M.; Douglas, P. S.; Ivanics, T.; Zhang, D. N.; Van Winkle, T. Creatinine kinase kinetics studied by phosphorus-31 nuclear magnetic resonance in a canine model of chronic hypertension-induced cardiac hypertrophy. *J Am Coll Cardiol* **19:**223-228; 1992.

[187] Meijles, D. N.; Cull, J. J.; Markou, T.; Cooper, S. T. E.; Haines, Z. H. R.; Fuller, S. J.; O'Gara, P.; Sheppard, M. N.; Harding, S. E.; Sugden, P. H.; Clerk, A. Redox Regulation of Cardiac ASK1 (Apoptosis Signal-Regulating Kinase 1) Controls p38-MAPK (Mitogen-Activated Protein Kinase) and Orchestrates Cardiac Remodeling to Hypertension. *Hypertension* **76:**1208-1218; 2020.
[188] Santos, C. X.; Anilkumar, N.; Zhang, M.; Brewer, A. C.; Shah, A. M. Redox signaling in cardiac myocytes. *Free Radic Biol Med* **50:**777-793; 2011.
[189] Tu, V. C.; Sun, H.; Bowden, G. T.; Chen, Q. M. Involvement of oxidants and AP-1 in angiotensin II-activated NFAT3 transcription factor. *Am J Physiol Cell Physiol* **292:**C1248-1255; 2007.
[190] Amin, J. K.; Xiao, L.; Pimental, D. R.; Pagano, P. J.; Singh, K.; Sawyer, D. B.; Colucci, W. S. Reactive oxygen species mediate alpha-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes. *J Mol Cell Cardiol* **33:**131-139; 2001.
[191] Dai, D. F.; Johnson, S. C.; Villarin, J. J.; Chin, M. T.; Nieves-Cintron, M.; Chen, T.; Marcinek, D. J.; Dorn, G. W., 2nd; Kang, Y. J.; Prolla, T. A.; Santana, L. F.; Rabinovitch, P. S. Mitochondrial oxidative stress mediates angiotensin II-induced cardiac hypertrophy and Galphaq overexpression-induced heart failure. *Circ Res* **108:**837-846; 2011.
[192] Bianchi, P.; Pimentel, D. R.; Murphy, M. P.; Colucci, W. S.; Parini, A. A new hypertrophic mechanism of serotonin in cardiac myocytes: receptor-independent ROS generation. *FASEB J* **19:**641-643; 2005.
[193] Kaludercic, N.; Carpi, A.; Nagayama, T.; Sivakumaran, V.; Zhu, G.; Lai, E. W.; Bedja, D.; De Mario, A.; Chen, K.; Gabrielson, K. L.; Lindsey, M. L.; Pacak, K.; Takimoto, E.; Shih, J. C.; Kass, D. A.; Di Lisa, F.; Paolocci, N. Monoamine oxidase B prompts mitochondrial and cardiac dysfunction in pressure overloaded hearts. *Antioxid Redox Signal* **20:**267-280; 2014.
[194] Schluter, K. D.; Kutsche, H. S.; Hirschhauser, C.; Schreckenberg, R.; Schulz, R. Review on Chamber-Specific Differences in Right and Left Heart Reactive Oxygen Species Handling. *Front Physiol* **9:**1799; 2018.
[195] Bhatti, S. N.; Li, J. M. Nox2 dependent redox-regulation of Akt and ERK1/2 to promote left ventricular hypertrophy in dietary obesity of mice. *Biochem Biophys Res Commun* **528:**506-513; 2020.
[196] Byrne, J. A.; Grieve, D. J.; Bendall, J. K.; Li, J. M.; Gove, C.; Lambeth, J. D.; Cave, A. C.; Shah, A. M. Contrasting roles of NADPH oxidase isoforms in pressure-overload versus angiotensin II-induced cardiac hypertrophy. *Circ Res* **93:**802-805; 2003.
[197] Zhao, G. J.; Zhao, C. L.; Ouyang, S.; Deng, K. Q.; Zhu, L.; Montezano, A. C.; Zhang, C.; Hu, F.; Zhu, X. Y.; Tian, S.; Liu, X.; Ji, Y. X.; Zhang, P.; Zhang, X. J.; She, Z. G.; Touyz, R. M.; Li, H. Ca(2+)-Dependent NOX5 (NADPH Oxidase 5) Exaggerates Cardiac Hypertrophy Through Reactive Oxygen Species Production. *Hypertension* **76:**827-838; 2020.
[198] Namai-Takahashi, A.; Sakuyama, A.; Nakamura, T.; Miura, T.; Takahashi, J.; Kurosawa, R.; Kohzuki, M.; Ito, O. Xanthine Oxidase Inhibitor, Febuxostat Ameliorates the High Salt Intake-Induced Cardiac Hypertrophy and Fibrosis in Dahl Salt-Sensitive Rats. *Am J Hypertens* **32:**26-33; 2019.
[199] Yamamoto, E.; Kataoka, K.; Yamashita, T.; Tokutomi, Y.; Dong, Y. F.; Matsuba, S.; Ogawa, H.; Kim-Mitsuyama, S. Role of xanthine oxidoreductase in the reversal of diastolic heart failure by candesartan in the salt-sensitive hypertensive rat. *Hypertension* **50:**657-662; 2007.

[200] Fosslien, E. Review: Mitochondrial medicine--cardiomyopathy caused by defective oxidative phosphorylation. *Ann Clin Lab Sci* **33:**371-395; 2003.
[201] Rosca, M. G.; Tandler, B.; Hoppel, C. L. Mitochondria in cardiac hypertrophy and heart failure. *J Mol Cell Cardiol* **55:**31-41; 2013.
[202] Kaludercic, N.; Takimoto, E.; Nagayama, T.; Feng, N.; Lai, E. W.; Bedja, D.; Chen, K.; Gabrielson, K. L.; Blakely, R. D.; Shih, J. C.; Pacak, K.; Kass, D. A.; Di Lisa, F.; Paolocci, N. Monoamine oxidase A-mediated enhanced catabolism of norepinephrine contributes to adverse remodeling and pump failure in hearts with pressure overload. *Circ Res* **106:**193-202; 2010.
[203] McMurray, J. J.; Pfeffer, M. A. Heart failure. *Lancet* **365:**1877-1889; 2005.
[204] Neubauer, S. The failing heart--an engine out of fuel. *N Engl J Med* **356:**1140-1151; 2007.
[205] Zhang, M.; Perino, A.; Ghigo, A.; Hirsch, E.; Shah, A. M. NADPH oxidases in heart failure: poachers or gamekeepers? *Antioxid Redox Signal* **18:**1024-1041; 2013.
[206] Dhalla, A. K.; Singal, P. K. Antioxidant changes in hypertrophied and failing guinea pig hearts. *Am J Physiol* **266:**H1280-1285; 1994.
[207] Yang, Y.; Ago, T.; Zhai, P.; Abdellatif, M.; Sadoshima, J. Thioredoxin 1 negatively regulates angiotensin II-induced cardiac hypertrophy through upregulation of miR-98/let-7. *Circ Res* **108:**305-313; 2011.
[208] Andreadou, I.; Schulz, R.; Papapetropoulos, A.; Turan, B.; Ytrehus, K.; Ferdinandy, P.; Daiber, A.; Di Lisa, F. The role of mitochondrial reactive oxygen species, NO and H2 S in ischaemia/reperfusion injury and cardioprotection. *J Cell Mol Med* **24:**6510-6522; 2020.
[209] Peleli, M.; Bibli, S. I.; Li, Z.; Chatzianastasiou, A.; Varela, A.; Katsouda, A.; Zukunft, S.; Bucci, M.; Vellecco, V.; Davos, C. H.; Nagahara, N.; Cirino, G.; Fleming, I.; Lefer, D. J.; Papapetropoulos, A. Cardiovascular phenotype of mice lacking 3-mercaptopyruvate sulfurtransferase. *Biochem Pharmacol* **176:**113833; 2020.
[210] Boardman, N. T.; Migally, B.; Pileggi, C.; Parmar, G. S.; Xuan, J. Y.; Menzies, K.; Harper, M. E. Glutaredoxin-2 and Sirtuin-3 deficiencies impair cardiac mitochondrial energetics but their effects are not additive. *Biochim Biophys Acta Mol Basis Dis* **1867:**165982; 2020.
[211] Meng, G.; Liu, J.; Liu, S.; Song, Q.; Liu, L.; Xie, L.; Han, Y.; Ji, Y. Hydrogen sulfide pretreatment improves mitochondrial function in myocardial hypertrophy via a SIRT3-dependent manner. *Br J Pharmacol* **175:**1126-1145; 2018.
[212] Molgaard, S.; Faricelli, B.; Salomonsson, M.; Engstrom, T.; Treiman, M. Increased myocardial vulnerability to ischemia-reperfusion injury in the presence of left ventricular hypertrophy. *J Hypertens* **34:**513-523; discussion 523; 2016.
[213] Yano, T.; Miki, T.; Tanno, M.; Kuno, A.; Itoh, T.; Takada, A.; Sato, T.; Kouzu, H.; Shimamoto, K.; Miura, T. Hypertensive hypertrophied myocardium is vulnerable to infarction and refractory to erythropoietin-induced protection. *Hypertension* **57:**110-115; 2011.
[214] Speechly-Dick, M. E.; Baxter, G. F.; Yellon, D. M. Ischaemic preconditioning protects hypertrophied myocardium. *Cardiovasc Res* **28:**1025-1029; 1994.
[215] Ebrahim, Z.; Yellon, D. M.; Baxter, G. F. Attenuated cardioprotective response to bradykinin, but not classical ischaemic preconditioning, in DOCA-salt hypertensive left ventricular hypertrophy. *Pharmacol Res* **55:**42-48; 2007.
[216] Galiuto, L.; Gabrielli, F. A.; Lanza, G. A.; Porfidia, A.; Paraggio, L.; Barchetta, S.; Locorotondo, G.; De Caterina, A. R.; Rebuzzi, A. G.; Crea, F. Influence of left ventricular hypertrophy on microvascular dysfunction and left ventricular remodelling after acute myocardial infarction. *Eur J Echocardiogr* **11:**677-682; 2010.

[217] Pantos, C. I.; Davos, C. H.; Carageorgiou, H. C.; Varonos, D. V.; Cokkinos, D. V. Ischaemic preconditioning protects against myocardial dysfunction caused by ischaemia in isolated hypertrophied rat hearts. *Basic Res Cardiol* **91:**444-449; 1996.
[218] Randall, M. D.; Gardiner, S. M.; Bennett, T. Enhanced cardiac preconditioning in the isolated heart of the transgenic ((mREN-2) 27) hypertensive rat. *Cardiovasc Res* **33:**400-409; 1997.
[219] Ebrahim, Z.; Yellon, D. M.; Baxter, G. F. Ischemic preconditioning is lost in aging hypertensive rat heart: independent effects of aging and longstanding hypertension. *Exp Gerontol* **42:**807-814; 2007.
[220] Moolman, J. A.; Genade, S.; Tromp, E.; Opie, L. H.; Lochner, A. Ischaemic preconditioning does not protect hypertrophied myocardium against ischaemia. *S Afr Med J* **87 Suppl 3:**C151-156; 1997.
[221] Penna, C.; Tullio, F.; Moro, F.; Folino, A.; Merlino, A.; Pagliaro, P. Effects of a protocol of ischemic postconditioning and/or captopril in hearts of normotensive and hypertensive rats. *Basic Res Cardiol* **105:**181-192; 2010.
[222] Penna, C.; Tullio, F.; Perrelli, M. G.; Moro, F.; Abbadessa, G.; Piccione, F.; Carriero, V.; Racca, S.; Pagliaro, P. Ischemia/reperfusion injury is increased and cardioprotection by a postconditioning protocol is lost as cardiac hypertrophy develops in nandrolone treated rats. *Basic Res Cardiol* **106:**409-420; 2011.
[223] Wagner, C.; Ebner, B.; Tillack, D.; Strasser, R. H.; Weinbrenner, C. Cardioprotection by ischemic postconditioning is abrogated in hypertrophied myocardium of spontaneously hypertensive rats. *J Cardiovasc Pharmacol* **61:**35-41; 2013.
[224] Hausenloy, D. J.; Schulz, R.; Girao, H.; Kwak, B. R.; De Stefani, D.; Rizzuto, R.; Bernardi, P.; Di Lisa, F. Mitochondrial ion channels as targets for cardioprotection. *J Cell Mol Med* **24:**7102-7114; 2020.
[225] Matas, J.; Young, N. T.; Bourcier-Lucas, C.; Ascah, A.; Marcil, M.; Deschepper, C. F.; Burelle, Y. Increased expression and intramitochondrial translocation of cyclophilin-D associates with increased vulnerability of the permeability transition pore to stress-induced opening during compensated ventricular hypertrophy. *J Mol Cell Cardiol* **46:**420-430; 2009.
[226] Chen, C. H.; Wu, C. W.; Shih, C. D.; Lien, W. H.; Huang, S. L.; Huang, C. C. Attenuation of Isoflurane Preconditioning-Induced Acute Cardioprotection in Hypertensive Hypertrophied Hearts. *J Cardiothorac Vasc Anesth* **30:**1317-1323; 2016.
[227] Fantinelli, J. C.; Perez Nunez, I. A.; Gonzalez Arbelaez, L. F.; Schinella, G. R.; Mosca, S. M. Participation of mitochondrial permeability transition pore in the effects of ischemic preconditioning in hypertrophied hearts: role of NO and mitoKATP. *Int J Cardiol* **166:**173-180; 2013.
[228] Siti, H. N.; Kamisah, Y.; Kamsiah, J. The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review). *Vascul Pharmacol* **71:**40-56; 2015.
[229] Szwejkowski, B. R.; Gandy, S. J.; Rekhraj, S.; Houston, J. G.; Lang, C. C.; Morris, A. D.; George, J.; Struthers, A. D. Allopurinol reduces left ventricular mass in patients with type 2 diabetes and left ventricular hypertrophy. *J Am Coll Cardiol* **62:**2284-2293; 2013.
[230] Weston, S. R.; Leyden, W.; Murphy, R.; Bass, N. M.; Bell, B. P.; Manos, M. M.; Terrault, N. A. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology* **41:**372-379; 2005.
[231] Farrell, G. C.; Larter, C. Z. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* **43:**S99-S112; 2006.

[232] Targher, G.; Day, C. P.; Bonora, E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* **363:**1341-1350; 2010.
[233] Oni, E. T.; Agatston, A. S.; Blaha, M. J.; Fialkow, J.; Cury, R.; Sposito, A.; Erbel, R.; Blankstein, R.; Feldman, T.; Al-Mallah, M. H.; Santos, R. D.; Budoff, M. J.; Nasir, K. A systematic review: burden and severity of subclinical cardiovascular disease among those with nonalcoholic fatty liver; should we care? *Atherosclerosis* **230:**258-267; 2013.
[234] Collaborators, G. B. D. C. The global, regional, and national burden of cirrhosis by cause in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* **5:**245-266; 2020.
[235] Gracia-Sancho, J.; Maeso-Diaz, R.; Fernandez-Iglesias, A.; Navarro-Zornoza, M.; Bosch, J. New cellular and molecular targets for the treatment of portal hypertension. *Hepatol Int* **9:**183-191; 2015.
[236] Hernandez-Guerra, M.; Garcia-Pagan, J. C.; Turnes, J.; Bellot, P.; Deulofeu, R.; Abraldes, J. G.; Bosch, J. Ascorbic acid improves the intrahepatic endothelial dysfunction of patients with cirrhosis and portal hypertension. *Hepatology* **43:**485-491; 2006.
[237] Hink, U.; Li, H.; Mollnau, H.; Oelze, M.; Matheis, E.; Hartmann, M.; Skatchkov, M.; Thaiss, F.; Stahl, R. A.; Warnholtz, A.; Meinertz, T.; Griendling, K.; Harrison, D. G.; Forstermann, U.; Munzel, T. Mechanisms Underlying Endothelial Dysfunction in Diabetes Mellitus. *Circ Res* **88:**E14-E22.; 2001.
[238] Smith, B. W.; Adams, L. A. Nonalcoholic fatty liver disease and diabetes mellitus: pathogenesis and treatment. *Nat Rev Endocrinol* **7:**456-465; 2011.
[239] Weng, S. Y.; Schuppan, D. AMPK regulates macrophage polarization in adipose tissue inflammation and NASH. *J Hepatol* **58:**619-621; 2013.
[240] Eckert, C.; Klein, N.; Kornek, M.; Lukacs-Kornek, V. The complex myeloid network of the liver with diverse functional capacity at steady state and in inflammation. *Front Immunol* **6:**179; 2015.
[241] Takaki, A.; Kawai, D.; Yamamoto, K. Multiple hits, including oxidative stress, as pathogenesis and treatment target in non-alcoholic steatohepatitis (NASH). *Int J Mol Sci* **14:**20704-20728; 2013.
[242] Wang, X.; Hausding, M.; Weng, S. Y.; Kim, Y. O.; Steven, S.; Klein, T.; Daiber, A.; Schuppan, D. Gliptins Suppress Inflammatory Macrophage Activation to Mitigate Inflammation, Fibrosis, Oxidative Stress, and Vascular Dysfunction in Models of Nonalcoholic Steatohepatitis and Liver Fibrosis. *Antioxid Redox Signal* **28:**87-109; 2018.
[243] Libby, P. Inflammation in atherosclerosis. *Nature* **420:**868-874; 2002.
[244] Hansson, G. K. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* **352:**1685-1695; 2005.
[245] Guzik, T. J.; Hoch, N. E.; Brown, K. A.; McCann, L. A.; Rahman, A.; Dikalov, S.; Goronzy, J.; Weyand, C.; Harrison, D. G. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. *J Exp Med* **204:**2449-2460; 2007.
[246] Wenzel, P.; Knorr, M.; Kossmann, S.; Stratmann, J.; Hausding, M.; Schuhmacher, S.; Karbach, S. H.; Schwenk, M.; Yogev, N.; Schulz, E.; Oelze, M.; Grabbe, S.; Jonuleit, H.; Becker, C.; Daiber, A.; Waisman, A.; Munzel, T. Lysozyme M-positive monocytes mediate angiotensin II-induced arterial hypertension and vascular dysfunction. *Circulation* **124:**1370-1381; 2011.
[247] Gonzalez, A.; Huerta-Salgado, C.; Orozco-Aguilar, J.; Aguirre, F.; Tacchi, F.; Simon, F.; Cabello-Verrugio, C. Role of Oxidative Stress in Hepatic and Extrahepatic

Dysfunctions during Nonalcoholic Fatty Liver Disease (NAFLD). *Oxid Med Cell Longev* **2020:**1617805; 2020.
[248] Serviddio, G.; Bellanti, F.; Vendemiale, G. Free radical biology for medicine: learning from nonalcoholic fatty liver disease. *Free Radic Biol Med* **65:**952-968; 2013.
[249] Paik, Y. H.; Kim, J.; Aoyama, T.; De Minicis, S.; Bataller, R.; Brenner, D. A. Role of NADPH oxidases in liver fibrosis. *Antioxid Redox Signal* **20:**2854-2872; 2014.
[250] Loffredo, L.; Zicari, A. M.; Perri, L.; Carnevale, R.; Nocella, C.; Angelico, F.; Del Ben, M.; Mosca, A.; Zaffina, S.; Panera, N.; Alisi, A.; Duse, M.; Violi, F.; Nobili, V. Does Nox2 Overactivate in Children with Nonalcoholic Fatty Liver Disease? *Antioxid Redox Signal* **30:**1325-1330; 2019.
[251] Carpino, G.; Pastori, D.; Baratta, F.; Overi, D.; Labbadia, G.; Polimeni, L.; Di Costanzo, A.; Pannitteri, G.; Carnevale, R.; Del Ben, M.; Arca, M.; Violi, F.; Angelico, F.; Gaudio, E. PNPLA3 variant and portal/periportal histological pattern in patients with biopsy-proven non-alcoholic fatty liver disease: a possible role for oxidative stress. *Sci Rep* **7:**15756; 2017.
[252] Baratta, F.; Pastori, D.; Bartimoccia, S.; Cammisotto, V.; Cocomello, N.; Colantoni, A.; Nocella, C.; Carnevale, R.; Ferro, D.; Angelico, F.; Violi, F.; Del Ben, M. Poor Adherence to Mediterranean Diet and Serum Lipopolysaccharide are Associated with Oxidative Stress in Patients with Non-Alcoholic Fatty Liver Disease. *Nutrients* **12**; 2020.
[253] Daiber, A.; Steven, S.; Weber, A.; Shuvaev, V. V.; Muzykantov, V. R.; Laher, I.; Li, H.; Lamas, S.; Munzel, T. Targeting vascular (endothelial) dysfunction. *Br J Pharmacol* **174:**1591-1619; 2017.
[254] Dornas, W.; Schuppan, D. Mitochondrial oxidative injury: a key player in nonalcoholic fatty liver disease. *Am J Physiol Gastrointest Liver Physiol* **319:**G400-G411; 2020.
[255] Rolo, A. P.; Teodoro, J. S.; Palmeira, C. M. Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. *Free Radic Biol Med* **52:**59-69; 2012.
[256] Tomita, K.; Teratani, T.; Suzuki, T.; Oshikawa, T.; Yokoyama, H.; Shimamura, K.; Nishiyama, K.; Mataki, N.; Irie, R.; Minamino, T.; Okada, Y.; Kurihara, C.; Ebinuma, H.; Saito, H.; Shimizu, I.; Yoshida, Y.; Hokari, R.; Sugiyama, K.; Hatsuse, K.; Yamamoto, J.; Kanai, T.; Miura, S.; Hibi, T. p53/p66Shc-mediated signaling contributes to the progression of non-alcoholic steatohepatitis in humans and mice. *J Hepatol* **57:**837-843; 2012.
[257] An, P.; Wei, L. L.; Zhao, S.; Sverdlov, D. Y.; Vaid, K. A.; Miyamoto, M.; Kuramitsu, K.; Lai, M.; Popov, Y. V. Hepatocyte mitochondria-derived danger signals directly activate hepatic stellate cells and drive progression of liver fibrosis. *Nat Commun* **11:**2362; 2020.
[258] Oliveira, P. J.; Rolo, A. P.; Seica, R.; Santos, M. S.; Palmeira, C. M.; Moreno, A. J. Chronic cholestasis and cardiac mitochondrial function in Wistar rats: a model for cardiovascular alterations in chronic liver disease? *Rev Port Cardiol* **22:**67-75; 2003.
[259] Nakatsu, Y.; Seno, Y.; Kushiyama, A.; Sakoda, H.; Fujishiro, M.; Katasako, A.; Mori, K.; Matsunaga, Y.; Fukushima, T.; Kanaoka, R.; Yamamotoya, T.; Kamata, H.; Asano, T. The xanthine oxidase inhibitor febuxostat suppresses development of nonalcoholic steatohepatitis in a rodent model. *Am J Physiol Gastrointest Liver Physiol* **309:**G42-51; 2015.
[260] Kushiyama, A.; Nakatsu, Y.; Matsunaga, Y.; Yamamotoya, T.; Mori, K.; Ueda, K.; Inoue, Y.; Sakoda, H.; Fujishiro, M.; Ono, H.; Asano, T. Role of Uric Acid Metabolism-Related Inflammation in the Pathogenesis of Metabolic Syndrome

Components Such as Atherosclerosis and Nonalcoholic Steatohepatitis. *Mediators Inflamm* **2016:**8603164; 2016.
[261] Mondal, A.; Bose, D.; Saha, P.; Sarkar, S.; Seth, R.; Kimono, D.; Albadrani, M.; Nagarkatti, M.; Nagarkatti, P.; Chatterjee, S. Lipocalin 2 induces neuroinflammation and blood-brain barrier dysfunction through liver-brain axis in murine model of nonalcoholic steatohepatitis. *J Neuroinflammation* **17:**201; 2020.
[262] Daiber, A.; Kroller-Schon, S.; Frenis, K.; Oelze, M.; Kalinovic, S.; Vujacic-Mirski, K.; Kuntic, M.; Bayo Jimenez, M. T.; Helmstadter, J.; Steven, S.; Korac, B.; Munzel, T. Environmental noise induces the release of stress hormones and inflammatory signaling molecules leading to oxidative stress and vascular dysfunction-Signatures of the internal exposome. *Biofactors* **45:**495-506; 2019.
[263] Sorescu, D.; Griendling, K. K. Reactive oxygen species, mitochondria, and NAD(P)H oxidases in the development and progression of heart failure. *Congest Heart Fail* **8:**132-140; 2002.
[264] Daiber, A.; Andreadou, I.; Oelze, M.; Davidson, S. M.; Hausenloy, D. J. Discovery of new therapeutic redox targets for cardioprotection against ischemia/reperfusion injury and heart failure. *Free Radic Biol Med*; 2020.
[265] Colak, Y.; Senates, E.; Yesil, A.; Yilmaz, Y.; Ozturk, O.; Doganay, L.; Coskunpinar, E.; Kahraman, O. T.; Mesci, B.; Ulasoglu, C.; Tuncer, I. Assessment of endothelial function in patients with nonalcoholic fatty liver disease. *Endocrine* **43:**100-107; 2013.
[266] Ozturk, K.; Uygun, A.; Guler, A. K.; Demirci, H.; Ozdemir, C.; Cakir, M.; Sakin, Y. S.; Turker, T.; Sari, S.; Demirbas, S.; Karslioglu, Y.; Saglam, M. Nonalcoholic fatty liver disease is an independent risk factor for atherosclerosis in young adult men. *Atherosclerosis* **240:**380-386; 2015.
[267] Loffredo, L.; Baratta, F.; Ludovica, P.; Battaglia, S.; Carnevale, R.; Nocella, C.; Novo, M.; Pannitteri, G.; Ceci, F.; Angelico, F.; Violi, F.; Del Ben, M. Effects of dark chocolate on endothelial function in patients with non-alcoholic steatohepatitis. *Nutr Metab Cardiovasc Dis* **28:**143-149; 2017.
[268] Labenz, C.; Huber, Y.; Michel, M.; Nagel, M.; Galle, P. R.; Kostev, K.; Schattenberg, J. M. Impact of NAFLD on the Incidence of Cardiovascular Diseases in a Primary Care Population in Germany. *Dig Dis Sci* **65:**2112-2119; 2020.
[269] Alexander, M.; Loomis, A. K.; van der Lei, J.; Duarte-Salles, T.; Prieto-Alhambra, D.; Ansell, D.; Pasqua, A.; Lapi, F.; Rijnbeek, P.; Mosseveld, M.; Avillach, P.; Egger, P.; Dhalwani, N. N.; Kendrick, S.; Celis-Morales, C.; Waterworth, D. M.; Alazawi, W.; Sattar, N. Non-alcoholic fatty liver disease and risk of incident acute myocardial infarction and stroke: findings from matched cohort study of 18 million European adults. *BMJ* **367:**l5367; 2019.
[270] Vadarlis, A.; Antza, C.; Bakaloudi, D. R.; Doundoulakis, I.; Kalopitas, G.; Samara, M.; Dardavessis, T.; Maris, T.; Chourdakis, M. Systematic review with meta-analysis: The effect of vitamin E supplementation in adult patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol*; 2020.
[271] Salamone, F.; Galvano, F.; Marino Gammazza, A.; Paternostro, C.; Tibullo, D.; Bucchieri, F.; Mangiameli, A.; Parola, M.; Bugianesi, E.; Li Volti, G. Silibinin improves hepatic and myocardial injury in mice with nonalcoholic steatohepatitis. *Dig Liver Dis* **44:**334-342; 2012.
[272] Li, L.; Hai, J.; Li, Z.; Zhang, Y.; Peng, H.; Li, K.; Weng, X. Resveratrol modulates autophagy and NF-kappaB activity in a murine model for treating non-alcoholic fatty liver disease. *Food Chem Toxicol* **63:**166-173; 2014.

[273] Xia, N.; Daiber, A.; Habermeier, A.; Closs, E. I.; Thum, T.; Spanier, G.; Lu, Q.; Oelze, M.; Torzewski, M.; Lackner, K. J.; Munzel, T.; Forstermann, U.; Li, H. Resveratrol reverses endothelial nitric-oxide synthase uncoupling in apolipoprotein E knockout mice. *J Pharmacol Exp Ther* **335:**149-154; 2010.
[274] Jung, Y. A.; Choi, Y. K.; Jung, G. S.; Seo, H. Y.; Kim, H. S.; Jang, B. K.; Kim, J. G.; Lee, I. K.; Kim, M. K.; Park, K. G. Sitagliptin attenuates methionine/choline-deficient diet-induced steatohepatitis. *Diabetes Res Clin Pract* **105:**47-57; 2014.
[275] Svegliati-Baroni, G.; Saccomanno, S.; Rychlicki, C.; Agostinelli, L.; De Minicis, S.; Candelaresi, C.; Faraci, G.; Pacetti, D.; Vivarelli, M.; Nicolini, D.; Garelli, P.; Casini, A.; Manco, M.; Mingrone, G.; Risaliti, A.; Frega, G. N.; Benedetti, A.; Gastaldelli, A. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. *Liver Int* **31:**1285-1297; 2011.
[276] Kim, Y. O.; Schuppan, D. When GLP-1 hits the liver: a novel approach for insulin resistance and NASH. *Am J Physiol Gastrointest Liver Physiol* **302:**G759-761; 2012.
[277] Wang, Y.; Parlevliet, E. T.; Geerling, J. J.; van der Tuin, S. J.; Zhang, H.; Bieghs, V.; Jawad, A. H.; Shiri-Sverdlov, R.; Bot, I.; de Jager, S. C.; Havekes, L. M.; Romijn, J. A.; Willems van Dijk, K.; Rensen, P. C. Exendin-4 decreases liver inflammation and atherosclerosis development simultaneously by reducing macrophage infiltration. *Br J Pharmacol* **171:**723-734; 2014.
[278] Batchuluun, B.; Inoguchi, T.; Sonoda, N.; Sasaki, S.; Inoue, T.; Fujimura, Y.; Miura, D.; Takayanagi, R. Metformin and liraglutide ameliorate high glucose-induced oxidative stress via inhibition of PKC-NAD(P)H oxidase pathway in human aortic endothelial cells. *Atherosclerosis* **232:**156-164; 2014.
[279] Steven, S.; Hausding, M.; Kroller-Schon, S.; Mader, M.; Mikhed, Y.; Stamm, P.; Zinssius, E.; Pfeffer, A.; Welschof, P.; Agdauletova, S.; Sudowe, S.; Li, H.; Oelze, M.; Schulz, E.; Klein, T.; Munzel, T.; Daiber, A. Gliptin and GLP-1 analog treatment improves survival and vascular inflammation/dysfunction in animals with lipopolysaccharide-induced endotoxemia. *Basic Res Cardiol* **110:**6; 2015.
[280] Matsubara, J.; Sugiyama, S.; Sugamura, K.; Nakamura, T.; Fujiwara, Y.; Akiyama, E.; Kurokawa, H.; Nozaki, T.; Ohba, K.; Konishi, M.; Maeda, H.; Izumiya, Y.; Kaikita, K.; Sumida, H.; Jinnouchi, H.; Matsui, K.; Kim-Mitsuyama, S.; Takeya, M.; Ogawa, H. A dipeptidyl peptidase-4 inhibitor, des-fluoro-sitagliptin, improves endothelial function and reduces atherosclerotic lesion formation in apolipoprotein E-deficient mice. *J Am Coll Cardiol* **59:**265-276; 2012.
[281] Shah, Z.; Kampfrath, T.; Deiuliis, J. A.; Zhong, J.; Pineda, C.; Ying, Z.; Xu, X.; Lu, B.; Moffatt-Bruce, S.; Durairaj, R.; Sun, Q.; Mihai, G.; Maiseyeu, A.; Rajagopalan, S. Long-term dipeptidyl-peptidase 4 inhibition reduces atherosclerosis and inflammation via effects on monocyte recruitment and chemotaxis. *Circulation* **124:**2338-2349; 2011.
[282] Kroller-Schon, S.; Knorr, M.; Hausding, M.; Oelze, M.; Schuff, A.; Schell, R.; Sudowe, S.; Scholz, A.; Daub, S.; Karbach, S.; Kossmann, S.; Gori, T.; Wenzel, P.; Schulz, E.; Grabbe, S.; Klein, T.; Munzel, T.; Daiber, A. Glucose-independent improvement of vascular dysfunction in experimental sepsis by dipeptidyl-peptidase 4 inhibition. *Cardiovasc Res* **96:**140-149; 2012.
[283] Chinda, K.; Palee, S.; Surinkaew, S.; Phornphutkul, M.; Chattipakorn, S.; Chattipakorn, N. Cardioprotective effect of dipeptidyl peptidase-4 inhibitor during ischemia-reperfusion injury. *Int J Cardiol* **167:**451-457; 2013.

[284] Vincent, R. K.; Williams, D. M.; Evans, M. A look to the future in non-alcoholic fatty liver disease: Are glucagon-like peptide-1 analogues or sodium-glucose co-transporter-2 inhibitors the answer? *Diabetes Obes Metab*; 2020.
[285] Shinozaki, S.; Tahara, T.; Lefor, A. K.; Ogura, M. Long-term empagliflozin therapy improves levels of hepatic fibrosis marker in patients with non-alcoholic fatty liver disease complicated by type 2 diabetes mellitus. *J Med Invest* **67:**280-284; 2020.
[286] Taheri, H.; Malek, M.; Ismail-Beigi, F.; Zamani, F.; Sohrabi, M.; Reza Babaei, M.; Khamseh, M. E. Effect of Empagliflozin on Liver Steatosis and Fibrosis in Patients With Non-Alcoholic Fatty Liver Disease Without Diabetes: A Randomized, Double-Blind, Placebo-Controlled Trial. *Adv Ther* **37:**4697-4708; 2020.
[287] Jojima, T.; Tomotsune, T.; Iijima, T.; Akimoto, K.; Suzuki, K.; Aso, Y. Empagliflozin (an SGLT2 inhibitor), alone or in combination with linagliptin (a DPP-4 inhibitor), prevents steatohepatitis in a novel mouse model of non-alcoholic steatohepatitis and diabetes. *Diabetol Metab Syndr* **8:**45; 2016.
[288] Zinman, B.; Wanner, C.; Lachin, J. M.; Fitchett, D.; Bluhmki, E.; Hantel, S.; Mattheus, M.; Devins, T.; Johansen, O. E.; Woerle, H. J.; Broedl, U. C.; Inzucchi, S. E.; Investigators, E.-R. O. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. *N Engl J Med* **373:**2117-2128; 2015.
[289] Steven, S.; Oelze, M.; Hanf, A.; Kroller-Schon, S.; Kashani, F.; Roohani, S.; Welschof, P.; Kopp, M.; Godtel-Armbrust, U.; Xia, N.; Li, H.; Schulz, E.; Lackner, K. J.; Wojnowski, L.; Bottari, S. P.; Wenzel, P.; Mayoux, E.; Munzel, T.; Daiber, A. The SGLT2 inhibitor empagliflozin improves the primary diabetic complications in ZDF rats. *Redox Biol* **13:**370-385; 2017.
[290] Oelze, M.; Kroller-Schon, S.; Welschof, P.; Jansen, T.; Hausding, M.; Mikhed, Y.; Stamm, P.; Mader, M.; Zinssius, E.; Agdauletova, S.; Gottschlich, A.; Steven, S.; Schulz, E.; Bottari, S. P.; Mayoux, E.; Munzel, T.; Daiber, A. The sodium-glucose co-transporter 2 inhibitor empagliflozin improves diabetes-induced vascular dysfunction in the streptozotocin diabetes rat model by interfering with oxidative stress and glucotoxicity. *PLoS One* **9:**e112394; 2014.
[291] Zelniker, T. A.; Braunwald, E. Mechanisms of Cardiorenal Effects of Sodium-Glucose Cotransporter 2 Inhibitors: JACC State-of-the-Art Review. *J Am Coll Cardiol* **75:**422-434; 2020.
[292] Qin, F.; Simeone, M.; Patel, R. Inhibition of NADPH oxidase reduces myocardial oxidative stress and apoptosis and improves cardiac function in heart failure after myocardial infarction. *Free Radic Biol Med* **43:**271-281; 2007.
[293] Cohen, M. V.; Yang, X. M.; Liu, G. S.; Heusch, G.; Downey, J. M. Acetylcholine, bradykinin, opioids, and phenylephrine, but not adenosine, trigger preconditioning by generating free radicals and opening mitochondrial K(ATP) channels. *Circ Res* **89:**273-278; 2001.
[294] Gross, G. J.; Auchampach, J. A. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. *Circ Res* **70:**223-233; 1992.
[295] Takashi, E.; Wang, Y.; Ashraf, M. Activation of mitochondrial K(ATP) channel elicits late preconditioning against myocardial infarction via protein kinase C signaling pathway. *Circ Res* **85:**1146-1153; 1999.
[296] Daiber, A. Redox signaling (cross-talk) from and to mitochondria involves mitochondrial pores and reactive oxygen species. *Biochim Biophys Acta* **1797:**897-906; 2010.
[297] Dikalov, S. Cross talk between mitochondria and NADPH oxidases. *Free Radic Biol Med* **51:**1289-1301; 2011.

[298] Schulz, E.; Wenzel, P.; Munzel, T.; Daiber, A. Mitochondrial redox signaling: Interaction of mitochondrial reactive oxygen species with other sources of oxidative stress. *Antioxid Redox Signal* **20:**308-324; 2014.
[299] Daiber, A.; Di Lisa, F.; Oelze, M.; Kroller-Schon, S.; Steven, S.; Schulz, E.; Munzel, T. Crosstalk of mitochondria with NADPH oxidase via reactive oxygen and nitrogen species signalling and its role for vascular function. *Br J Pharmacol* **174:**1670-1689; 2017.
[300] Wenzel, P.; Kossmann, S.; Munzel, T.; Daiber, A. Redox regulation of cardiovascular inflammation - Immunomodulatory function of mitochondrial and Nox-derived reactive oxygen and nitrogen species. *Free Radic Biol Med* **109:**48-60; 2017.
[301] Cominacini, L.; Mozzini, C.; Garbin, U.; Pasini, A.; Stranieri, C.; Solani, E.; Vallerio, P.; Tinelli, I. A.; Fratta Pasini, A. Endoplasmic reticulum stress and Nrf2 signaling in cardiovascular diseases. *Free Radic Biol Med* **88:**233-242; 2015.
[302] Zhao, D.; Yang, J.; Yang, L. Insights for Oxidative Stress and mTOR Signaling in Myocardial Ischemia/Reperfusion Injury under Diabetes. *Oxid Med Cell Longev* **2017:**6437467; 2017.
[303] Mei, Y.; Thompson, M. D.; Cohen, R. A.; Tong, X. Autophagy and oxidative stress in cardiovascular diseases. *Biochim Biophys Acta* **1852:**243-251; 2015.
[304] Godfraind, T. Antioxidant effects and the therapeutic mode of action of calcium channel blockers in hypertension and atherosclerosis. *Philos Trans R Soc Lond B Biol Sci* **360:**2259-2272; 2005.
[305] DiNicolantonio, J. J.; Lavie, C. J.; Fares, H.; Menezes, A. R.; O'Keefe, J. H. Meta-analysis of carvedilol versus beta 1 selective beta-blockers (atenolol, bisoprolol, metoprolol, and nebivolol). *Am J Cardiol* **111:**765-769; 2013.
[306] Niccoli, G.; Montone, R. A.; Ibanez, B.; Thiele, H.; Crea, F.; Heusch, G.; Bulluck, H.; Hausenloy, D. J.; Berry, C.; Stiermaier, T.; Camici, P. G.; Eitel, I. Optimized Treatment of ST-Elevation Myocardial Infarction. *Circ Res* **125:**245-258; 2019.
[307] Kiyuna, L. A.; Albuquerque, R. P. E.; Chen, C. H.; Mochly-Rosen, D.; Ferreira, J. C. B. Targeting mitochondrial dysfunction and oxidative stress in heart failure: Challenges and opportunities. *Free Radic Biol Med* **129:**155-168; 2018.
[308] Munzel, T.; Gori, T.; Keaney, J. F., Jr.; Maack, C.; Daiber, A. Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications. *Eur Heart J* **36:**2555-2564; 2015.
[309] Landmesser, U.; Spiekermann, S.; Dikalov, S.; Tatge, H.; Wilke, R.; Kohler, C.; Harrison, D. G.; Hornig, B.; Drexler, H. Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. *Circulation* **106:**3073-3078; 2002.
[310] Maack, C.; Bohm, M. Targeting mitochondrial oxidative stress in heart failure throttling the afterburner. *J Am Coll Cardiol* **58:**83-86; 2011.
[311] Braunwald, E. Biomarkers in heart failure. *N Engl J Med* **358:**2148-2159; 2008.
[312] Bencsik, P.; Kupai, K.; Gorbe, A.; Kenyeres, E.; Varga, Z. V.; Paloczi, J.; Gaspar, R.; Kovacs, L.; Weber, L.; Takacs, F.; Hajdu, I.; Fabo, G.; Cseh, S.; Barna, L.; Csont, T.; Csonka, C.; Dorman, G.; Ferdinandy, P. Development of Matrix Metalloproteinase-2 Inhibitors for Cardioprotection. *Front Pharmacol* **9:**296; 2018.
[313] Botker, H. E.; Hausenloy, D.; Andreadou, I.; Antonucci, S.; Boengler, K.; Davidson, S. M.; Deshwal, S.; Devaux, Y.; Di Lisa, F.; Di Sante, M.; Efentakis, P.; Femmino, S.; Garcia-Dorado, D.; Giricz, Z.; Ibanez, B.; Iliodromitis, E.; Kaludercic, N.; Kleinbongard, P.; Neuhauser, M.; Ovize, M.; Pagliaro, P.; Rahbek-Schmidt, M.; Ruiz-Meana, M.; Schluter, K. D.; Schulz, R.; Skyschally, A.; Wilder, C.; Yellon, D.

M.; Ferdinandy, P.; Heusch, G. Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. *Basic Res Cardiol* **113:**39; 2018.
[314] Davidson, S. M.; Ferdinandy, P.; Andreadou, I.; Botker, H. E.; Heusch, G.; Ibanez, B.; Ovize, M.; Schulz, R.; Yellon, D. M.; Hausenloy, D. J.; Garcia-Dorado, D.; Action, C. C. Multitarget Strategies to Reduce Myocardial Ischemia/Reperfusion Injury: JACC Review Topic of the Week. *J Am Coll Cardiol* **73:**89-99; 2019.
[315] Zhang, Q.; Zhao, D.; Xie, W.; Xie, X.; Guo, M.; Wang, M.; Wang, W.; Liu, W.; Liu, J. Recent Trends in Hospitalization for Acute Myocardial Infarction in Beijing: Increasing Overall Burden and a Transition From ST-Segment Elevation to Non-ST-Segment Elevation Myocardial Infarction in a Population-Based Study. *Medicine (Baltimore)* **95:**e2677; 2016.
[316] Yu, L. M.; Di, W. C.; Dong, X.; Li, Z.; Zhang, Y.; Xue, X. D.; Xu, Y. L.; Zhang, J.; Xiao, X.; Han, J. S.; Liu, Y.; Yang, Y.; Wang, H. S. Melatonin protects diabetic heart against ischemia-reperfusion injury, role of membrane receptor-dependent cGMP-PKG activation. *Biochim Biophys Acta Mol Basis Dis* **1864:**563-578; 2018.
[317] Xiao, C.; Xia, M. L.; Wang, J.; Zhou, X. R.; Lou, Y. Y.; Tang, L. H.; Zhang, F. J.; Yang, J. T.; Qian, L. B. Luteolin Attenuates Cardiac Ischemia/Reperfusion Injury in Diabetic Rats by Modulating Nrf2 Antioxidative Function. *Oxid Med Cell Longev* **2019:**2719252; 2019.
[318] Thirunavukkarasu, M.; Penumathsa, S. V.; Koneru, S.; Juhasz, B.; Zhan, L.; Otani, H.; Bagchi, D.; Das, D. K.; Maulik, N. Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: Role of nitric oxide, thioredoxin, and heme oxygenase. *Free Radic Biol Med* **43:**720-729; 2007.
[319] Fourny, N.; Lan, C.; Seree, E.; Bernard, M.; Desrois, M. Protective Effect of Resveratrol against Ischemia-Reperfusion Injury via Enhanced High Energy Compounds and eNOS-SIRT1 Expression in Type 2 Diabetic Female Rat Heart. *Nutrients* **11**; 2019.

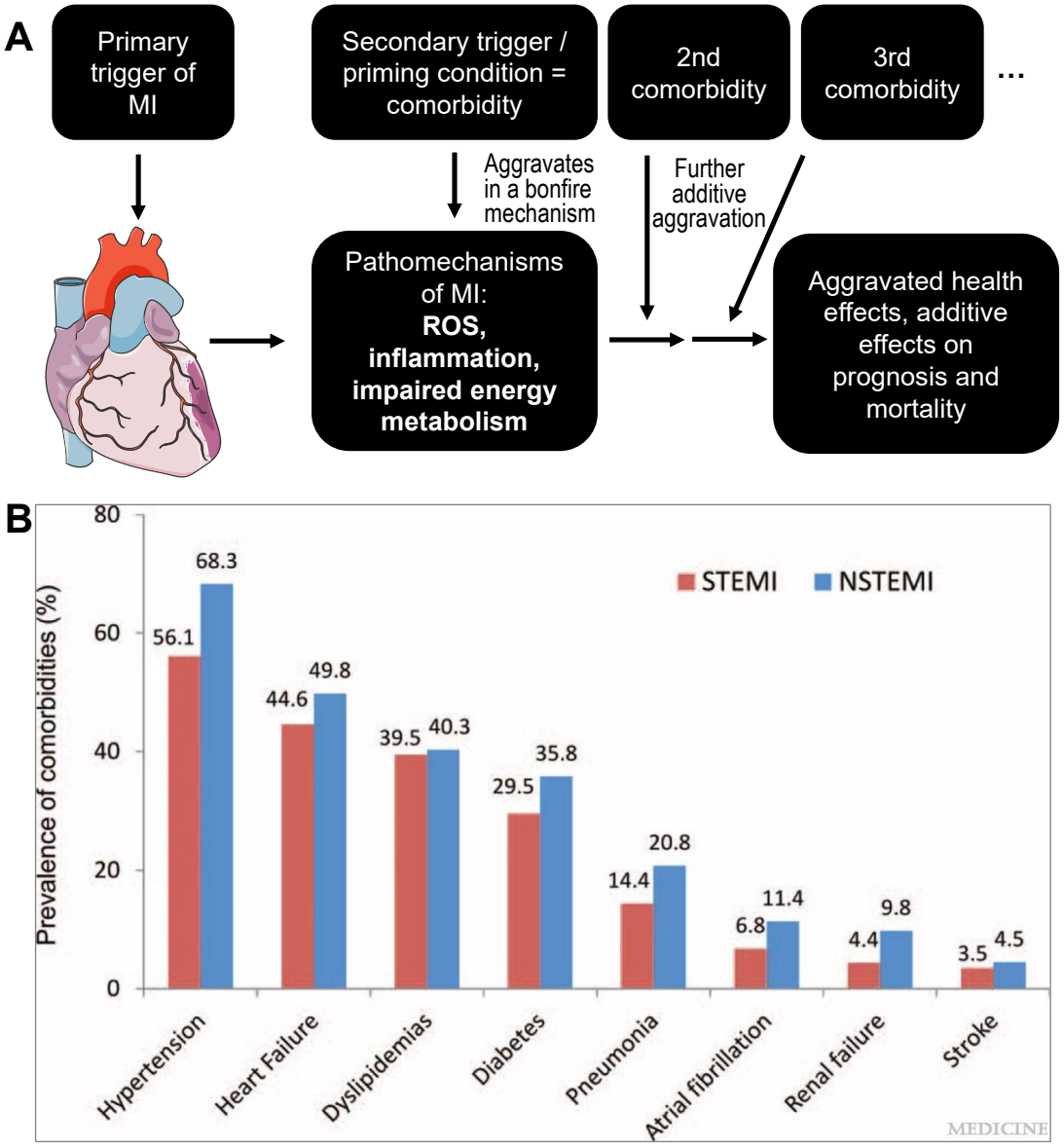

Figure 1. Proposed concept of comorbidities in myocardial infarction (MI) with oxidative stress and inflammation as central pathomechanisms. (A) Comorbidities aggravate adverse health outcomes of MI. Cartoon taken from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License. **(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 [315] with permission. Copyright © 2016, Wolters Kluwer Health.

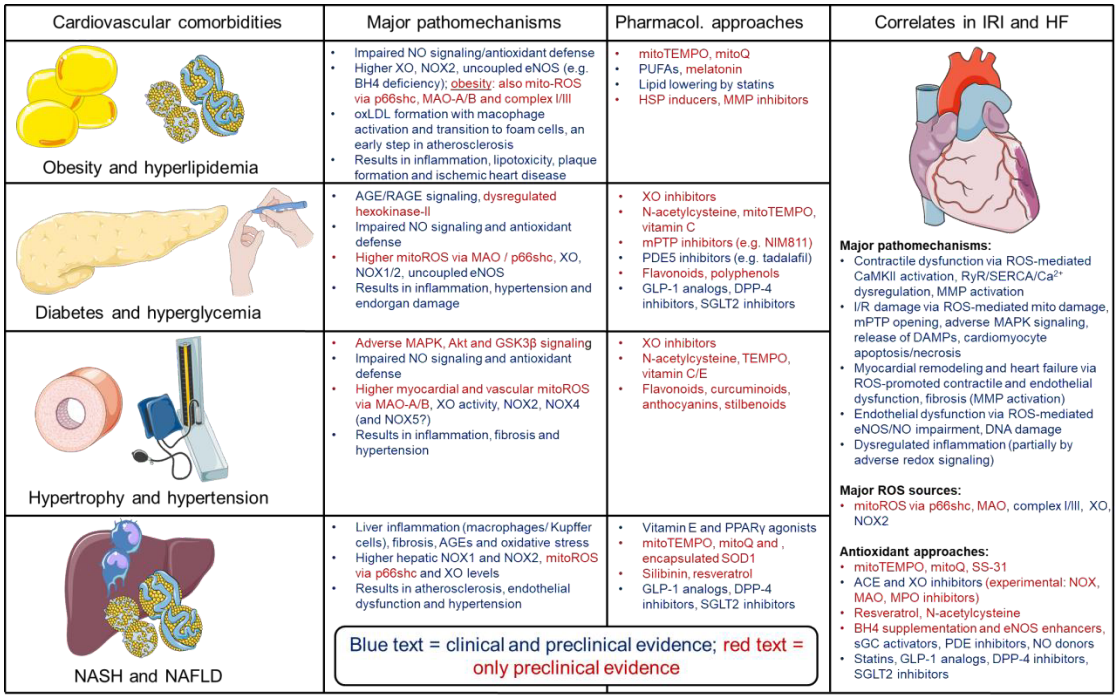

Figure 2. Similarities in pathomechanisms, oxidative stress pathways and pharmacotherapy of cardiovascular comorbidities and IRI damage or heart failure. Abbreviations: Akt, protein kinase B; AGE, advanced glycation end-products; BH4, tetrahydrobiopterin; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; DAMP, damage-associated molecular patterns; DPP4, dipeptidyl protease 4; eNOS, endothelial nitric oxide synthase; GLP-1, glucagon-like peptide-1; GSK-3β, glycogen synthase kinase-3β; HF, heart failure; HSP, heat shock protein; IRI, ischemia/reperfusion injury; MAO-A/B, monoamine oxidase-A/B; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; mPTP, mitochondrial permeability transition pore; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NOX, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase; OxLDL, oxidised low-density lipoprotein (LDL); PDE5, phosphodiesterase-5; PPARγ, peroxisome proliferator activated receptor γ; PUFA, polyunsaturated fatty acid; RAGE, receptor of advanced glycation end-products (AGE); ROS, reactive oxygen species; Ryr, ryanodine; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; sGC, soluble guanylate cyclase; SGLT2, sodium-glucose cotransporter-2; SOD, superoxide dismutase; XO, xanthine oxidase. Cartoons taken from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License. This is a summary of the discussed references in the present work.

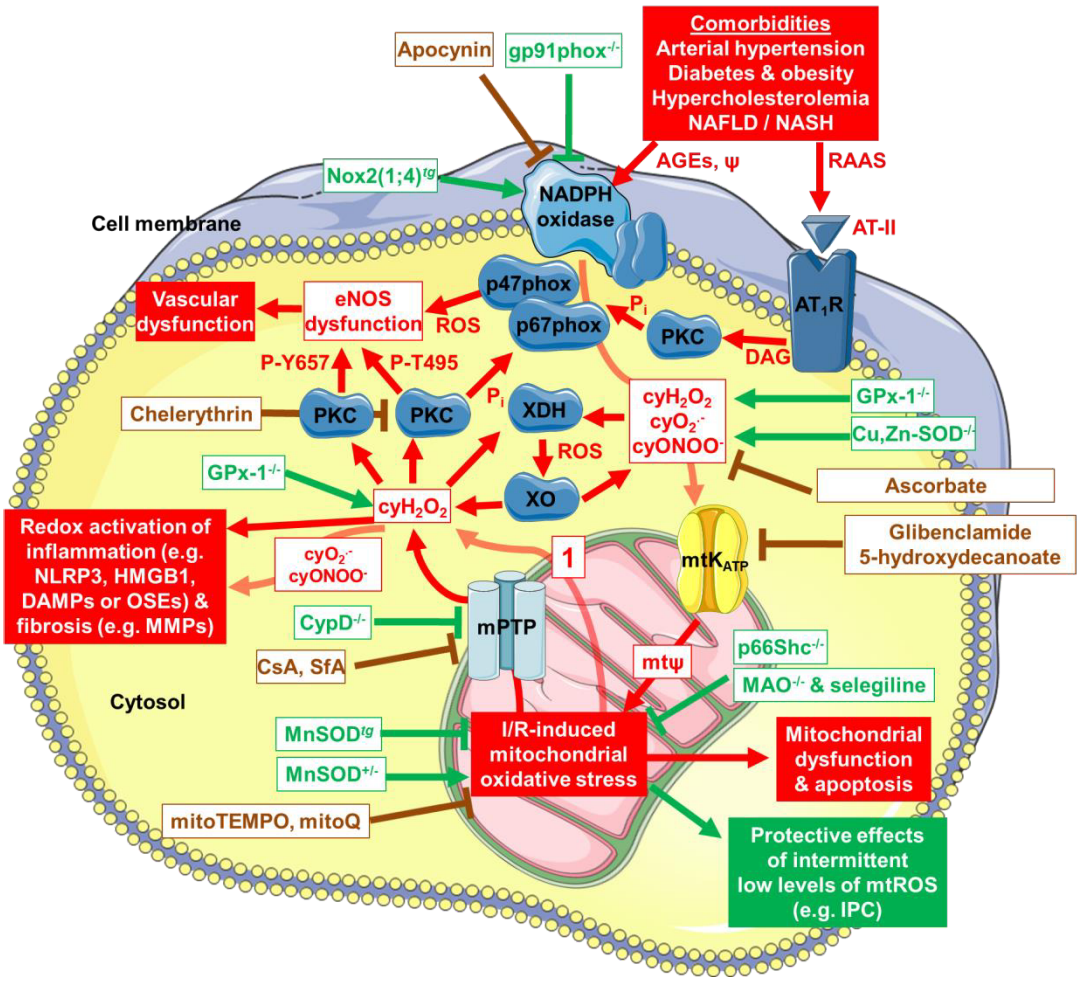

Figure 3. Proposed mechanism of the redox crosstalk between different ROS sources explaining the aggravation of ischemia/reperfusion damage by comorbidity factors. The green and brown boxes represent novel/unexplored genetic or pharmacological redox approaches to interfere with the vicious cycle between comorbidities and IRI (as observed during MI). 1: mitochondrial permeability due to other pathways than mPTP. **Abbreviations:** AT_1R, angiotensin-II receptor (type 1); cy, cytosolic; CsA, cyclosporine A; CypD, cyclophilin D; DAG, diacylglycerol; gp91phox, NOX2; MMPs, matrix metalloproteinases; 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 [296, 298, 299]. Cartoons taken from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License.

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) [62]	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) [63]	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) [64]	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) [65]	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) [66]	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) [67]	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) [106]	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) [107]	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) [108]	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) [116]	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) [152]	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) [153]	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) [162]	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) [165]	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) [164]	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) [163]	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) [316]	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) [156]	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) [168]	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) [317]	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) [170]	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) [169]	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) [172]	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) [318]	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) [319]	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) [171]	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.*