



Modulation of the nitric oxide/cGMP pathway in cardiac contraction and relaxation: Potential role in heart failure treatment

Rocco Mollace^{a,b,1}, Federica Scarano^{a,1}, Irene Bava^a, Cristina Carresi^c, Jessica Maiuolo^d, Annamaria Tavernese^a, Micaela Gliozzi^a, Vincenzo Musolino^d, Saverio Muscoli^e, Ernesto Palma^c, Carolina Muscoli^a, Daniela Salvemini^f, Massimo Federici^b, Roberta Macri^{a,*}, Vincenzo Mollace^{a,g,**}

^a Pharmacology Laboratory, Institute of Research for Food Safety and Health IRC-FSH, Department of Health Sciences, University Magna Graecia of Catanzaro, Catanzaro 88100, Italy

^b Department of Systems Medicine, University of Rome Tor Vergata, Italy

^c Veterinary Pharmacology Laboratory, Institute of Research for Food Safety and Health IRC-FSH, Department of Health Sciences, University Magna Graecia of Catanzaro, Catanzaro 88100, Italy

^d Pharmaceutical Biology Laboratory, Institute of Research for Food Safety and Health IRC-FSH, Department of Health Sciences, University Magna Graecia of Catanzaro, Catanzaro 88100, Italy

^e Division of Cardiology, Foundation PTV Polyclinic Tor Vergata, Rome 00133, Italy

^f Department of Pharmacology and Physiology, Saint Louis University School of Medicine, St. Louis, MO 63104, USA

Abbreviations: 3-MST, 3-mercaptopyruvate sulfurtransferase; 6-keto PGF1 α , 6-ketoprostaglandin F1alpha; 6MWT, six minute walking test; A/D, Active/De-active; AA, arachidonic acid; ACE-I, angiotensin-converting enzyme inhibitors; ADHF, acute decompensated heart failure; ADMA, asymmetric dimethylarginine; AMPK, 5'-AMP activated protein kinase; ARNI, angiotensin receptor-neprilysin inhibitors; BH2, dihydrobiopterin; BH4, tetrahydrobiopterin; BK, large conductance Ca²⁺ + -activated K⁺; BNP, brain natriuretic peptide; CAD, coronary artery disease; CaMKII, calcium/calmodulin-dependent protein kinase type II; cAMP, cyclic adenosine monophosphate; CBS, cystathionine- β -synthase; cGC, cytosolic guanylate cyclase; cGMP, cyclic guanosine 3',5'-monophosphate; CoQ10, Coenzyme Q10; COX, cyclooxygenase; COX-1, cyclooxygenase-1; COX-2, Cyclooxygenase-2; CSE, cystathionine- γ -lyase; CTEPH, chronic thromboembolic pulmonary hypertension; CVD, cardiovascular diseases; CYB5R3, cytochrome b5 reductase 3; Cys, cysteine; DBP, diastolic blood pressure; DEA/NO, NO donor diethylamine-NONOate; DHFR, dihydrofolate reductase; DOCA, deoxycorticosterone acetate; eNOS, endothelial nitric oxide synthase; F2-IsoPs, F2-isoprostanes; FMD, flow-mediated dilatation; GOSPEL, Goal Oriented Strategy to Preserve Ejection Fraction Trial; GPx, glutathione peroxidase; GSH, glutathione; GSNO, S-nitrosoglutathione; GSNOR, S-nitrosoglutathione reductase; GTP, guanosine-5'-triphosphate; H₂O₂, Hydrogen peroxide; H₂S, hydrogen sulfide; Hb, haemoglobin; HF, heart failure; HFpEF, preserved ejection fraction; HFrEF, reduced ejection fraction; HIF-1 α , Hypoxia-inducible factor 1-alpha; HNO, Nitroxyl; HPAEC, human pulmonary artery endothelial cells; HSP90, heat shock protein 90; I/R, ischemia/reperfusion; ID, Iron deficiency; IMT, intima-media thickness; iNOS, Inducible nitric oxide synthase; iPSC, induced pluripotent stem cell; KLF2, Krüppel type 2 factor; KO, knockout; LAD, left anterior descending coronary artery; LDL, low-density lipoprotein; LOX-1, low-density lipoprotein receptor-1; LTCC, L-type calcium channel; LVEF, Left ventricular ejection fraction; LVH, left ventricular hypertrophy; LVMI, Left ventricular mass index; MAP, mean arterial pressure; MDA, malondialdehyde; MI, myocardial infarction; mitoKATP, mitochondrial ATP-sensitive potassium; MLC, myosin light chain; MLCK, Ca²⁺ /calmodulin-dependent myosin light chain kinase; MLCP, myosin light chain phosphatase; MPTP, mitochondrial permeability transition pore; MRA, mineralocorticoid receptor antagonists; MYBPC, myosin-binding protein C; NADPH, nicotinamide adenine dinucleotide phosphate; NaHS, Sodium hydrosulfide; NF- κ B, Nuclear factor- κ B; nNOS, Neuronal nitric oxide synthase; NO/sGC/cGMP, NO/soluble guanylyl cyclase/cyclic GMP; NO, nitric oxide; NOS, nitric oxide synthase; O₂⁻, superoxide; O₂²⁻, superoxide anion; OH⁻, hydroxide; ONOO⁻, peroxynitrite; PAH, pulmonary arterial hypertension; PDE5A, Phosphodiesterase 5A; PDE5i, phosphodiesterase type 5 inhibitors; PDEs, phosphodiesterase enzymes; PGE2, prostaglandin E2; PGF2, prostaglandin F2; PGH2, prostaglandin H2; PGI2, prostaglandin G2; PGs, prostaglandins; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PKG-I, cGMP-dependent protein kinase type-I; PP1, protein phosphatase 1; PWV, pulse wave velocity; PYK2, Proline-rich tyrosine kinase 2; REPLACE, Riociguat Replacing PDE5 Inhibitor Therapy Evaluated Against Continued PDE5i" Therapy; RNS, reactive nitrogen species; ROCKs, Rho-associated protein kinases; ROS, reactive oxygen species; RyR, ryanodine receptor; SBP, systolic blood pressure; SGLT2, sodium glucose cotransporter 2; SERCA2a, sarcoplasmic/endoplasmic reticulum Ca²⁺ ATPase 2a; SERVE, SystEmic Right VEntricular size; sGC, soluble guanylate cyclase; SIDAMI, Sildenafil and Diastolic Dysfunction After Acute Myocardial Infarction; SNO, S-nitrosothiol; SOD, superoxide dismutase; SR, sarcoplasmic reticulum; STEMI, ST-elevation myocardial infarction; SVR, systemic vascular resistance; TAC, Transverse aortic constriction; TIMI, Thrombolysis In Myocardial Infarction; TLR4, Toll-like receptor 4; TRPC6, transient receptor potential channel 6; TXA2, thromboxane A2; Tyr, tyrosine; UBIAD1, UbiA Prenyltransferase Domain Containing 1; VEGF, vascular endothelial growth factor; VOCC, voltage-operated calcium channels; VSMCs, Vascular smooth muscle cells; XOR, xanthine oxidoreductase; β 3-Ars, β 3-adrenergic receptors; β -AR, β -adrenergic receptor.

* Corresponding author.

** Corresponding author at: Pharmacology Laboratory, Institute of Research for Food Safety and Health IRC-FSH, Department of Health Sciences, University Magna Graecia of Catanzaro, Catanzaro 88100, Italy.

E-mail addresses: robertamacri85@gmail.com (R. Macri), mollace@libero.it (V. Mollace).

<https://doi.org/10.1016/j.phrs.2023.106931>

Received 31 May 2023; Received in revised form 9 September 2023; Accepted 15 September 2023

Available online 16 September 2023

1043-6618/© 2023 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

ARTICLE INFO

Keywords:

NO/cGMP signalling
 NOS uncoupling
 Cardiac contraction/relaxation
 Oxidative/nitrosative stress
 Heart failure
 Therapeutic strategies

ABSTRACT

Evidence exists that heart failure (HF) has an overall impact of 1–2 % in the global population being often associated with comorbidities that contribute to increased disease prevalence, hospitalization, and mortality. Recent advances in pharmacological approaches have significantly improved clinical outcomes for patients with vascular injury and HF. Nevertheless, there remains an unmet need to clarify the crucial role of nitric oxide/cyclic guanosine 3',5'-monophosphate (NO/cGMP) signalling in cardiac contraction and relaxation, to better identify the key mechanisms involved in the pathophysiology of myocardial dysfunction both with reduced (HFrEF) as well as preserved ejection fraction (HFpEF). Indeed, NO signalling plays a crucial role in cardiovascular homeostasis and its dysregulation induces a significant increase in oxidative and nitrosative stress, producing anatomical and physiological cardiac alterations that can lead to heart failure. The present review aims to examine the molecular mechanisms involved in the bioavailability of NO and its modulation of downstream pathways. In particular, we focus on the main therapeutic targets and emphasize the recent evidence of preclinical and clinical studies, describing the different emerging therapeutic strategies developed to counteract NO impaired signalling and cardiovascular disease (CVD) development.

1. Introduction

Heart failure (HF) is a clinical syndrome whose diagnosis requires the presence of structural and/or functional cardiac anomaly resulting in elevated intracardiac pressure and/or insufficient cardiac output at rest and/or during exercise. Typical symptoms comprise fatigue, breathlessness and ankle swelling. In addition, elevated levels of natriuretic peptide and evidence of systemic or pulmonary congestion are present. Diagnosis is more expected in patients with a story of myocardial infarction, diabetes, coronary artery disease, hypertension and chronic kidney disease [1]. A classification of HF based on left ventricular ejection fraction (LVEF) has recently been introduced. This comprises HF with reduced EF (HFrEF, with LVEF ≤ 40 %), HF with mildly EF (HFmrEF, with LVEF 41–49 %) and HF with preserved ejection fraction (HFpEF, with LVEF ≥ 50 %) [2]. In developed countries, thanks to better management of cardiovascular disease (CVD), HF incidence is approximately 3\1000 person-year, while this data tends to increase with age [3].

The prevalence also increases with age, from 1 % for patients <55 years to 10 % for those ≥ 70 years. A recent observational study, “the European Society of Cardiology Heart Failure Long-Term (ESC-HF-LT) Registry”, showed that in an ambulatory setting, most of HF patients have HFrEF. Studies on hospitalized patients, on the other hand, highlighted a uniform distribution of patients with HFrEF and HFpEF [3,4].

Treatment options for patients with HFrEF have expanded significantly over the past few decades.

The core therapeutic approach that has been shown to improve survival, decrease risk of hospitalizations and reduce symptoms in patients with HFrEF, includes a triple therapy: angiotensin-converting enzyme inhibitors (ACE-I), or an angiotensin receptor-neprilysin inhibitors (ARNI), associated to beta-blockers and mineralocorticoid receptor antagonists (MRA) [1,5].

Neurohormonal modulation is central to the management of patients with HFrEF, but other pathways were targeted by drugs with different mechanisms of action. Among these, the antidiabetic medications, sodium glucose cotransporter 2 (SGLT2) inhibitors appear effective in preventing CVD [6]. Specifically, in the EMPEROR-Reduced and DAPA-HF trials, Empagliflozin and Dapagliflozin, respectively, were shown to reduce the risk of cardiovascular death or hospitalization in patients with HFrEF, with or without diabetes [7,8].

To date, treatment of patients with HFpEF with drugs commonly used in HFrEF has not been shown to convincingly reduce mortality and morbidity, displaying the contribution of non-cardiac comorbidities as

LVEF increases. Therefore, management of HFpEF comprise lifestyle interventions, (diet, exercise training), management of risk factors and comorbidities such as hypertension, diabetes, coronary artery disease, obesity, etc., associated with pharmacological therapies [1,9].

Loop diuretics remain the cornerstone of HFpEF management as they help attenuate volume overload, though thiazide diuretics and/or MRAs are helpful in patients with diuretic resistance [10]. More recently, large clinical trials showed the beneficial effects of SGLT2 inhibitors on outcomes in HFpEF patients. In particular, the results of EMPEROR-Preserved and DELIVER trials revealed that empagliflozin and dapagliflozin, respectively, reduce the risk of cardiovascular death and hospitalization, suggesting their potential use in patients with symptomatic stable HFpEF, regardless of diabetes mellitus [11–13].

The mechanisms underlying HFpEF are not yet fully understood and many factors are thought to be involved. While HFrEF is primarily due to a state of volume overloaded and systolic dysfunction, HFpEF results from systemic inflammation induced by concurrent comorbidities [14]. This condition leads to the production of reactive oxygen species (ROS) by endothelial cells, reduced nitric oxide (NO) availability and decreased activation of cyclic guanosine 3',5'-monophosphate (cGMP)-dependent protein kinase (PKG), resulting in concentric myocardial hypertrophy and diastolic dysfunction observed in HFpEF [15].

Evidence of low NO bioavailability and decreased cGMP is also present in patients with HFrEF, resulting in impaired vasodilation and aerobic exercise capacity, as well as decreased muscle power [16].

The identification and characterization of the complex machinery generating NO by endothelial cells and its role in endothelium-dependent vascular relaxation in 1990 by Salvador Moncada [17] represents a milestone in defining the fundamental mechanisms regulating vascular physiology and accommodation of regional blood flow. Indeed, the evidence that NO released by endothelial cells is a key player in the endogenous relaxation of vascular smooth muscle cells and that this is accompanied by a potent anti-platelet action has proven essential to understanding the pathophysiology of relevant disease states, including atherothrombosis, vascular injury and HF [18]. Furthermore, the identification of the crucial role of NO/cGMP signalling in cardiac contraction and relaxation has contributed to the assessment of intimate processes contributing to the pathophysiology of myocardial dysfunction with both reduced and preserved ejection fraction [19,20].

Finally, the recent development of NO-modulating molecules that potentiate cGMP through the inhibition of specific phosphodiesterases (PDEs), has renewed interest in this area, thereby representing potential tools in the approach to the treatment of HF [21,22].

The present review aims to re-evaluate the role of the NO/cGMP pathway in the mechanisms of cardiac contraction and relaxation under

¹ These authors contributed equally to this work.

basal conditions as well as in myocardial dysfunction and the potential for compounds targeting NO/cGMP in the treatment of HF. Finally, the emerging role of nutraceutical supplementation in oxidative and nitrosative stress and NO impairment are reviewed. In particular, we examined the recent preclinical and clinical evidence about the emerging therapeutic strategies developed to improve HF outcomes through the modulation of altered NO signalling.

2. Biomolecular mechanisms regulating NO release

NO is generated via the bioconversion of L-arginine to L-citrulline in a reaction catalysed by heme-containing enzymes identified as nitric oxide synthase (NOS). This occurs constitutively and Ca^{2+} -dependently in endothelial cells (eNOS) and neurons (nNOS), which release nM concentrations of this radical. On the other hand, an inducible, Ca^{2+} -independent isoform of NOS (iNOS) is expressed in inflammatory cells as a consequence of cytokine stimulation, leading to the release of mM concentrations of NO [23,24].

The regulation of constitutive nNOS and eNOS isoform expression is mediated by several mechanisms involving transcriptional, post-transcriptional and post-translational modifications, such as acetylation, protein-protein interaction, phosphorylation, S-nitrosylation, and S-glutathionylation (Fig. 1) [18,25,26].

On the other hand, regulation of iNOS isoform is mainly mediated by gene transcription under oxidative stress and pro-inflammatory conditions [27].

Furthermore, NO generation is known to occur in many tissues, including cardiomyocytes, via specific pathways that do not involve the bioconversion of L-arginine to L-citrulline [28].

The role of NOSs in vascular and non-vascular mechanisms of

cardiovascular regulation still needs to be clarified.

2.1. Biosynthesis of NO via the NO-synthase(s) system

eNOS is a 133 kDa protein encoded by the NOS3 gene, located in the 7q35–7q36 region of human chromosome 7. At the cardiovascular level, eNOS is mainly expressed in vascular and endocardial endothelial cells, in cardiac myocytes and also in platelets [29–31].

eNOS expression is regulated at different levels. Transcriptional activators include shear stress and stretch that induce transcription factors Nuclear factor- κ B (NF- κ B) and Krüppel type 2 factor (KLF2), as well as ROS, through oxidation of sensitive kinases (i.e., p38 mitogen-activated protein kinase) [32,33].

Under pathophysiological conditions, DNA methylation is involved in the epigenetic regulation of eNOS expression, and several microRNAs are involved in post-transcriptional regulation [34,35].

In addition, mechanisms such as acetylation, protein-protein interaction, phosphorylation, S-glutathionylation and S-nitrosylation, are involved in post-translational regulation of eNOS activity [18,26,36].

Under basal conditions, eNOS is anchored to plasma membranes and the perinuclear/Golgi region by co-translational N-myristoylation and post-translational palmitoylation of cysteine and is maintained in an inactive state within the caveolae, where it can interact with the scaffolding domain of caveolin 1 (principally expressed in endothelial cells) or caveolin 3 (principally expressed in cardiac myocytes) [37,38].

Stimulating factors, such as bradykinin, acetylcholine or vascular endothelial growth factor (VEGF), result in deacetylation of eNOS and an increase of intracellular Ca^{2+} , with detachment of the caveolin–eNOS inhibitory interaction. This is followed by Ca^{2+} -calmodulin complex binding, recruitment of heat shock protein 90 (HSP90) and serine/

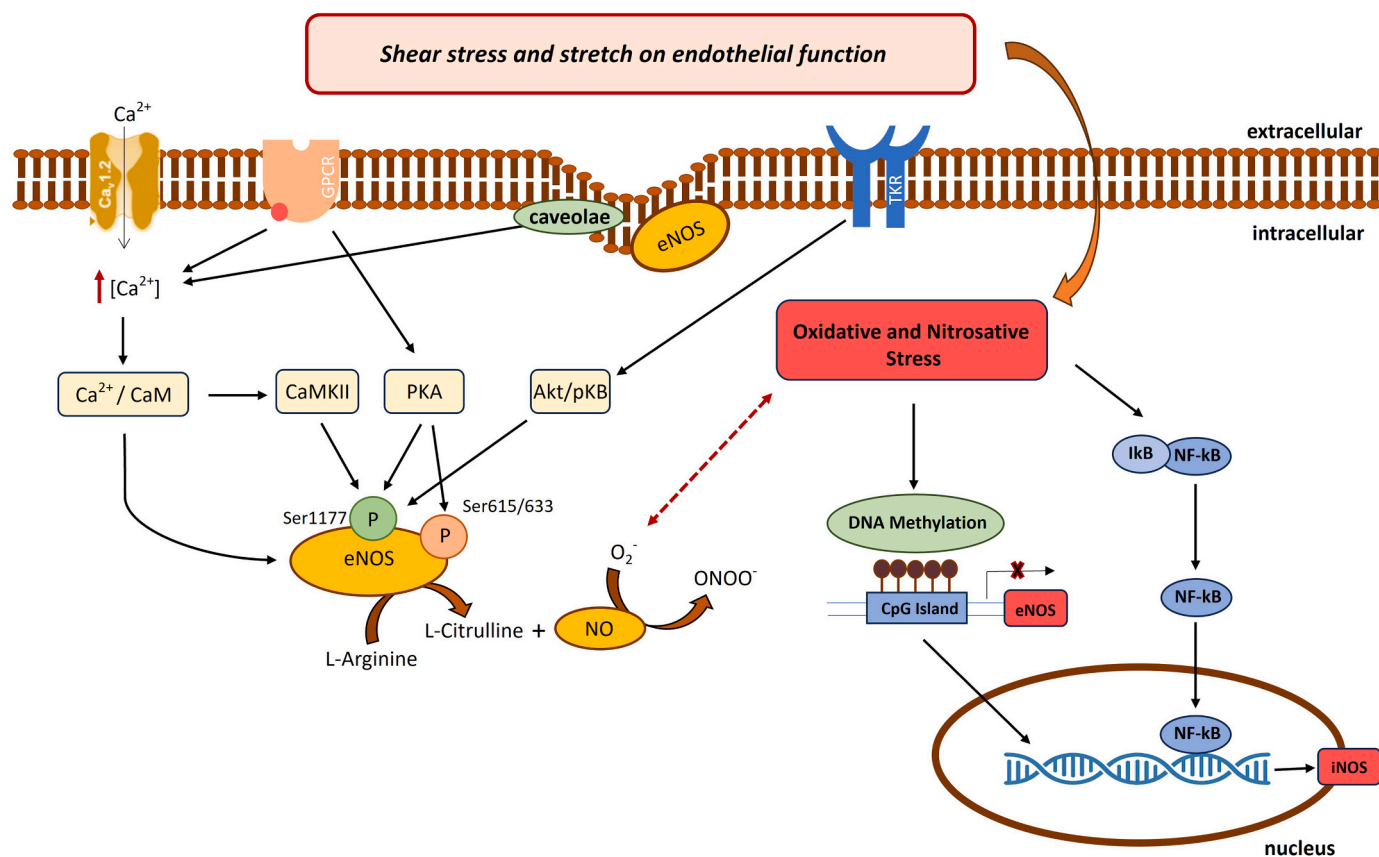


Fig. 1. Shear stress and stretch on endothelial function: oxidative/nitrosative stress and eNOS/iNOS modulation. The figure shows the interaction between oxidative and nitrosative stress induced by shear stress on endothelial cells and the role of transcriptional and post-translational modulation of eNOS and iNOS in the enhancement of endothelial cells damage.

threonine protein kinase AKT/PKB. AKT/PKB phosphorylates eNOS on Ser1177 (human), which lead to enhanced activity of the enzyme and, thus, increased NO production [39,40]. Ser1177 can be phosphorylated by other kinases, such as 5'-AMP activated protein kinase (AMPK), cAMP-dependent protein kinase (PKA) and calcium/calmodulin-dependent protein kinase type II (CaMKII). Phosphorylation sites Ser615 and Ser633 (by PKA) or Src-dependent phosphorylation of eNOS on Tyr81, are also associated with increased NO production [40,41]. Among negative regulatory sites, phosphorylation at Ser114 and Tyr657 have been associated with attenuation in NO production [42]. In addition, S-nitrosylation of cysteine residues (Cys94 and Cys99) suppresses the activity of eNOS enzyme and under agonist stimulation, eNOS is quickly denitrosylated with kinetics reflecting the observed increase in eNOS activity [43]. Finally, under oxidative stress, S-glutathionylation of eNOS reversibly decreases eNOS activity, with a concomitant increase in superoxide anion (O_2^-) generation [44].

2.2. Inducible nitric oxide synthase (iNOS)

iNOS is a 130 kDa protein encoded by the NOS2 gene located on 17q11.2-q12 of human chromosome 17. The level of NO produced by iNOS is mainly regulated at the transcriptional level [45]. Oxidative stress and inflammation are major transcriptional regulators of iNOS in various cell types, including endothelial cells, cardiac myocytes, VSMCs, nerve cells, and fibroblasts [46,47]. Unlike eNOS and nNOS, active iNOS dimer generates NO independently of intracellular Ca^{2+} concentrations and preserves a high NO production until substrate and cofactor depletion or enzyme degradation [48]. Thus, in the cardiovascular system, iNOS expression is mainly associated with pathological remodelling [49].

2.3. NOS-independent release of NO

NO can be produced independently from NOS by nitrate-nitrite pathway. After an intake of nitrate-rich diet (i.e., vegetables such as spinach or beet roots), nitrate is rapidly absorbed in the gastrointestinal tract. Approximately 25 % of the circulating nitrate is actively extracted by the salivary glands and then concentrated and reduced to nitrite by nitrate reductase enzymes. The nitrite formed is continuously swallowed and may enter the systemic circulation after absorption [50,51]. Subsequently, nitrite may be reduced through the nitrite reductase activity of different proteins, such as deoxymyoglobin in myocardium and vascular wall, deoxyhaemoglobin in erythrocytes, mitochondrial electron transport system, xanthine oxidoreductase (XOR) or even NOSs themselves [52–54].

In this way, nitrites as a pool of NO can influence different parameters and processes including platelet aggregation, blood pressure and cardioprotection both in HF and ischemia [55,56]. Under ischemic conditions, both homogenized rat and human myocardium generate NO from nitrite in a XOR activity-dependent reaction [57]. A protective role for XOR-catalysed NO production was also demonstrated in a rat model of renal ischemia/reperfusion (I/R) injury, in which topical sodium nitrite administration during reperfusion after bilateral renal ischemia significantly attenuated renal dysfunction and injury [58]. This protection was also observed in clinical setting, where low-dose of sodium nitrite before ischemia prevented the peak flow-mediated dilation decrease [59]. The cardioprotection has been due to mitochondrial respiration inhibition and ROS production, and to revascularization increase through mechanisms involving the mobilization and migration of regenerating endothelial cells, associated with an attenuation of myoblast apoptosis [53,60]. Furthermore, mechanisms that may provide beneficial effects of nitrite in I/R injury include decreased platelet reactivity and inflammatory cell recruitment, as platelet thrombus formation, endothelial protrusion and inflammation underlie of microvascular injury after reperfusion [55,61,62].

HF with preserved ejection fraction (HFpEF) is characterized by

exercise intolerance and poor long-term prognosis [63]. Of note, nitrite therapy improved LV dimensions and attenuated circulating and cardiac brain natriuretic peptide (BNP) levels in mice with Transverse aortic constriction (TAC) [64]. In a double-blind, randomized clinical trial, acute sodium nitrite infusion in subjects with HFpEF attenuated exercise haemodynamic derangements and improved ventricular performance with stress [65]. Further nitrite and nitrate clinical applications are treated below. Interestingly, in addition to the effect of the gut microbiome on nitrate reduction, oral supplementation of probiotics such as *Akkermansia muciniphila* improved the endothelial dysfunction in ApoE^{-/-} mice through activation of the eNOS\NO pathway [66].

3. NO-induced modulation of cardiovascular responses

NO maintains cardiovascular health by activating two distinct pathways: an indirect pathway through the stimulation of soluble guanylate cyclase (sGC), which catalyses the intracellular synthesis of cyclic guanosine 3',5'-monophosphate (cGMP) and the activation of PKG, or through direct S-nitrosylation of proteins [67,68]. NO is a paracrine signalling molecule that can diffuse from endothelial cells to vascular smooth muscle cells (VSMCs), vessel lumen, or cardiac myocytes; but it can also act as an autocrine signal, especially in cardiac myocytes [69]. Paracrine NO signalling is regulated by haemoglobin (Hb) α , expressed in human and mouse arterial endothelial cells and in particular at the level of myoendothelial junction. There, endothelial Hb α haem iron in the Fe³⁺ state allows NO diffusion, while this signalling is disrupted when Hb α is reduced to the Fe²⁺ state by endothelial cytochrome b5 reductase 3 (CYB5R3) [70]. Given the high reactivity of NO rather than the free radical itself, nitrosylated intermediates have been suggested, such as Fe-nitrosyl-heme complexes that could be exchangeable between different heme-containing proteins among which sGC, ensuring safer and coordinate delivery of the signal within and between cells [71]. In addition to the NOS\NO\GC signalling pathway, a direct mechanism of S-nitrosylation has also emerged as a complementary pathway in vascular regulation by NO. In the following sections, we analysed the role and the regulation of sGC, PKG, and S-nitrosylation and the interplay between hydrogen sulfide (H₂S) and NOS\NO signalling, as well as the crosstalk between the COX and NOS pathways.

3.1. Soluble guanylate cyclase (sGC)

sGC is the main NO receptor involved in regulation of several cardiovascular signalling pathways. sGC is an ~150-kDa heterodimer composed of α and β subunits, with $\alpha1/\beta1$ isoform predominating in vascular tissue and an $\alpha2/\beta1$ isoform predominating in cardiac and nerve cells [72]. Binding of NO to the heme group of sGC induces a conformational change that activates the catalytic domain of sGC and triggers the formation of the second messenger cGMP from GTP. At the intracellular level, cGMP exerts its effects through interaction with a group of proteins known as intracellular cGMP receptor proteins [73]. The cGMP amount regulation is exerted through PDEs, which in turn hydrolyse the phosphodiester bond within cGMP to GMP [74]. In total, 11 different isoenzymes have been identified, where some PDEs (PDE5, PDE6 and PDE9) are selective for cGMP, some are specific for hydrolysis of cyclic adenosine monophosphate (cAMP), others hydrolyse both (PDE1, PDE2, and PDE3) [75]. The role of cGMP in promoting cardiomyocyte relaxation has increased interest in the development of pharmacological interventions to prolong or potentiate the effects of cGMP-mediated processes by inhibiting its degradation [76]. Of note, PDE2 is the only cAMP hydrolysing PDE that is allosterically activated by cGMP [77]. PDE2 inhibition is associated with an antihypertrophic effect both *in vitro* and *in vivo*, due to cAMP-dependent activation of PKA [78]. Several studies have shown that inhibition of cGMP hydrolysis has a beneficial effect against cardiac remodelling (reviewed below), including in human HF and in mouse models, where PDE5 inhibition attenuated cardiac remodelling induced by pressure overload after TAC

[79,80]. Importantly, oxidative stress can limit the binding of NO to sGC, either through oxidation of its heme Fe^{2+} to the Fe^{3+} state or through loss of heme, resulting in the accumulation of inactive sGC [81, 82].

3.2. cGMP-dependent protein kinase activation

cGMP-dependent protein kinase type-I (PKG-I) is a serine/threonine-specific protein kinase that is activated by cGMP. PKG-I is implicated in the regulation of vascular tone, platelet aggregation and VSMC proliferation, through the expression of two isoforms, PKG-I α (mainly in VCMCs and cardiac myocytes) and PKG-I β (in platelets) [83]. The cGMP-induced smooth muscle relaxation is primarily mediated by cGMP-dependent PKG activation that involves different molecular events culminating in a decrease in intracellular Ca^{2+} concentration and a reduction in the sensitivity of the contractile system [84,85].

An important factor in the regulation of vascular tone is the level of myosin light chain (MLC) phosphorylation, which is controlled by the balance between Ca^{2+} /calmodulin-dependent myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP) [86]. PKG-I α exerts its effect on vasodilation through intracellular Ca^{2+} concentration reduction, which decreases myosin light chain kinase activity, and instead increases myosin light chain phosphatase activity, both directly and by transforming protein RhoA phosphorylation in order to inhibit Rho-associated protein kinases (ROCKs); these events lead to a decrease of myosin light chain-actin binding [87]. In addition, in VSMC, PKG-I α mediates reduction of intracellular Ca^{2+} concentration through stimulation of large conductance Ca^{2+} -activated K^{+} (BK) channels, leading to a rapid efflux of K^{+} and membrane hyperpolarization, and inhibition of voltage-operated calcium channels (VOCC) [88]. Another mechanism by which PKG-I α mediates dilatation of VSMC is the phosphorylation of phospholamban, first demonstrated in isolated rat aorta precontracted with norepinephrine, resulting in increased Ca^{2+} /ATPase pump activity and reuptake of Ca^{2+} into the sarcoplasmic reticulum (SR) [88,89]. Cardiac PKG-I α induces relaxation through phosphorylation of both L-type calcium channel (LTCC), with decreased calcium currents, and phospholamban, with increased Ca^{2+} reuptake into the sarcoplasmic reticulum (SR). Furthermore, NO donor diethylamine-NONOate (DEA/NO) was shown to exert negative inotropic and relaxant effects in rat ventricular myocytes through a reduction in myofilament Ca^{2+} sensitivity, mediated exclusively by PKG phosphorylation of troponin I [90]. Another phosphorylation site was identified after pharmacological activation of PKG-I α in mice subjected to LV pressure overload (TAC). Indeed, PKG-I α is able to inhibit pathological cardiac remodelling through the increase of phosphorylation of myosin-binding protein C (MYBPC) at Ser 273 [91]. Of particular note, PKG-I exerts a negative feedback control on cGMP concentration, limiting its accumulation in VSMCs and in cardiomyocytes induced by NO-donors through PDE5 activation. Binding of cGMP to a noncatalytic GAF domain of PDE5 and subsequent phosphorylation by PKG-I has been shown to enhance PDE5 activity and maintain activation [92,93]. In human VSMCs, PDE5 activation by 8-Br-cGMP-induced PKG-I activation is associated with PDE5 dephosphorylation by protein phosphatase 1 (PP1), suggesting that phosphorylation/dephosphorylation mechanisms may be key steps in the regulation of SM relaxation/contraction cycles [94]. Furthermore, a rapid increase in cGMP levels, followed by a rapid cGMP decline, in NO-stimulated human platelets, demonstrated that PDE5 phosphorylation is also involved in NO-induced desensitization of the cGMP response [95,96].

An alternative pathway of PKG-I α activation was identified in which PKG-I α is activated in a cGMP-independent manner through the oxidation of the Cys42 sites of the dimer, resulting in the formation of disulfide bonds [97]. The oxidative activation of PKG-I α and its role in the regulation of blood pressure homeostasis was studied in the knock-in (KI) mouse expressing only a "redox-dead" C42S version of PKG-I α , where single atom substitution prevented the vasodilator effect of

Hydrogen peroxide (H_2O_2) on resistance vessels and caused hypertension *in vivo* [98]. Furthermore, it has been observed that the dilatory effect of oxidant-induced PKG-I α activation was due to PKG-I α translocation to the cell membrane, opening of smooth muscle BK channels and subsequent hyperpolarization and dilation of coronary arterioles [99]. In cardiomyocytes, oxidative activation of PKG-I α maintains oxidized PKG-I α in the cytosol, increasing PDE5 activity. Conversely, redox-dead PKG-I α translocates to the plasma membrane, enhancing suppression of the transient receptor potential channel 6 (TRPC6), with less hypertrophy and fibrosis [100].

3.3. Protein S-nitrosylation

S-nitrosylation is a post-translational modification characterized by the covalent linkage between a nitrosyl group and a reactive thiol group of a cysteine to form S-nitrosothiol (SNO), which plays a key role in NO-mediated signal transfer [101]. Furthermore, in the presence of low ROS levels, S-nitrosylation not only prevents the binding between NO and ROS, but also protects the thiol groups of cysteine from ROS-mediated oxidation. However, this protective effect is lost in the presence of high levels of ROS, so NO could react with ROS to form reactive nitrogen species (for example, peroxynitrite (ONOO^-)) [101]. There are emerging data suggesting that S-nitrosylation of several protein targets plays an important role in cardioprotection [102]. Indeed, S-nitrosylation is associated with reduced I/R injury through inhibition of LTCC and consequent reduced Ca^{2+} uptake into myocytes, and activation of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a), with further reduction of cytosolic Ca^{2+} [103]. At the vascular level, the protective effect of S-nitrosylation is mediated by the downregulation of $\text{Ca}_v1.2$ channels, resulting in a significant reduction in blood pressure [104].

Each NOS isoform can mediate selective S-nitrosylation of target proteins and specificity of S-nitrosylation is related to intracellular compartmentalization of NOS [105]. For example, eNOS localized within the caveolae is close to LTCC, resulting in modulation of Ca^{2+} flux after LTCC S-nitrosylation, whereas nNOS colocalization with the ryanodine receptor (RyR) modulates SR Ca^{2+} cycling [106]. In particular, in skeletal muscle, hyper-nitrosylation of RyR has been shown to cause SR calcium leak and calstabin-1 depletion, contributing to muscle weakness in muscular dystrophy [107]. The importance of cardiac RyR S-nitrosylation was highlighted in a study by Gonzalez et al., in which nNOS depletion was associated with decreased S-nitrosylation and increased RyR oxidation, resulting in increased diastolic Ca^{2+} and depression of myocardial contractility [108].

Furthermore, S-nitrosylation has a directly effect on NOS signalling. S-nitrosylated sGC showed reduced sensitivity to NO-induced activation [109]. Of note, sGC desensitization, through S-nitrosylation nitroglycerin-dependent, is the basis of tolerance to nitrates [110]. An *in vitro* study showed that S-nitrosylation of dihydrofolate reductase (DHFR), mediated by S-nitrosoglutathione (GSNO), a NO donor, prevented DHFR degradation and eNOS uncoupling via regeneration of tetrahydrobiopterin (BH4), an essential eNOS cofactor [111]. The balance between S-nitrosylation and denitrosylation is modulated through NOS and S-nitrosoglutathione reductase (GSNOR) enzyme activity, respectively. Mice lacking GSNOR (GSNOR $^{-/-}$ mice), showed increased cardiomyocyte proliferation and recovered better than wild type mice post-myocardial infarction (MI) [112]. Furthermore, GSNOR deficiency was recently shown to promote cardiomyocyte differentiation and maturation, and to accelerate induced pluripotent stem cell (iPSC) maturation, further supporting the cardioprotective role of S-nitrosylation [113]. Conversely, GSNOR overexpression prevents pathological left ventricular hypertrophy (LVH) induced by chronic β -adrenergic receptor (β -AR) activation [114]. Therefore, maintaining the balance between S-nitrosylation and denitrosylation is essential for proper NOS \NO regulation of vascular tone and cardiac contractility [115].

3.4. H₂S and NOS signalling modulation

In the last decade, the role of H₂S, a gaseous mediator, has been recognized as an important regulator of the vascular system and in particular, as an enhancer of vascular NO signalling [116]. At the vascular level, H₂S production is mainly catalysed by three enzymes: cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), 3-mercaptopyruvate sulfurtransferase (3-MST) using L-cysteine or homocysteine as substrates [117,118].

H₂S has been shown to acts as an enhancer of the eNOS\ sGC\ cGMP\ PKG pathway by targeting NO production and its downstream signalling. In endothelial cells, H₂S doubles the NO generation from eNOS, promoting eNOS activation through phosphorylation at Ser1177 by AKT [119]. In addition, H₂S is able to increase NO production in an eNOS-independent manner, involving XOR-reduction of nitrite in NO, suggesting a potential role of H₂S in the therapeutic enhancement of nitrite supplementation [120]. An *in vitro* study showed that H₂S also exerts an antioxidant effect, reducing the amount of ferric cytosolic guanylate cyclase (cGC) and increasing the amount of NO-sensitive ferrous cGC [121]. Acting on downstream signalling mediators, evidence indicates that H₂S exerts a vasodilatory effect through inhibition of cGMP degradation, specifically by acting as a PDE5i [122,123]. Another layer of interaction between H₂S and NO signalling occurs at the level of PKG activation, through H₂S-catalyzed formation of an activating interprotein disulfide within PKG-α [124].

This cooperative interaction between H₂S and NOS signalling appears to enhance the NOS-dependent cardioprotective and vasodilatory effects, as demonstrated in a mouse model of I/R injury, in which H₂S pre-treatment reduced infarct size, thus suggesting a potential therapeutic approach for H₂S precursors (reviewed below) [125].

3.5. COX-modulation by NO

The NOS and cyclooxygenase (COX) pathways produce important mediators of tissue homeostasis and pathophysiological processes, sharing several similarities. Both NOS and COX have constitutive and inducible isoforms. Cyclooxygenase-2 (COX-2) is the inducible form of the COX enzyme, whose synthesis is triggered by the same proinflammatory prostaglandins (PGs) at the site of inflammation [126,127].

The COX pathway catalyses the conversion of arachidonic acid to prostaglandin H₂ (PGH₂), mediated by both cyclooxygenase-1 (COX-1) and COX-2, which is subsequently converted to a variety of prostaglandins, such as prostaglandin E₂ (PGE₂), prostaglandin F₂ (PGF₂), thromboxane A₂ (TXA₂) and prostaglandin G₂ (PGI₂), by specific isomerase enzymes [128].

Therefore, in inflammatory states, NO and PGs are released simultaneously in micromolar amounts, suggesting the involvement of both in the pathogenesis of many disease states.

Furthermore, several lines of evidence suggest a constant crosstalk between NO and PG release that occurs at different levels [129].

Indeed, NO can directly interfere with COX expression and PG biosynthesis and PGs generated from COX isoforms can interfere with NOS activity [130].

In 1993, Salvemini et al. demonstrated for the first time that COX activity is modulated by NO [131]. In this study, exogenous NO application was shown to increase COX-1 activity both *in vitro* and *in vivo*, leading to a 7-fold increase in PGE₂ formation. In the same study, exposure of IL-1β-stimulated fibroblasts to NO gas or NO donors increased COX-2 activity at least 4-fold, suggesting that COX regulation by NO is a potent mechanism used to amplify the inflammatory response [131]. Subsequently, exposure of endothelial cells to NO donors (glyceryl trinitrate, sodium nitroprusside, or 3'-morpholininosydnonimine) was shown to increase COX activity and PGI₂ release, inhibiting thrombin-induced platelet aggregation at least 10-fold [132]. Therefore, NO (and NO donors) exert vasodilatory and antithrombotic effects both

by activation of sGC and cGMP increase and by activation of COX-1 in endothelial cells with prostacyclin formation and cAMP increase [133]. Given the increased COX-1 and COX-2 activity NO induced, the study investigated whether the endogenously produced NO was also able to modulate COX-2 activity. For this purpose, RAW-264.7, a macrophage cell line, was stimulated with lipopolysaccharide (LPS) to induce iNOS and COX-2 activity and the consequent production of a large amount of NO and PGs, respectively [134,135].

Treatment with selective and non-selective iNOS inhibitors, as expected, reduced NO production from these cells. Of notable importance was the observation that inhibition of NO production was associated with inhibition of PG production, suggesting that NO endogenously released from macrophages stimulated COX-2 activity independently of its known effects on sGC. In fact, methylene blue-mediated sGC inhibition reduced cGMP production in fibroblasts without influencing the ability of NO to induce COX activity and PGs production [131,132].

Several studies have shown divergent effects of NO on COX isoforms. Indeed, NO was observed to down-regulate PGE₂ release, associated with reduced COX-2 expression, both in LPS-stimulated macrophages and in COX-1 deficient cells [136].

On the other hand, COX inhibition by aspirin or indomethacin, has been associated with reduced NOS activity in human platelets. This effect was mediated by thromboxane A₂ inhibition or reduction in intracellular Ca²⁺ [137].

The evidence above indicates that the modulatory effect of NO on COX activity may differ according to the cell type used for experimental procedures and to the nature and intensity of the stimulus leading to activation of PGs biosynthesis [129].

Therefore, knowledge of the molecular mechanisms underlying NOS and COX activation is fundamental for understanding the mutual interactions between NO and COX. As first reported by Tsai et al., there is no evidence of a significant direct interaction between NO and the heme prosthetic group of COX enzyme [138]. Other mechanisms have been

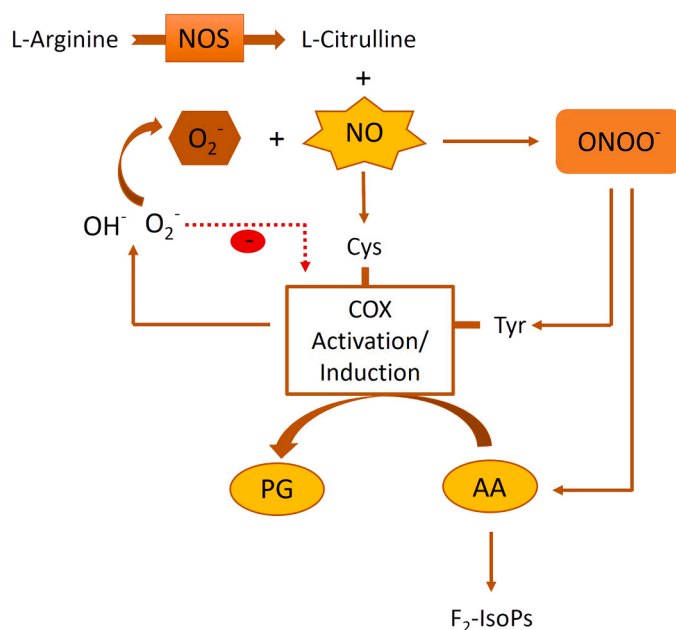


Fig. 2. Modulation of COX activity by NO. NO increases COX activity through the interaction with superoxide anion and the subsequent reduction of negative feedback mechanism, the formation of nitrothiols by S-nitrosylation of cysteine residues in the catalytic domain of the enzyme and the formation of ONOO⁻, which in turn enhances COX activity and PGs production. In addition, ONOO⁻ mediates the activation of COX enzymes through the oxidative modification of tyrosine residues in the polypeptide backbone. Furthermore, ONOO⁻ can oxidize the COX substrate arachidonic acid, producing vasoconstrictors F₂-isoprostanes, and consequently reduce prostacyclin production.

proposed to explain the modulation of COX activity by NO (Fig. 2).

Since COX activity is associated with the production of superoxide anions, which through a negative feedback mechanism inhibits its activation; it has been proposed that NO, acting as an antioxidant, increases COX activity [139]. In particular NO, interacting with superoxide anion, reduces the amount of radical normally necessary for COX auto-inactivation.

A second proposed mechanism is the formation of nitrothiols by NO-mediated S-nitrosylation of cysteine residues in the catalytic domain of COX enzymes. This mechanism determines COX activation in a heme-independent manner [140]. The third mechanism proposed is the formation of ONOO⁻, the product of reaction of NO with superoxide radical [141]. ONOO⁻ activates *in vitro* COX-1 and COX-2 activity [142]. In arterial smooth muscle cells, addition of exogenous ONOO⁻ activates COX-1 and PGE₂ production [143]. The mechanisms involved in ONOO⁻-mediated activation of COX enzymes have yet to be defined but could involve oxidative inactivation or modification of key amino acid residues in the polypeptide backbone. Furthermore, ONOO⁻ can oxidize the COX substrate arachidonic acid, producing vasoconstricting F₂-isoprostanes, and can block prostacyclin synthase activity and thereby reduce prostacyclin production [144]. ONOO⁻ is also involved in NF-κB activation that is a potent inducer of COX-2 and iNOS in inflamed cells [129]. A final proposed mechanism is that iNOS itself binds to COX-2 and S-nitrosylates at the Cys-526 residue, improving its catalytic activity. This interaction is specific for COX-2, because iNOS does not have an interaction with COX-1 [144]. It has been highlighted that iNOS is essential for the enzymatic activity of newly synthesized COX-2 in cardiac tissue during myocardial ischemia. In particular, in morphine-induced delayed cardioprotection model, the infarct-sparing effect after morphine administration was completely abolished by the COX-2-specific inhibitor. Furthermore, knockout (KO) of the iNOS gene or iNOS selective inhibitor administration did not attenuate the increased COX-2 expression after morphine pre-treatment, but completely abolished the upregulation of myocardial PGE₂ and 6-keto-prostaglandin F₁α (6-keto PGF₁α) [145,146].

Since NO-mediated COX regulation is a potent mechanism for NO modulation of inflammatory response progression, a more complete understanding of the molecular mechanisms involved in COX-NO crosstalk regulation will allow the identification of important molecular targets for future pharmacological interventions.

NOS, nitric oxide synthase; NO, nitric oxide; COX, cyclooxygenase; ONOO⁻, peroxyxynitrite; PG, prostaglandin; O₂⁻, superoxide; OH⁻, hydroxide; Cys, cysteine; Tyr, tyrosine; AA, arachidonic acid; F₂-IsoPs, F₂-isoprostanes.

4. Role of NO in myocardial contraction/relaxation

Several *in vivo* studies using pharmacological mechanisms of inhibition or genetic deletion with loss of NOS (nNOS and eNOS) function have shown more severe myocardial damage after I/R injury [147–149].

In contrast, upregulation of cardiac-myocyte NOS expression produces significant protection to counteract the I/R damage, by the prevention of mitochondrial permeability transition pores opening [150]; in particular, eNOS [151] and nNOS [152] overexpression results in improved left ventricular (LV) function and in the reduction of infarct size [148].

In vitro and *in vivo* evidence suggests that the eNOS inhibition in I/R injury is due to the activation of cardiac Proline-rich tyrosine kinase 2 (PYK2), a redox-sensitive kinase [153]; that leads to the phosphorylation of a tyrosine residue (Tyr656 mouse sequence; Tyr657 human sequence) of eNOS [154]; consequently, the pharmacological inactivation of PYK2 could represent a potential target in cardiac I/R damage reduction [23,155,156].

Protection from myocardial damage following I/R injury, exerted by NOS activity, is achieved via different overlapping mechanisms and molecular pathways. These include inhibition of the LTCC mediated

through Cav1.2 and the consequent reduction of Ca²⁺ accumulation [157], mitochondrial reduction of reactive oxygen species production by cytochrome-c oxidase (CcO) activity [158–161], reduction of reactive oxygen species production induced through the modulation of XOR activity [162] and upregulation of mitochondrial ATP-sensitive potassium (mitoK_{ATP}) channels (Fig. 3) [163,164].

Indeed, *in vivo* studies demonstrate that the opening of mitoK_{ATP} channels plays a key role in cardioprotection during late ischemic preconditioning through Akt/PI3 kinase signalling and iNOS and eNOS activation, which produce significant cardiac function improvement [145,165,166].

Various studies showed that nNOS exerts its cardioprotective activity, following I/R injury and MI, through translocation from the sarcoplasmic reticulum (SR) to different subcellular compartments [167], such as mitochondria or sarcolemma [168]. In particular nNOS plays a key role in the regulation of SR Ca²⁺ release and reuptake [169].

In addition, nNOS translocation to the plasma membrane suggests an adaptive mechanism to reduce (by LTCC inhibition) the pathological remodelling of the left ventricle in HF due to the harmful effects of chronic β-adrenergic stimulation [103,167].

However, modulation of NOS/NO signalling in myocardial damage following I/R injury is controversial; both beneficial and adverse effects of NOS activity have been described [145].

Indeed, since ONOO⁻ synthesis reduction prevents the increase of NO production in early reperfusion, the dysregulation of NOS and the NOS uncoupling could exacerbate cardiac tissue damage during I/R injury, thus producing superoxide anion (O₂⁻ instead of NO), with the subsequent production of ONOO⁻ [170,171].

In this context, oxidation of BH₄ to dihydrobiopterin (BH₂) induced by ROS and reactive nitrogen species (RNS) and the imbalance of BH₄/BH₂ ratio leads to the amplification of myocardial injury, propagating NOS uncoupling [172,173]. Several studies have suggested that pharmacological supplementation of BH₄ could play a key role in the NOS coupling improvement, thus maintaining nitric oxide bioavailability [18,174–179].

In this case, the role of iNOS remains unclear. iNOS could be responsible for RNS in MI and in hypertrophic remodelling that results from pressure overload [180,181]. However, iNOS can exert its activity through the inhibition of mitochondrial oxidative stress production, mainly by the down-regulation of opening and swelling of the mitochondrial permeability transition pore (MPTP) [150,182,183].

In support of iNOS protective activity, there are several studies showing that constitutive NOS and iNOS are able to confer beneficial effects in ischaemic preconditioning through regulation of different mechanisms ranging from inhibition of cytosolic Ca²⁺ accumulation exerted by the S-nitrosylation of many Ca²⁺ channels [184,185], such as RYR2 [186,187], LTCC [158–161] and SERCA2a, to upregulation of mitoK_{ATP} channel opening [163,164].

Evidence suggests that SNO inactivates mitochondrial Complex I, which is the entry point for nicotinamide adenine dinucleotide phosphate (NADPH) electrons into the respiratory chain through various mechanisms [188,189]. Indeed, peroxyxynitrite is able to oxidize different amino acid residues, with the highest reactivity versus the cysteine residue. In particular, since rapid complex I reactivation plays a crucial role in the pathological onset of I/R tissue damage, reversible inhibition of Complex I through the Active/De-active (A/D) transition [188,190] represents a protective mechanism and a potential therapeutic strategy for *in vivo* reduction of myocardial, brain, and skeletal muscle damage after I/R injury [191–193]. Again, eNOS exerts beneficial effects in cardiac remodelling, preventing myocyte hypertrophy development and cardiac fibrosis [194]. Regarding the effects of nNOS and eNOS activity in myocardial tissue, several studies showed that modulation of NOS enzymes has a beneficial role in atrial fibrillation reduction [195]. Myocardial nNOS depletion might represent a molecular mechanism upstream of atrial fibrillation maintenance in humans [196]. In addition, nNOS has a protective *in vivo* activity, reducing ventricular

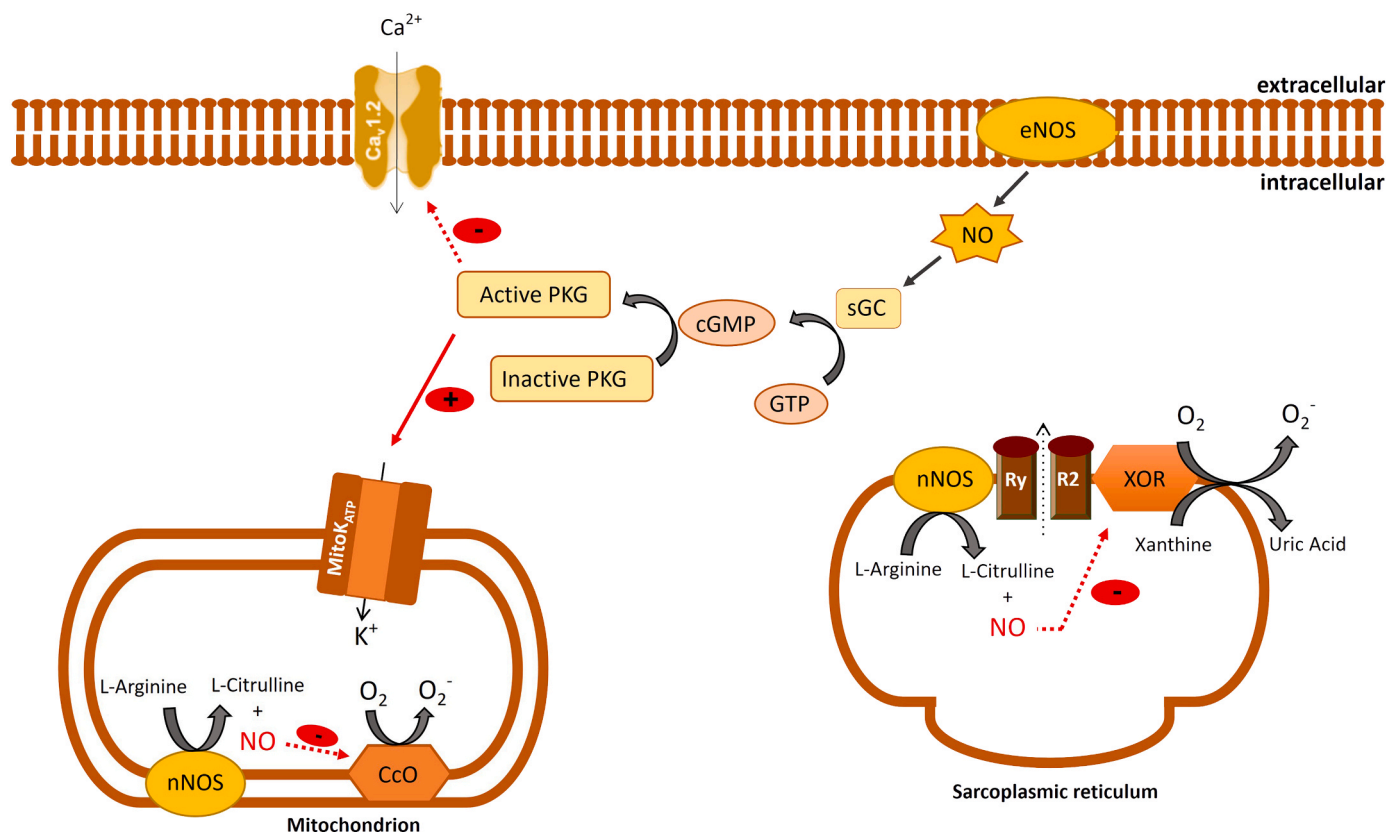


Fig. 3. NOS activity and mechanisms of myocardial damage protection following ischemia/reperfusion (I/R) injury. eNOS reduces myocardial damage through the reduction of Ca^{2+} accumulation due to the inhibition of the LTCC mediated by Cav1.2 and induces the opening upregulation of mitoK_{ATP} channels, which play a key role in the cardioprotection through the Akt/PI3 kinase signalling. In addition, nNOS produce a decrease of mitochondrial-derived and sarcoplasmic reticulum-derived Reactive Oxygen Species production by the enhancement of cytochrome-c oxidase activity and the modulation of XOR activity, respectively. eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; sGC, soluble guanylyl cyclase; GTP, guanosine-5'-triphosphate; cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; mitoK_{ATP}, mitochondrial ATP-sensitive potassium; O₂⁻, superoxide; O₂, oxygen; K⁺, potassium ion; LTCC, L-type calcium current; RyR2, ryanodin receptor 2; XOR, xanthine oxidoreductase.

arrhythmias induced by MI [197]. The beneficial effect of nNOS is mainly due to the inhibition of LTCC opening and S-nitrosylation protein induction [197]. The dual role of nNOS and eNOS in pathological remodelling reduction or aggravation, respectively, might be due to the haemodynamic stress degree and the consequent NOS uncoupling exacerbated by oxidative stress [198]. Moreover, *in vivo* data suggest that constitutive NOS could modulate the myocardial reaction to oxidative stress and β_3 -adrenergic stimulation, showing a loss of myocardial function and a significant enhancement of pathological left ventricular remodelling due to nNOS deletion [198,199]. In cardiomyocytes, the β_3 -adrenergic receptors (β_3 -ARs) are coupled to constitutive NOS and play a crucial role in cardiovascular function regulation: their activation produces LTCC stimulation and increases the atrial contractility [200,201]. An *in vivo* study that examined the regulation of molecular mechanisms involved in the β_3 -NO pathway highlighted the key role of β_3 -adrenergic receptors in direct negative inotropic NO-mediated responses [202].

4.1. Dysfunctional NO signalling in cardiovascular system

RNS production that includes ONOO⁻ and NOS uncoupling represents the main mechanisms involved in dysfunctional NO signalling [203,204] (Fig. 4).

4.1.1. NOS uncoupling mechanisms

NOS is an enzyme that in physiological conditions, when is coupled to substrate L-arginine and its cofactors such as BH₄, plays a crucial role in cardiovascular homeostasis. Its uncoupling, due to the oxidation of

BH₄ to BH₂, results in the superoxide production and the consequent formation of RNS [205,206].

Experimental *in vitro* and *in vivo* studies suggest that rather than the BH₄ oxidation to BH₂, the BH₄:BH₂ ratio could play a pivotal role in NOS uncoupling [191]. Indeed, in NIH 3T3 murine fibroblasts which express eNOS with low biopterin levels, the reduced BH₄:BH₂ ratio amplified eNOS uncoupling [207,208].

A recent clinical study conducted in coronary artery disease (CAD) patients highlighted the role of intracellular BH₄ oxidation and BH₄:BH₂ ratio, showing the adverse effects of superoxide and peroxynitrite on cellular activity, due to NOS uncoupling [174]. Additionally, severe NADPH depletion caused by CD38 activation occurring in the post-ischemic heart can affect NOS function and impair NADPH-dependent BH₄ synthesis, leading to endothelial dysfunction [209].

However, *in vitro* and *in vivo* studies have demonstrated that BH₄ also exhibits a direct NOS-independent antioxidant activity that could be essential for adaptive vascular endothelial function secondary to inflammation and O₂⁻ production through Complex I of the mitochondrial respiratory chain [210]. Indeed, it has been observed that BH₄ supplementation *in vivo* was able to produce cardioprotective effects, reducing myocardial inflammation [211].

4.1.2. Iron deficiency (ID) and reactive nitrogen species

Under physiological conditions, there is a balance between formation and scavenging of ROS/RNS, in which iron plays a crucial role: indeed, the dysregulation of iron homeostasis, such as iron overload and iron deficiency, induces oxidative and nitrosative stress through different mechanisms [212].

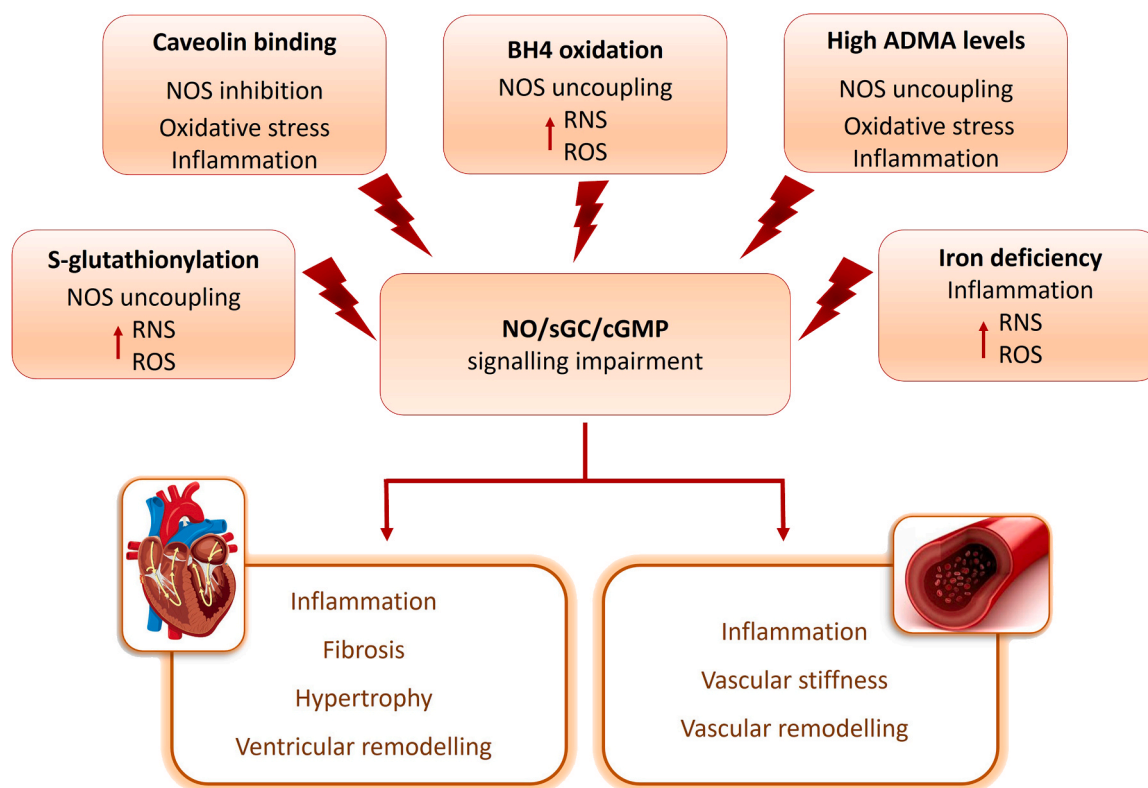


Fig. 4. NO/sGC/cGMP signalling impairment and cardiac and endothelial dysfunction. RNS production, that includes ONOO^- and O_2^- , NOS uncoupling, NOS inhibition and inflammation, induced by S-glutathionylation, Caveolin binding, BH4 oxidation, High ADMA levels and Iron deficiency, are the mainly mechanisms involved in dysfunctional NO/sGC/cGMP signalling. The impairment of NO/sGC/cGMP signalling is responsible for inflammation, fibrosis, hypertrophy and ventricular remodelling at cardiac level and inflammation, vascular stiffness and vascular remodelling at endothelia level. NOS, nitric oxide synthase; NO, nitric oxide; sGC, soluble guanylyl cyclase; cGMP, cyclic guanosine monophosphate; RNS, reactive nitrogen species; ROS, reactive oxygen species; BH4, tetrahydrobiopterin; ADMA, asymmetric dimethylarginine.

In particular, ID can induce the dysregulation of several iron-based enzymes [213]; *in vitro* evidence suggests that ID is associated with mitochondrial Complex IV activity reduction, thus inducing Complexes I–III reduction [214] and the consequent ROS formation [215]. In addition, the increase in inflammatory mediators caused by ID leads to activation of leukocytes, resulting in ROS and RNS production [216].

Several *in vivo* and clinical studies showed that ID produces NOS-upregulation and increases NO levels [217,218]. In addition, anaemic rats show high concentrations and activities of vascular and renal eNOS and iNOS [218] and enhanced NADPH oxidase, suggesting that the ONOO^- formed as a result of ID could induce nitrosative stress, inferred from the high nitrotyrosine levels [219].

Further clinical studies have been observed the significant inhibition of antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in patients with ID and the consequent restoration of their normal levels following iron replacement therapy [220].

4.1.3. Asymmetric dimethyl arginine (ADMA)

In cardiomyocytes, the maintenance of homeostatic conditions and NOS coupling is established for an extracellular L-arginine concentration at least of $100\mu\text{mol/l}$ [221].

However, uncoupling of all NOS isoforms could also be a result of competition for L-arginine with arginase and arginine methyltransferase, which in turn transforms L-arginine in urea and L-ornithine or ADMA [222], a product that attenuates NO production by NOS, inducing superoxide production [222,223].

Several studies showed that increased ADMA levels were responsible for eNOS uncoupling, oxidative stress and inflammation. Again, high plasma levels of ADMA were associated with different cardiometabolic

dysfunctions, such as congestive heart failure, coronary artery disease, hypertension, atherothrombosis and diabetes mellitus [224,225]. Therefore, the ADMA value might be an independent, prognostic biomarker of CVD [226].

4.1.4. S-glutathionylation

Under pro-oxidative conditions, the dysregulation of vascular function can be caused by NOS uncoupling triggered by S-glutathionylation, which consists of the reversible post-translational formation of a mixed tripeptide glutathione (GSH) and a thiol protein through the creation of a disulfide bond [227], the S-glutathionylation site in human NOS reductase domain, Cys689 and Cys908, which prevents the electron transfer between the flavins and prompts the reductase domain to produce O_2^- . When the GSH: Glutathione disulfide (GSSG) is restored, S-glutathionylation can be reversed, suggesting that the NOS uncoupling produced by S-glutathionylation may be a temporary adaptive mechanism to decrease NO synthesis, thus avoiding the irreversible nitrosative stress induced by ONOO^- overproduction [228].

4.1.5. Caveolin and NOS inhibition

In the modulation of endothelial and cardiac function, a crucial role is exerted by caveolin, a structural protein of caveolae involved in the regulation of inflammation and NO-mediated oxidative stress [229].

The caveolin scaffolding domain binds to different molecules, including eNOS, Akt, protein kinase C (PKC) and PKA, which play a key role in the vascular wall regulation and myocardium inflammation. [230].

It has been shown that Caveolin-1 could have a dual activity in vascular modulation: it is known that Caveolin-1 inactivates eNOS through its calcium-calmodulin site binding, with subsequent inhibition

of its translocation and phosphorylation [231], thus reducing NO generation and increasing endothelial dysfunction [232,233].

Moreover, Caveolin-1 knockdown induces NO over-production that leads to the generation of RNS, thereby impairing endothelial function [232,233].

Toll-like receptor 4 (TLR4) represents the main regulator of Caveolin-1 activity, playing a key role in vascular inflammation through the phosphorylation of its Tyr14 residue and the subsequent inhibition of NO production by eNOS. On the other hand, TLR4 induces NF- κ B activation, which in turn activates iNOS, leading to vascular inflammation initiation and VSMCs proliferation [234].

Evidence suggests that the increased production of oxidized low-density lipoprotein in metabolic disorders upregulates Caveolin-1 [235]. The consequent over-expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is associated with the translocation of NF- κ B, which in turn activates iNOS and COX enzymes [235].

In addition, Sirtuin-1, a NAD-dependent protein deacetylase, plays a key role in Caveolin-1 activity modulation and endothelial dysfunction, both regulating Caveolin-1 expression and mediating NOS deacetylation at residues of lysine, with a reduction of inhibitory eNOS in caveolae [236]. The deletion or the inhibition of Sirtuin-1 leads to CVD development [237].

4.1.6. NO/sGC/cGMP signalling impairment

Under physiological conditions, NO binds the heme group of the β -subunit of heterodimer sGC. Oxidative stress induces oxidation of the sGC heme group (from Fe²⁺ to Fe³⁺), thereby desensitizing the enzyme to NO stimulus. Furthermore, ROS production is NO-dependent, inducing thiol-oxidation and cysteine nitrosylation of sGC, thus decreasing its activity. Both mechanisms interfere with NO/soluble guanylyl cyclase/cyclic GMP (NO/sGC/cGMP) signalling, as observed in several diseases [238,239].

A study conducted in rat aortic VSMC cells showed that CYB5R3

regulates sGC activity through the reduction from Fe³⁺ to Fe²⁺, restoring NO/sGC/cGMP signalling, thereby sensitizing the enzyme to NO binding [240,241].

On the other hand, in a smooth muscle cell-specific CYB5R3 KO mouse model, loss of CYB5R3 exacerbates angiotensin II-induced hypertension through the increase of sGC heme oxidation [242].

Furthermore, a recent study tested the protective role of CYB5R3 in chronic hypoxia caused by biventricular hypertrophy, blunted vasodilation to NO-dependent activation of sGC in coronary and pulmonary arteries and decreased cardiac function in CYB5R3 KO mice; the results showed cardiac remodelling and functional changes with impaired cardiac function in KO mice [243].

In addition, in Apo-sGC mice, an *in vivo* model to study the consequences of sGC oxidation and the therapeutic effects of sGC activators, the activation of heme-containing reduced sGC is a fundamental mechanism to induce vasorelaxation, platelet aggregation inhibition and NO-mediated blood pressure modulation [244].

Moreover, *in vitro* and *in vivo* studies demonstrated that sGC S-nitrosylation of the β -subunit (Cys122) could be another mechanism able to disrupt the sensitivity of sGC to exogenous NO donors, thus producing nitrate tolerance [110,245,246]; therefore, in the cardiovascular oxidative disease development, the nitrosylation of sGC could represent the connecting thread between NO-related oxidative stress and NO tolerance [109].

5. Emerging therapeutic strategies in NO signalling modulation

The sections below will describe different therapeutic approaches used to modulate impaired NO signalling underlying CVD development, in order to restore its physiological production and regulate downstream pathways (Fig. 5) (Table 1).

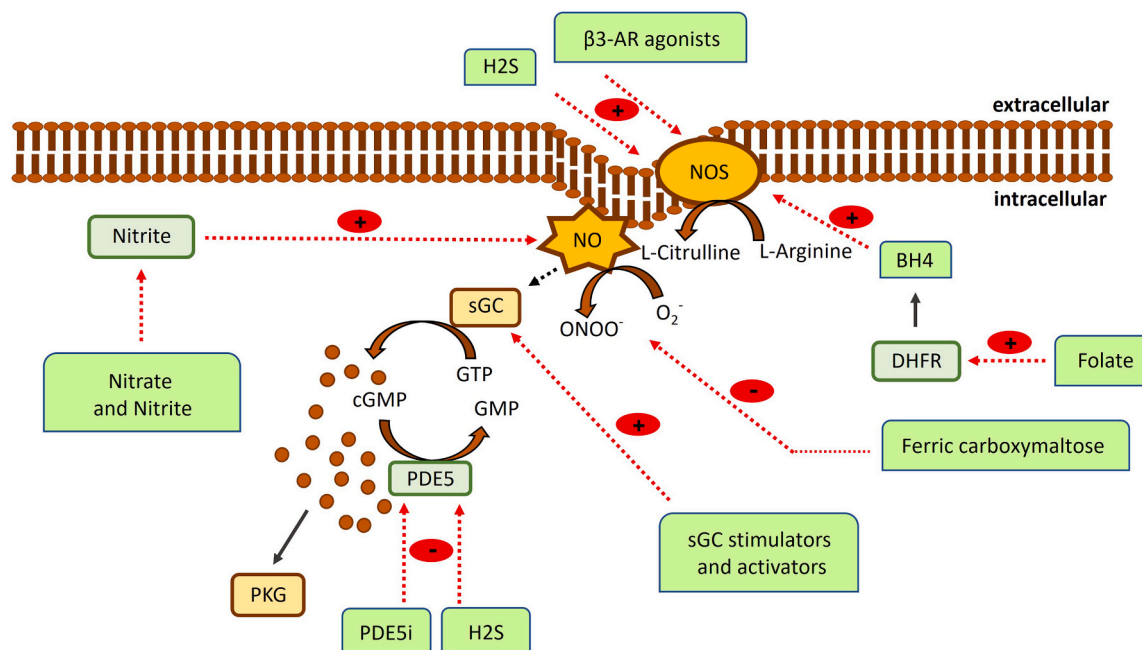


Fig. 5. Emerging therapeutic strategies in NO/sGC/cGMP signalling modulation. Different therapeutic approaches are used to modulate impaired NO signalling and to regulate downstream pathways, underlying cardiovascular diseases development, in order to restore NO production, bioavailability and physiological activity. Folate and BH₄ supplementation, as well as β₃-AR agonists and H₂S, act by restoring and stimulating NOS activity; nitrate and nitrite supplementation acts directly by improving NO bioavailability; sGC stimulators and activators play a key role in the modulation of sGC activity and cGMP production; PDE5i and H₂S inhibit PDE5 activity, thus increasing cGMP bioavailability and PKG production. NOS, nitric oxide synthase; NO, nitric oxide; sGC, soluble guanylyl cyclase; GTP, guanosine-5'-triphosphate; cGMP, cyclic guanosine monophosphate; GMP, guanosine monophosphate; PKG, protein kinase G; BH₄, tetrahydrobiopterin; DHFR, dihydrofolate reductase; H₂S, hydrogen sulfide; PDE5i, phosphodiesterase type 5 inhibitors; β₃-AR, beta 3 adrenergic receptor; ONOO⁻, peroxynitrite; O₂⁻, superoxide.

Table 1

Schematic representation of emerging therapeutic strategies in NO/sGC/cGMP signalling modulation in preclinical and clinical studies.

| Therapeutic strategies | Study Type | Properties | Molecular mechanism | References |
|--|-----------------|---|---|-------------------------------------|
| PDE5i-Sildenafil | Clinical | Improvement of myocardial contractility; Attenuation of myocardial remodelling; Modulation of haemodynamic state | ↑ cAMP/PKA activation; ↑ [Ca ²⁺] _i ; ↓ cGMP degradation | [250],[260],[262] |
| | <i>In vivo</i> | Cardioprotective activity against I/R injury; Attenuation of TAC-induced myocardial oxidative stress | iNOS-mediated mitoK _{ATP} channel opening; PKG activation; Inhibition of Rho/Rho-kinase pathway; ↓PDE5 expression | [251–254],[256] |
| PDE5i-Icarin | <i>In vitro</i> | Cardioprotective effects against doxorubicin-induced cardiotoxicity | Restoration of eNOS/iNOS rate | [257] |
| PDE5i-Tadalafil sGC activator-Cinaciguat | Clinical | Cardioprotection | PKG-dependent generation of H ₂ S | [258],[259] |
| | <i>In vivo</i> | ↓ Cardiac preload and afterload; ↑ Cardiac output; ↓ Right ventricular hypertrophy remodelling | Direct binding to oxidized, heme-free sGC; ↑cGMP | [274],[276],[277],[278] |
| sGC activator-Ataciguat | <i>In vivo</i> | Improvement of endothelial function and platelet hyperaggregation; ↓Atherosclerosis | | [259],[275] |
| sGC stimulator-Riociguat | <i>In vivo</i> | ↓ Infarct size; Improvement in LV systolic function | Direct binding to reduced, heme-containing, sGC; | [79] |
| | Clinical | Prolongation of 6MWT distance; ↓NT-proBNP; ↓ Pulmonary vascular resistance; ↓ Right heart size; Improvement of diastolic function | ↑cGMP | [280,282–284] |
| sGC stimulator-Vericiguat BH4 supplementation | Clinical | ↓ HF hospitalization and mortality in HFrEF patients | | [285,287] |
| | <i>In vivo</i> | ↓Oxidative stress; ↓ Cardiac hypertrophy; ↓ Endothelial dysfunction; ↓ Ventricular dysfunction eNOS-dependent coronary flow restoration | ↓ eNOS uncoupling | [289],[290],[291],[292],[206] |
| Folate supplementation | Clinical | Improvement of endothelial-dependent vasodilatation; ↓ Macrovascular dysfunction in patients with HFpEF | Restoration of NO activity | [293],[294],[295] |
| | <i>In vitro</i> | ↓ Endothelial dysfunction | ↓ Homocysteine; ↑ BH4 and NO bioavailability; ↓ Superoxide anion; BH4 stabilization or regeneration from BH ₂ ; Direct binding to the active site of eNOS enzyme | [300],[301] |
| Iron supplementation- Ferric carboxymaltose | Clinical | Improvement of NO-dependent vasodilation; ↓Risk of stroke | ↑ NOS coupling and NO bioavailability; ↓ Vascular oxidative stress; ↓ Oxidative and nitrosative stress | [302],[303],[305] |
| | Clinical | Improvement of functional capacity; ↓inflammation and re-hospitalization in HFrEF patients; Improvement of exercise capacity and endothelial function in HFpEF patients | | [310],[311],[313] |
| Nitroxyl (HNO) | <i>In vivo</i> | Inotropic and lusitropic effects; Vasodilation | ↑ SERCA activity and activation of RyR2; | [317],[318],[319],[320],[321],[322] |
| Nitroxyl (HNO) donors-CXL-1020 | Clinical | ↓ Left and right heart filling pressures; ↓ Systemic vascular resistance | ↑Ca ²⁺ responsiveness of cardiac myofilament proteins; | [324] |
| HNO donors (BMS-986231) | Clinical | ↓ Pulmonary capillary wedge pressure (PCWP) in HFrEF patients; ↓Pulmonary arterial diastolic pressure (PADP); ↓ Pulmonary arterial systolic pressure (PASP) | Activation of sGC/cGMP signalling; Superoxide-suppressing activity | [325],[326],[327] |
| Nitrate–nitrite supplementation | Clinical | ↓ blood pressure; ↓ carotid intima-media thickness (IMT); ↓ MI size | ↑ NO production independently from NOS; ↓ ROS production; ↓ pro-inflammatory neutrophil activation | [56],[329],[330],[333],[334] |
| Hydrogen sulfide | <i>In vivo</i> | Restored the ischemic blood flow; ↑ Angiogenesis; ↓ Infarct size; | ↑ NOS expression; ↑ VEGF and HIF-1α activity; ↓ PDE5 activity; | [348],[350],[351],[352],[353] |
| | Clinical | ↓ BNP levels in patients with HF | ↑ cGMP bioavailability and PKG production | [354],[355] |
| β3-Adrenergic agonists-Mirabegron | Clinical | ↓ Hypertrophic or fibrotic remodelling; ↑ Cardiac index and ↓ Pulmonary vascular resistance in HFrEF patients; ↓ Na ⁺ overload in HF | NOS activation; Antioxidant activity; ↓ Oxidative inactivation of the Na ⁺ -K ⁺ -ATPase pump | [359],[360],[366],[367] |

↑= Increase; ↓= Decrease

5.1. Inhibitors of phosphodiesterase type 5 and NO signalling modulation

Phosphodiesterase type 5 inhibitors (PDE5i) are selective and powerful cGMP-specific PDE5i, which, as discussed above, catalyse the hydrolysis of cGMP that exerts a powerful vasodilatory activity and acts as a NO donor. Since PDE5 is an enzyme ubiquitously present in tissues including blood vessels and heart, it has been hypothesized that PDE5i, usually employed in erectile dysfunction treatment [247], could produce beneficial effects in patients suffering from CVD, pulmonary arterial hypertension [248], heart failure and diabetes [249]. Indeed, in patients with HF, the PDE5i sildenafil induces increased cAMP production and a consequent activation of PKA, that in turn leads to the improvement of myocardial contractility through intracellular calcium concentration increase [250]. *In vivo* studies demonstrated that sildenafil administration exerts a significant cardioprotective activity against I/R injury, comparable to preconditioning induced by sublethal ischemia or adenosine. The observed cardioprotection could be a result of iNOS-mediated mitoK_{ATP} channel opening [251]. Indeed, NO production catalysed by iNOS could activate cGC, that in turn leads to cGMP formation and the consequent activation of PKG, which induces mitoK_{ATP} channel opening and the related cardioprotective effects [252].

In addition, it has been shown that chronic administration of sildenafil in mice after permanent occlusion of the left anterior descending coronary artery (LAD) led to ischemic cardiomyopathy attenuation and left ventricular function improvement through PKG activation [253]. However, in a mouse model, it has been observed that sildenafil treatment is also able to mitigate heart failure progression through the inhibition of Rho/Rho-kinase pathway [254].

Furthermore, preclinical studies described the PDE5 upregulation and the NO signalling dysregulation under oxidative stress conditions [251,255], demonstrating that PDE5 inhibitor administration is involved in a significant reduction of oxidative stress in heart failure [256].

Based on the crucial role played by PDE5 upregulation and oxidative stress in cardiac damage, a recent *in vitro* study carried out on cardiomyocytes exposed to doxorubicin suggested that PDE5 inhibition by natural extract Icarin could restore the eNOS/iNOS rate, thereby promoting cardioprotective effects against doxorubicin-induced cardiotoxicity [257].

Since PDE5i treatment, in preclinical studies, showed a significant cardioprotective effects, several clinical trials evaluated the cardioprotective action of phosphodiesterase inhibitors, especially as regards the beneficial effects carried out in the damage from cardiac ischemia, showing that Tadalafil, a novel long-acting inhibitor of phosphodiesterase-5, exerted its cardioprotective action through PKG-dependent generation of H₂S and via the phosphatidylinositol 3-kinase/Akt signalling pathway [258,259]. Nevertheless, while different clinical studies observed that sildenafil, a potent PDE5 inhibitor, acts through the suppression of cGMP degradation, attenuating myocardial remodelling [260] and endothelium vasodilatation [261], and simultaneously modulating haemodynamic state avoiding systemic hypotension [79,262] in cardiovascular injury, further trials have shown conflicting results [18].

Indeed, in the Sildenafil and Diastolic Dysfunction After Acute Myocardial Infarction (SIDAMI) Trial, including patients with recent MI and diastolic dysfunction, it was shown that oral treatment with sildenafil did not affect increased filling pressure and pulmonary artery hypertension, but there were effects on secondary end points [263]; the improvement in cardiac output is in line with previous studies performed in patients with HFpEF [264] and HFrEF, respectively [265].

In addition, other studies demonstrated that chronic sildenafil therapy, in chronic HF patients, was able to improve oxygen uptake, exercise haemodynamic and functional exercise capacity, through PDE5i-induced NO signalling regulation [266–268].

Sildenafil effects are also being examined in larger clinical trials: the HFpEF RELAX trial showed arterial pressure reduction, without the

improvement of clinical status and exercise tolerance in patients with HFpEF, probably due to the high dosage or to the lack of cardiac PDE5 upregulation [253]. Indeed, PDE5i activity is subordinate to cGMP production, and the haemodynamic stress might be due to molecular mechanisms upstream of the potential PDE5i effects [269].

The SystEmic Right VEntricular size (SERVE) trial is an on-going, multi-center, double-blind, randomized, placebo-controlled clinical study intended to evaluate the PDE5i (Tadalafil) effects on right ventricular volume and function, with the aim of provide information about PDE5 inhibition as a new therapeutic target in right ventricular failure [270].

Furthermore, the GOSPEL (Goal Oriented Strategy to Preserve Ejection Fraction Trial) clinical trial aims to evaluate the PDE5i effects of on right ventricular function and clinical outcome in patients with pulmonary arterial hypertension (PAH) [271].

The recent “Riociguat Replacing PDE5 Inhibitor Therapy Evaluated Against Continued PDE5i” Therapy (REPLACE) Trial, a prospective, randomized, controlled, international, multicenter, double arm study, suggested the potential use of riociguat in patients with PAH to directly stimulate sGC to counter PDE5i, which blocks the cGMP degradation [272].

5.2. sGC stimulators or activators and NO signalling

Since, as described above, the alteration of NO-cGMP signalling and the impaired cGMP production induce endothelial dysregulation, fibrosis and ventricular hypertrophy, resulting in the development of heart failure, the NO-cGMP pathway represent an important treatment target to improve these outcomes. The therapy with cGC stimulators and activators can enhance cGMP production by the upregulation of the enzymatic activity of sGC, through the direct binding to reduced, heme-containing, sGC and to oxidized, heme-free sGC, respectively [273].

Several *in vivo* models of pulmonary hypertension, MI, chronic renal and heart failure have evaluated the pharmacological effects of cinaciguat, a sGC activator. In particular, a study that used an experimental canine model of HF showed that cinaciguat administration causes a dose-dependent reduction in cardiac preload and afterload, and an increase of cardiac output [274].

Moreover, treatment with the sGC activator ataciguat led to endothelial function and platelet hyperaggregation improvement in an *in vivo* model of diabetes, including rats in chronic treatment with streptozotocin [259]. In addition, treatment of ApoE^{-/-} mice with ataciguat was able to significantly reduce atherosclerosis and improve endothelium-dependent vasorelaxation [275].

Several preclinical studies demonstrated the role of cinaciguat in right ventricular hypertrophy remodelling reduction caused by severe pulmonary hypertension [276]. In addition, cinaciguat treatment produced a significant improvement of cardiac hypertrophy in cardiomyopathy diabetic models and a significant prevention of cardiac remodelling and fibrosis in pressure-overload models [277,278].

Based on preclinical evidence, a phase 2 study was conducted in patients suffering from acute decompensated heart failure (ADHF), showing an improvement in cardiopulmonary functions. However, clinical development of three different randomized, double-blind, placebo-controlled phase 2b trials, including patients with ADHF, were blocked due to hypotensive effects produced, without beneficial effects [273]. Although the role of cinaciguat has been defined in acute HF, to the best of our knowledge there are no clinical data on sGC activator activity in chronic HF.

Similarly to sGC activators, *in vivo* treatment with sGC stimulators in post-MI models, in Dahl salt-sensitive rats and in angiotensin II pressure-overload models, showed beneficial activities to prevent ventricular stress, pathological cardiac remodelling and fibrosis, thus preserving cardiac function [275,279]. In particular, mice treated at the reperfusion onset with riociguat, a sGC stimulator, showed a significant reduction in infarct size and an improvement in LV systolic function [79], suggesting

the potential therapeutic effect of sGC stimulation during reperfusion to prevent HF after MI.

Several clinical studies were conducted to evaluate the safety and efficacy of chronic riociguat therapy: the PATENT-2 study showed good tolerability of riociguat treatment that improves cardiac function with the prolongation of six minute walking test (6MWT) distance [280]. Subsequently, the potential additive effect of PDE5i and riociguat was evaluated in the PATENT PLUS study, but no significant differences were observed in exercise capacity and haemodynamic markers between the combination of the two drugs and the use of sildenafil alone [281].

In the CHEST-1 multicenter study, riociguat treatment led to 6MWT distance improvement in patients suffering from chronic thromboembolic pulmonary hypertension (CTEPH), with a significant decrease of NT-proBNP and pulmonary vascular resistance. To test the long-term efficacy of riociguat, the CHEST-2 clinical study was carried out, and the results obtained confirm the improvements in 6MWT in patients with CTEPH after one year of treatment [282].

In the RIVER study, Marra et al. demonstrated that long-term treatment with riociguat caused a reduction of right heart size and an improvement of right ventricular function in patients suffering from PAH and CTEPH [283]. Furthermore, the DILATE-1, a randomized, double blind, placebo-controlled study, evaluated the effects of single doses of riociguat in patients with HFpEF and PH, suggesting that riociguat, in addition to the vasodilatory effect exerted at systemic level, could ameliorate diastolic function [284].

Two phase IIb trials were carried out to evaluate the effect of sGC stimulators in HFrEF (SOCRATES-Reduced) and HFpEF (SOCRATES-Preserved) patients respectively, and the data obtained have shown mixed results [285,286]. In particular, the primary end-points of SOCRATES-Preserved trial were not achieved and the patients treated with vericiguat (a soluble sGC stimulator) did not show a reduction in the N-terminal pro-BNP levels and in left atrial volume at 12 weeks of treatment. Nevertheless, vericiguat treatment was well-tolerated and was able to improve the quality of life in patients with chronic HFpEF [286]. Similarly, in the SOCRATES-Reduced clinical study, the primary end point, consisting in N-terminal pro-BNP reduction, was not obtained, but a significant improvement in clinical outcomes was observed, with a decrease of HF hospitalization and cardiovascular mortality [285]. The results of the pivotal phase III VICTORIA (Vericiguat Global Study in Subjects with Heart Failure with Reduced Ejection Fraction) trial, carried-out to verify the SOCRATES-REDUCED trial, confirm that the incidence of cardiovascular mortality and HF hospitalization was lower in patients with high risk of heart failure treated with vericiguat than patients receiving placebo [287].

5.3. BH4 or folate supplementation and NOS activity improvement

NO synthase co-factor BH4 plays a key role in NO regulation and oxidative stress reduction. Endothelial BH4 depletion results in NO loss and increased ROS production by NO synthase, leading to development of CVD [288]. Since the oxidative stress produced is associated with BH4 oxidation into BH2 and eNOS uncoupling, it has been hypothesized that BH4 supplementation could represent a valuable therapeutic option for NO-mediated endothelial dysfunction [173].

Indeed, *in vivo* studies demonstrated that BH4 supplementation was able to reverse NOS uncoupling and cardiac dysfunction [173,211]. In particular, BH4 supplementation in a mouse model of chronic ventricular pressure, obtained through the transaortic constriction, counteracted the increase of oxidative stress, the cardiac hypertrophy and the subsequent ventricular dysfunction development caused by NOS uncoupling [289].

Further evidence in a mouse model of hypertension, obtained by deoxycorticosterone acetate (DOCA) treatment, demonstrated that BH4 supplementation reversed endothelial dysfunction through NOS recoupling [290].

Moreover, BH4 supplementation was able to produce the partial

inhibition of ROS production and restore NO production in isolated rat hearts [291]. In addition, BH4 treatment improved ventricular function following I/R injury in isolated perfused rat hearts [292].

Following the promising preclinical results, the first clinical studies suggested the beneficial effects of BH4 supplementation on cardiac function, showing an improvement of endothelial-dependent vasodilatation due to restoration of NO activity in patients suffering from type II diabetes mellitus or hypertension [293,294]. In addition, a randomized, double-blind crossover study has identified a potential role of short-term BH4 supplementation to mitigate macrovascular dysfunction in patients with HFpEF, through NO pathway modulation [295].

Although later trials observed that oral BH4 supplementation in patients with coronary artery disease increased plasma levels of BH4, the simultaneous increase of the oxidation product BH2 led to the loss of therapeutic effect exerted through eNOS recoupling, with the subsequent inability to counteract systemic and vascular oxidation [296].

Due to the deleterious amplifying effect of BH2 production caused by BH4 supplementation, additional treatments able to reduce BH2 and restore NO activity have been evaluated. Among these, folic acid or its derivatives are able to reverse endothelial dysfunction through different mechanisms. The first mechanism consists of homocysteine lowering, since 5-methyltetrahydrofolate (5MeTHF) can methylate homocysteine to methionine. The second is direct antioxidant activity, through the O₂ scavenging effect exerted by 5MeTHF. The third mechanism involves chemical BH4 stabilization or regeneration from BH2 [297]. The last mechanism concerns the direct effects on eNOS, through the enhancement of BH4 binding. In addition, folates contain a characteristic pterin ring structure similar to BH4, and thus may directly bind the active site of eNOS enzyme, mimicking BH4 activity [298,299]. An *in vitro* study investigated the role exerted by folic acid on endothelial cell dysfunction, showing a significant increase of BH4 and NO bioavailability and a decreased levels of homocysteine in cells treated with high doses of folic acid [300]. In addition, folic acid treatment restores NO bioavailability and BH4 and DHFR levels in human pulmonary artery endothelial cells (HPAEC) and murine pulmonary arteries under hypoxia, through the promotion of BH4 recycling and eNOS recoupling [301].

Clinical evidence suggests that treatment with folic acid at 5 mg/daily is effective in improving NO-dependent vasodilation in patients suffering from endothelial dysfunction, through increased NOS coupling and NO bioavailability, independent of decreased plasma homocysteine [302]. In addition, a placebo-controlled, double-blind, parallel design in which patients with coronary artery disease received 5 mg/daily (high-dose) folic acid, 400 µg/daily (low-dose) folic acid, or placebo for 7 weeks, showed that low doses of folic acid treatment counteract vascular dysfunction through the improvement of NOS activity and reduction of vascular oxidative stress. Treatment with high doses of folic acid did not show greater benefit than low doses, since the direct beneficial effect was due to the level of 5-methyltetrahydrofolate in vascular tissue rather than in plasma [303].

Nevertheless, in a clinical study that enrolled women with either a history of CVD or three or more coronary risk factors, it was observed that after 7.3 years of treatment and follow-up, the combined treatment with folic acid, vitamin B6, and vitamin B12 did not reduce the total cardiovascular risk, despite significant homocysteine lowering [304].

The divergent results above suggested a potential dose-dependent activity of folic acid treatment in CVD. A meta-analysis study conducted in 2016 showed that folic acid supplementation reduced by 10 % the risk of stroke and by 4 % the risk of overall CVD, with a significant correlation with lower plasma folate levels, absence of preexisting CVD and decreased homocysteine levels [305]. In the meta-analysis, there is no evidence of benefits on endothelial dysfunction due to the folate supplementation [305]. However, it has been shown that the chronic treatment with high doses of folic acid could lead to the development and progression of cancer, thus requiring a careful risk-benefit assessment [306]. In particular, although the previous evidence is conflicting [307], a recent study confirmed that the treatment with folic acid and

vitamin B12 was related to a significant colorectal cancer risk increase [308].

Further *in vivo* studies using Sprague–Dawley rats, C57BL/6 J mice and eNOS^{-/-} mice were conducted to evaluate the potential role of *in tandem* NADPH and BH4 supplementation in postischemic endothelial dysfunction, observing that the association between NADPH and BH4 supplementation produce a complete eNOS-dependent coronary flow restoration [209]. In fact, I/R damage causes NADP(H) depletion, which impairs eNOS function and reduces the amount of BH4 that can be recycled by NADPH-dependent pathways. Specifically, since activation of CD38 was demonstrated to be the cause of the strong endothelium NADP(H) depletion, it has been observed that CD38 suppression increased recovery of ventricular systolic function, decreased post-ischemic cardiac infarction, and preserved endothelium-dependent coronary flow, eNOS coupling, and NO production [209].

5.4. Iron supplementation

As described above, iron deficiency, through the dysregulation of several iron-based enzymes, plays a crucial role in oxidative and nitrosative stress induction in cardiomyocytes, thus producing mitochondrial metabolism impairment and left ventricular dysfunction [212,213]. Since patients with heart failure frequently suffer from iron deficiency, which is a predictor of bad clinical outcomes, the correction of anaemia could play a key role in heart failure management [309]. To this goal, several clinical studies have been performed, showing the benefit of intravenous supplementation with ferric carboxymaltose in patients with HFpEF, in which significant functional capacity improvement, inflammation decrease and subsequent re-hospitalization reduction have been observed without a significant decrease in mortality [310].

In the trial FAIR-HFpEF, conducted to evaluate the efficacy of intravenous iron carboxymaltose treatment in patients with HFpEF, iron carboxymaltose supplementation improved functional capacity, symptoms and the quality of life [311]. Furthermore, these results were confirmed by the CONFIRM-HF trial, carried out to evaluate the benefits and safety of long-term intravenous iron treatment; a significant reduction of hospitalization risk was observed, but there was no evidence of mortality prevention [312].

In addition to these data, a recent clinical trial in a cohort of patients with HFpEF showed for the first time that intravenous ferric carboxymaltose therapy supplementation was able to improve cardiac performance derived from 6MWT, mainly in patients in which the diastolic function was severely compromised [313]. The amelioration of exercise capacity was associated with improvement of endothelial function and oxidative status of the patients enrolled, evaluated by Endopat study of vascular reactivity and malondialdehyde (MDA) level determinations, respectively [313].

5.5. Nitroxyl (HNO)

HNO is a well-known pharmacological agent for the prevention and treatment of I/R injury and HF [314]. Endogenous HNO can be synthesized from NOS-dependent and -independent pathways. In the first case, HNO can arise from NOS itself or from the oxidation of NOS intermediates including N-hydroxy-L-arginine and hydroxylamine. NOS-independent pathways involve NO reduction by xanthine oxidase, ubiquinol, haemoglobin, mitochondrial cytochrome c, and manganese superoxide dismutase (MnSOD) [315]. Furthermore, HNO derives from the redox interaction between H₂S and NO and plays a specific effective role within the cardiovascular system [316]. Indeed, it has been demonstrated that HNO resulting from this chemical interaction shows inotropic and lusitropic effects under normal and congestive heart failure conditions in animal models [317]. HNO reacts with negatively charged thiols, converting them reversibly to disulfide residues or, less reversibly, to sulfonamides. This chemical reaction determines in isolated cardiomyocytes the increase in SERCA activity and activation of

RyR2, increasing Ca²⁺ re-uptake into the SR and eliciting a rapid release of Ca²⁺, respectively. This results in an optimization of diastolic and systolic function. In addition, HNO directly modifies cardiac myofilament proteins to increase their Ca²⁺ responsiveness and thereby systolic force generation [318]. Interestingly, in rodent cardiomyocytes, the HNO donor Angeli's salt (AS) increases Ca²⁺ transients exclusively from changes in SR Ca²⁺-cycling, and not from L-type Ca²⁺ current (I_{Ca}), thus counteracting the detrimental effects of enhanced extracellular Ca²⁺ influx via I_{Ca} (adverse remodelling, increased arrhythmogenesis and increased apoptosis) observed after prolonged use of classical pharmacological agents used in the treatment of HF (i.e., β-AR agonists, phosphodiesterase inhibitors, etc.) [319]. Thus, this observation supports the potential therapeutic use of HNO donors in HF treatment, as HNO works independently of I_{Ca}. Furthermore, preclinical studies have shown that HNO exerts vasodilation like NO through the activation of sGC/cGMP signalling but does not develop tolerance usually associated with traditional nitrates, likely due to its superoxide-suppressing activity [320–322].

The clinical use of HNO donors is limited, due to their high alkaline properties. Therefore, HNO was generated using CXL-1020, whose decomposition generates pure HNO, mimicking all cardiac activities of the classical HNO donors without NO generation or the need for an alkaline vehicle [323]. CXL-1020 efficacy was tested in a phase IIB trial in patients with systolic HF (NCT01096043). An intravenous infusion (6 h) of CXL-1020 (1–20 μg/kg/min) in hospitalized patients was able to reduce left and right heart filling pressures and systemic vascular resistance. In addition, the highest dose tested augmented cardiac and stroke volume, while heart rate was unchanged at all doses [324]. Prolonged infusion of higher doses of CXL-1020 showed inflammatory irritation at the site of infusion. Therefore, a novel second-generation of HNO donors (BMS-986231) was developed and tested in a phase 2a dose-escalation study in hospitalized patients with HF with reduced ejection fraction (NCT02157506). In particular, a BMS-986231 intravenous infusion (6 h) rapidly reduced pulmonary capillary wedge pressure (PCWP), which was one of the primary endpoints of the study. The effect was sustained throughout the duration of infusion in all dose groups. Moreover, time-averaged reductions in pulmonary arterial diastolic pressure (PADP) and pulmonary arterial systolic pressure (PASP) were observed in all BMS-986231 dose groups, compared with placebo [325]. Among the adverse effects, hypotension and headache were evidenced more frequently in patients treated with BMS-986231 compared with the placebo group (range:42.9–83.3 % vs. 25 %), although no dose dependence was observed [325]. Subsequently, the STAND-UP AHF trial, a multicenter, randomized, double-blind, placebo-controlled, phase 2b study trial evaluated the safety and efficacy of continuous 48 h intravenous infusions of BMS-986231 in hospitalized patients with HF and impaired systolic function (NCT03016325). Though BMS-986231 was able to reduce congestion markers, this effect did not persist beyond the treatment period and did not confirm a long-term benefit [326]. Other ongoing trials are comparing the BMS-986231 haemodynamic effects respect to nitrates and placebo treatment (StandUP-Imaging study), and the additive effects with loop diuretics to improve decongestion in patients suffering from HF (StandUP-Kidney study) [327].

5.6. Nitrate–nitrite supplementation in cardiovascular disease

The protective role of nitrate and nitrite supplementation in *in vivo* models of I/R injury, hypertension and HFpEF have been shown in numerous clinical studies. Dietary nitrate supplementation (250 mL/die of beetroot juice for 4 weeks) decreased blood pressure in both drug-naïve and treated patients with hypertension, with absence of signs of tolerance and an improvement of parameters of vascular function, including aortic pulse wave velocity (PWV), augmentation index and flow-mediated dilatation (FMD) [56]. These beneficial effects seem to be dependent on the initial degree of blood pressure elevation and vascular

dysfunction, and not on the antihypertensive medication status [328]. In addition, nitrate supplementation can significantly decrease diastolic blood pressure (DBP) and systolic blood pressure (SBP) in older adults, especially in whose age ≥ 65 [329]. Recent evidence suggests that nitrate and nitrite supplementation could be involved in the regulation of glucose-insulin homeostasis. To this end, the efficacy of chronic oral nitrite therapy (sodium nitrite at a dose of 40 mg 3 \times daily for 12 weeks) was evaluated in a phase 2 study, in patients suffering from hypertension and metabolic syndrome (NCT01681810). Nitrite therapy significantly reduced SBP and DBP, but tolerance was observed after 10 weeks of therapy [330]. Beyond the antihypertensive properties of oral nitrite, this study highlighted a significant reduction of carotid intima-media thickness (IMT) and a marker of carotid atherosclerosis, and a trend towards improved insulin sensitivity [330]. Larger and blinded placebo-controlled studies are needed to confirm these findings. *In vivo* studies of myocardial I/R injury, nitrate and nitrite supplementation has shown beneficial effects, reducing reperfusion injury and the consequent MI size [57,331]. These findings have been translated in two independent clinical trials. In NIAMI clinical trial, intravenous infusion of sodium nitrite immediately prior to reperfusion in patients with acute ST-elevation myocardial infarction (STEMI), did not reduce infarct size [332]. Although in the phase 2 NITRITE-AMI study, intra-coronary nitrite infusion did not alter infarct size, in a sub-group of patients with Thrombolysis In Myocardial Infarction (TIMI) flow ≤ 1 , there was a significant reduction in MI size compared to the placebo group [333]. This beneficial effect could be attributed at least in part to the suppression of pro-inflammatory neutrophil activation during reperfusion [334].

Several studies suggest that low NO bioavailability is responsible of compromised exercise vasodilatory reserve and reduced skeletal muscle perfusion during exercise intolerance of HFpEF [335,336]. Recently, it has been observed that the nitrate-nitrite-NO pathway is a significant NO source that can improve haemodynamic imbalance induced during exercise in patients with HFpEF. In particular, acidosis and tissue hypoxia, increased during exercise, may improve the reduction of nitrite to NO [65,337]. The effects of acute and short-term administration of inorganic nitrate/nitrite have been evaluated in several randomized controlled trials but with conflicting results. Some studies showed positive effects of inorganic nitrate/nitrite in amelioration of cardiac haemodynamics and exercise capacity in patients suffering from HFpEF [65, 337–339], while others did not [340–342].

A recent meta-analysis of randomized controlled trials conducted in patient with HFpEF showed no benefit of inorganic nitrate/nitrite treatment on exercise capacity. Notably, peak oxygen consumption (peak VO_2), respiratory exchange ratio (VCO_2/VO_2) during exercise and exercise time did not increase compared with the placebo group. However, analysis of haemodynamic parameters showed that inorganic nitrate/nitrite could reduce rest SBP, rest/exercise DBP, rest/exercise mean arterial pressure (MAP), and exercise systemic vascular resistance (SVR) [343].

The plasmatic half-life of nitrite, too short to sustain high levels of plasmatic cGMP, can be a reason for the absence of beneficial clinical results. In addition, nitrite therapeutic effects can be influenced by the rate of administration and by the influence of dietary constituents or concomitant drugs. Indeed, treatment with proton-pump inhibitors abolishes the hypotensive effect of orally ingested nitrite [344]. Furthermore, nitrite administration with conjugated linoleic acid suppresses the inhibitory effects of nitrite on platelet activation and vasodilatory actions [345]. Therefore, these observations show that metabolic and physiological responses to oral nitrate and nitrite can be significantly modulated by interaction with diet or concomitant medications.

5.7. Hydrogen sulfide in NO/sGC/PKG signalling

H_2S plays a crucial role in NO/sGC/PKG signalling through the

upregulation of Ca^{2+} entry, p38 MAPK/Akt stimulation and oxidative stress reduction in endothelial cells, with the subsequent increase in eNOS phosphorylation and NO production [346,347].

In a preclinical study of hind-limb ischemia, which was carried out using unilateral permanent femoral artery ligation, it was demonstrated that H_2S interacts with nitric oxide metabolism in vascular remodelling caused by ischemia; in particular, H_2S treatment restored the ischemic hind-limb blood flow, increased NOS expression and stimulated NO-mediated nitrite reduction, and stimulated angiogenesis through the increase of VEGF and Hypoxia-inducible factor 1- α (HIF-1 α) activity. These data highlight that H_2S is able to increase tissue NO bioavailability, which is significantly decreased in CVD and chronically ischemic tissues [348]. This study suggests that H_2S supplementation could potentiate the beneficial effects of nitrite supplementation [348].

In addition, H_2S administration can increase cGMP through the inhibition of PDE [349]. A recent *in vivo* study observed that Sodium hydrosulfide (NaHS) is able to decrease Phosphodiesterase 5 A (PDE5A) activity in a dose-dependent manner, suggesting that the increase of cGMP in endothelial cells may be due to its reduced degradation, with a mechanism similar to the pharmacological activity of PDE5i [350,351].

The diallyl sulfide is a constituent of garlic oil that is able to release H_2S ; *in vivo* investigation of diallyl trisulfide intravenous and intraperitoneal treatment in I/R injury highlighted the cardioprotective activity exerted through eNOS activation and the maintenance of mitochondrial membrane potential, followed by myocardial inflammation reduction [352].

Taken together these data showed the beneficial effects of H_2S treatment during I/R injury, resulting in infarct size reduction, cardiac function and remodelling improvement [353].

A phase I clinical trial, carried out following the preclinical data obtained, used a novel H_2S prodrug (SG1002) to evaluate the safety and the modification of H_2S and NO bioavailability in healthy and HF subjects. SG1002 was well tolerated at all doses in both groups. The results showed a significant increase of blood H_2S levels and NO bioavailability, with a parallel reduction of BNP levels in patients with HF [354].

The recent GIPS-IV study, a double-blind, randomized, placebo-controlled, multicenter trial, which enrolled 380 patients with STEMI, examined the efficacy and safety of H_2S -donor sodium thiosulfate. The primary endpoint was the evaluation of H_2S -donor sodium thiosulfate effects on MI size, while the secondary endpoints consist of the evaluation of the effect of H_2S -donor sodium thiosulfate on different parameters such as CK-MB levels, LVEF and NT-proBNP levels [355]. However, unpublished results presented at the Annual Scientific Session, ACC22, showed that, at four months, although there was an absence of side effects and major adverse cardiovascular events, the clinical study did not meet the primary endpoint and no significant differences in both groups in the secondary endpoints were observed [356].

5.8. β 3-Adrenergic agonists and NO in the cardiovascular system

Different evidence has highlighted the innovative role of the β 3-AR, traditionally known as a modulator of lipolysis in adipose tissue, in the regulation of vascular tone [357]. Furthermore, β 3-ARs have been detected not only in human endothelial cells, but also in cardiac myocytes, where their stimulation is associated with a negative cardiac inotropic effect and protection against hypertrophic or fibrotic remodelling [358,359]. Notably, these cardiovascular effects related to β 3-AR stimulation are associated with NO release through NOS activation [360]. Together, these findings make β 3-AR agonists an attractive target for the development of new clinical strategies against CVD [361].

The effect of mirabegron, a β 3-AR agonist currently used for the treatment of overactive bladder disease, was first examined in a pilot trial (BEAT-HF), in which changes in LVEF after 6-month of treatment of patients with HF and reduced LVEF were evaluated [362]. Changes in LVEF after six months between treatment groups were not significantly different, except in a subgroup of patients with LVEF $< 40\%$, suggesting

that the beneficial decrease in cardiac myocyte $[Na^+]_i$ depend on the severity of HF [362].

Preclinical findings on the reduction of β_3 -AR agonist-mediated myocardial hypertrophy and fibrosis, due to haemodynamic or neuro-hormonal stresses, have been translated into the phase IIb Beta3-LVH clinical trial (NCT02599480) [359,363]. In this multicentre, randomized, placebo-controlled study, left ventricular mass index (LVMI) and diastolic function were measured in patients with LVH, randomly assigned to receive 50 mg of mirabegron ($n = 147$) or placebo ($n = 149$) for 12 months [364]. Unpublished results presented at the American Heart Association Scientific Sessions 2022 affirm that mirabegron did not meet primary and secondary endpoints, although safety at the dose used has been demonstrated [365]. Since β_3 -ARs increase in heart during the later stages of HF, its expression was probably low in this study and only the standard dose of the drug was used. Indeed, a small pilot study showed that high dose of mirabegron (300 mg/day for 1 week) in patients with severe HFrEF (New York Heart Association functional class III–IV) increased cardiac index and decreased pulmonary vascular resistance compared to the placebo group; these results are useful in patients with worsening or terminal HF [366]. Therefore, preliminary results showed that mirabegron was able to enhance contractility in the more dilated left ventricles state, instead of remodeling induction in condition of reduced diastolic dimensions.

In addition to the increased activation of NOS/NO signalling restricted to receptor-expressing damaged myocardium and vascular tissues, mediating physiological signalling and preventing side effects such as hypotension, β_3 -ARs also exert antioxidant activity [359]. Indeed, β_3 -AR activation not only protects the NO/cGMP pathway from oxidative damage, preserving its beneficial role in myocardial remodeling, but also reduces oxidative inactivation of the Na^+K^+ -ATPase pump, thereby decreasing Na^+ overload in HF [367].

6. Emerging role of nutraceutical supplementation in NO signalling impairment and HF management

Several evidence on pathophysiology of HF and other CVD suggests a crucial role of oxidative and nitrosative stress, due to the imbalance between free radical production and antioxidant defence activity [368, 369]. Notably, the key antioxidant role of nutraceutical supplementation in human health and cardioprotection, including the reduction of congestive HF incidence, has been highlighted [370–373].

6.1. Coenzyme Q10 and NO signalling modulation

Coenzyme Q10 (CoQ10), ubiquitous in mammalian tissues, plays a crucial role in cardiac mitochondrial function and NO signalling [374]. Preclinical data obtained in different models suggest that the supplementation with CoQ10 improved outcomes of cardiovascular diseases [375]. Studies conducted in Zebrafish models have shown that the lack of UbiA Prenyltransferase Domain Containing 1 (UBIAD1), an antioxidant enzyme that regulates eNOS activity through CoQ10 synthesis, resulted in cardiovascular impairment due to the cellular damage consequent to ROS accumulation [376]. Indeed, since CoQ10 regulates eNOS activity in cellular membranes, its depletion can trigger eNOS uncoupling and the subsequent ROS increase, shifting the nitrous–redox balance towards oxidation [377].

In a randomized double-blind trial (Q-SYMBIO trial), the effects of CoQ10 supplementation were evaluated in 420 patients suffering from systolic HF, highlighting a significant improvement of HF symptoms and a significant reduction in major adverse cardiovascular events [378], although a recent meta-analysis showed that there are not sufficient data to support the safety and efficacy of CoQ10 in HF [379].

Since there is a need for randomized controlled trials with large sample size, comparing coenzyme Q10 to placebo, CoQ10 is not currently recommended in the treatment of HF [378].

6.2. Flavonoids supplementation counteracts NO signalling impairment

Flavonoids are polyphenolic antioxidants widely present in vegetables, leaves, flowers, fruits, bark and seeds, commonly used in traditional medicine [380]. It has been proven that dietary flavonoid intake is able to reduce cardiovascular risk, due to its antioxidant and free-radical scavenging properties and beneficial effects on endothelial function, exerted by the inhibition of low-density lipoprotein (LDL) oxidation, platelet aggregation and vasoconstriction. Several experimental studies and clinical trials evaluated the effect of flavonoid supplementation on HF [380–383].

In particular, underlying mechanisms for black tea cardioprotective activity involve antioxidant, anti-inflammatory, vasculo-protective, lipid-lowering and antithrombotic properties of flavonoids. The endothelium-dependent vasodilation observed after supplementation with polyphenolic fraction of black tea was due to eNOS phosphorylation and activation PKA- and Akt-dependent [384,385].

However, although the promising experimental data, the results obtained on vasorelaxation in isolated aortic rings are not consistent and the results of clinical studies are divergent and inconclusive [383,386].

A similar modulation was obtained by *Olea europaea* L. extract administration, since oleuropein was able to attenuate the Ang-II-mediated oxidative stress in vascular progenitor cells by its radical scavenger intrinsic property and the regulation of Akt/eNOS signalling pathway [387]. Furthermore, oleuropein counteracted cardiac damage in normal and cholesterol-fed rabbits exposed to I/R injury [388]. Although the promising preclinical data, the clinical results do not uniquely support the experimental data and more studies are required to fully explain the cardioprotective role of oleuropein [389,390].

Resveratrol is a natural phytoalexin extracted from grapes, peanuts and red wine and plays a key role on ROS scavenging [391]. Preclinical evidence showed that resveratrol was able to improve cardiac function in I/R through the modulation of NO pathway, endogenous redox signalling and autophagy in diabetic rats.

Cardioprotective effects of resveratrol in I/R injury are well documented in the presence of cardiometabolic diseases. Indeed, it has been observed that resveratrol alleviated cardiac dysfunction due to I/R through induction of proteins involved in NO pathway, autophagy, and endogenous redox signalling molecules in diabetic rats [392,393]. However, in contrast to preclinical data, only few clinical studies highlighted the cardioprotective effects of resveratrol in patients suffering from diabetes [394]. Clinical trials with a large cohort are needed to validate the results obtained in the previous clinical studies, which enrolled a small number of patients.

Interestingly, a recent *in vivo* study demonstrated the cardioprotective effect of berberine, a main bioactive compound contained in *Rhizoma coptidis* and other herbs, used for the treatment of several diseases, such as hypertension, cancer, diabetes and atherosclerosis [395,396]. The observed cardioprotective effects of berberine were mediated by the increase of cardiac total NOS activity and the upregulation of Akt/eNOS pathway [397]. Furthermore, berberin significantly ameliorated cardiac function in a mouse model of chronic HF [398].

A randomized, double-blind controlled trial showed that berberin supplementation significantly ameliorated exercise capacity and LVEF in patients suffering from chronic HF [399]. Although these preclinical and clinical evidence, there is a lack of clinical data demonstrating the direct role of berberin on ROS scavenging activity, primarily tested in patients with MI and not in patients with chronic HF [400,401].

However, in experimental preclinical and clinical investigations, it has been extensively examined the potential antioxidant and cardioprotective role of *Citrus bergamia* Risso&Poiteau polyphenols [380, 402–404]. In particular, the *in vitro* data obtained highlighted the significant cardioprotective effects of Bergamot Polyphenolic Fraction (BPF) to counteract Doxorubicin-induced cardiomyopathy through its direct scavenging and antioxidant activity, reducing excessive autophagy activation and apoptosis, thus preventing the pathological cardiac

remodelling [402].

Furthermore, several experimental studies demonstrated that hypercholesterolemia produce an increase in infarct size in animal models. The decrease of cardiac content of NO and the increase of oxidative and nitrosative stress, the upregulation of apoptosis and cardiac gene expression profile modifications, induce myocardial impairment in hyperlipidemic condition [405].

The effect of BPF treatment in hyperlipidemic rats was associated to serum MDA levels reduction, a recognized biomarker of oxidative stress and PCSK9 expression decrease, suggesting the role of PCSK9 in the regulation of cholesterol metabolism and the relative cardioprotective action [406].

In addition, the results obtained in a clinical study suggest that the increase of NO release in response to BPF supplementation could play a key role in cardiovascular adaptive mechanisms in athletes, enhancing the exercise performance [407]. The study was carried out on athletes demonstrating the amelioration on serum ADMA, NO, Endopat indices of endothelial function and maximal oxygen uptake, apported by BPF supplementation. [407].

Nevertheless, although the nutraceutical supplementation is a promising field in cardiovascular disease prevention and treatment, the potential for its use in HF still needs to be extensively and adequately assessed. The limiting factors in the performance of translational studies include the antioxidant bioavailability, the choice of the correct clinical dosage of bioactive compounds and the design of experimental investigations, which is often unable to reflect comorbidity conditions in humans [380].

More studies are needed to assess and validate the best combination of nutraceutical supplementation to counteract myocardial dysfunction in patients suffering from HF.

7. Conclusion and future perspective

HF has an overall impact in the global population whose diagnosis requires the existence of structural and/or functional cardiac impairment resulting in elevated intracardiac pressure and/or insufficient cardiac output at rest and/or during exercise.

Clinical development and progression of HF is significantly affected by alteration of the oxidative environment and NO bioavailability. Notably, NOS uncoupling and the subsequent reduced NO production is involved in both vascular and cardiac cell damage.

The understanding of the molecular mechanisms underlying endothelial and myocardial dysfunction plays a crucial role in the identification of new therapeutic targets.

In particular, the mechanisms underlying HFpEF are not yet completely recognised and several factors are involved. HFpEF is a consequence of systemic inflammation induced by coexisting comorbidities that leads to ROS production by endothelial cells, reduced NO availability and decreased activation of cGMP-dependent PKG, triggering to myocardial hypertrophy and diastolic dysfunction. In addition, a low NO bioavailability and cGMP reduction are present in HFREF patients, leading to vasodilation impairment, aerobic exercise capacity and muscle power decrease.

There is currently a gap in the HFpEF treatment and most of the drugs approved for HFREF are ineffective for HFpEF. Different clinical investigations are ongoing, apporting new medical evidence for future treatment of HFpEF.

It has been highlighted that NOS/NO and NO/cGMP signalling dysregulation may exacerbate cardiovascular damage. Based on this evidence, therapeutic approaches aimed at improving NOS signalling appear to enhance the NOS-dependent cardioprotective and vasodilatory effects. Many of the reviewed clinical trials showed an amelioration of primary and advanced haemodynamic parameters as well as congestive HF markers, though these beneficial effects did not persist beyond the treatment period. Conversely, different clinical studies failed to meet primary and secondary endpoints, although good

tolerability and safety were observed at the doses used.

Therefore, further high-quality basic experiments and large-scale and long-term clinical trials are needed to explore the efficacy and safety of new therapeutical strategies able to improve the unmet clinical endpoints in patients with HF, achieving the goal of reducing mortality and improving quality of life. Indeed, to achieve these outcomes multidisciplinary and multifaceted approaches are needed, based on a therapeutic regimen, including multiple drug combinations, to obtain a symptomatic and prognostic improvement in all patients.

In addition, novel drugs and nutraceuticals have been discovered to modulate NO/cGMP pathway, thereby representing potential innovative solutions in approaching heart failure treatment. Finally, the contribution of nutritional sources of active ingredients interacting with NO/cGMP pathway appears to shed new light in the stimulation of endogenous biomolecular mechanisms of failing myocardial cells.

Nevertheless, the potential use of nutraceutical supplementation still needs to be extensively and adequately assessed to validate the best combination in HF prevention and treatment.

Funding

The work was supported by the public resources from the Italian Ministry of Research. PON-MIUR 03PE000_78.1 and PONMIUR 03PE000_78.2. POR Calabria FESR FSE 2014-2020 Asse 12-Azioni 10.5.6 e 10.5.12.

CRedit authorship contribution statement

Vincenzo Mollace, Rocco Mollace, Roberta Macrì, Federica Scarano: Conceptualization and design. Rocco Mollace, Roberta Macrì, Federica Scarano: Writing—Original Draft Preparation. Irene Bava, Cristina Carresi, Jessica Maiuolo, Annamaria Tavernese, Micaela Gliozzi, Vincenzo Musolino, Saverio Muscoli, Ernesto Palma, Carolina Muscoli: Writing—Review & Editing. Daniela Salvemini, Massimo Federici: Supervision, D.S.; M.F. Vincenzo Mollace: Funding Acquisition, V.M. (Vincenzo Mollace).

Declaration of Competing Interest

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

Acknowledgements

We would like to thank Dr Joel Eissenberg (Saint Louis University) for his input and editorial help.

References

- [1] A. McDonagh, M. Metra, M. Adamo, R.S. Gardner, A. Baumach, M. Böhm, H. Burri, J. Butler, J. Čelutkienė, O. Chioncel, J.G.F. Cleland, A.J.S. Coats, M. G. Crespo-Leiro, D. Farmakis, M. Gilard, S. Heymans, A.W. Hoes, T. Jaarsma, E. A. Jankowska, M. Lainscak, C.S.P. Lam, A.R. Lyon, J.J.V. McMurray, A. Mebazaa, R. Mindham, C. Muneretto, M. Francesco Piepoli, S. Price, G.M.C. Rosano, F. Ruschitzka, A. Kathrine Skibelund, 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure, ESC Sci. Doc. Group. Eur. Heart J. 42 (2021) 3599–3726, <https://doi.org/10.1093/eurheartj/ehab368>.
- [2] B. Bozkurt, A.J.S. Coats, H. Tsutsui, C.M. Abdelhamid, S. Adamopoulos, N. Albert, S.D. Anker, J. Atherton, M. Böhm, J. Butler, M.H. Drazner, G. Michael Felker, G. Filippatos, M. Fiuzat, G.C. Fonarow, J.E. Gomez-Mesa, P. Heidenreich, T. Imamura, E.A. Jankowska, J. Januzzi, P. Khazanie, K. Kinugawa, C.S.P. Lam, Y. Matsue, M. Metra, T. Ohtani, M. Francesco Piepoli, P. Ponikowski, G.M. C. Rosano, Y. Sakata, P. Seferović, R.C. Starling, J.R. Teerlink, O. Vardeny, K. Yamamoto, C. Yancy, J. Zhang, S. Zieroth, Universal definition and classification of heart failure: a report of the Heart Failure Society of America, Heart Failure Association of the European Society of Cardiology, Japanese Heart Failure Society and Writing Committee of the Universal Definition of Heart Failure: Endorsed by the Canadian Heart Failure Society, Heart Failure Association of India, Cardiac Society of Australia and New Zealand, and Chinese

- Heart Failure Association, *Eur. J. Heart Fail* 23 (2021) 352–380, <https://doi.org/10.1002/ehfj.2115>.
- [3] G. Savarese, P.M. Becher, L.H. Lund, P. Seferovic, G.M.C. Rosano, A.J.S. Coats, Global burden of heart failure: a comprehensive and updated review of epidemiology, *Cardiovasc. Res.* 118 (2023) 3272–3287, <https://doi.org/10.1093/cvr/cvac013>.
- [4] O. Chioncel, M. Lainscak, P.M. Seferovic, S.D. Anker, M.G. Crespo-Leiro, V. P. Harjola, J. Parissis, C. Laroche, M.F. Piepoli, C. Fonseca, A. Mebazaa, L. Lund, G.A. Ambrosio, A.J. Coats, R. Ferrari, F. Ruschitzka, A.P. Maggioni, G. Filippatos, Epidemiology and one-year outcomes in patients with chronic heart failure and preserved, mid-range and reduced ejection fraction: an analysis of the ESC heart failure long-term registry, *Eur. J. Heart Fail.* 19 (2017) 1574–1585, <https://doi.org/10.1002/ehfj.813>.
- [5] B. Greenberg, Medical management of patients with heart failure and reduced ejection fraction, *Korean Circ. J.* 52 (2022) 173–197, <https://doi.org/10.4070/kcj.2021.0401>.
- [6] H.D. Larkin, FDA expands empagliflozin heart failure indication, *JAMA* 327 (2022) 1219, <https://doi.org/10.1001/jama.2022.3970>.
- [7] J.J.V. McMurray, S.D. Solomon, S.E. Inzucchi, L. Køber, M.N. Kosiborod, F. A. Martinez, P. Ponikowski, M.S. Sabatine, I.S. Anand, J. Böhöhlávek, M. Böhm, C. E. Chiang, V.K. Chopra, R.A. de Boer, A.S. Desai, M. Diez, J. Drozd, A. Dukát, J. Ge, J.G. Howlett, T. Katova, M. Kitakaze, C.E.A. Ljungman, B. Merkely, J. C. Nicolau, E. O'Meara, M.C. Petrie, P.N. Vinh, M. Schou, S. Tereshchenko, S. Verma, C. Held, D.L. DeMets, K.F. Docherty, P.S. Jhund, O. Bengtsson, M. Sjöstrand, A.M. Langkilde, DAPA-HF Trial Committees and Investigators, Dapagliflozin in patients with heart failure and reduced ejection fraction, *N. Engl. J. Med.* 381 (2019) 1995–2008, <https://doi.org/10.1056/NEJMoa1911303>.
- [8] M. Packer, S.D. Anker, J. Butler, G. Filippatos, J.P. Ferreira, S.J. Pocock, P. Carson, I. Anand, W. Doehner, M. Haass, M. Komajda, A. Miller, S. Pehrson, J. R. Teerlink, M. Brueckmann, W. Jamal, C. Zeller, S. Schneid, F. Zannad, Effect of empagliflozin on the clinical stability of patients with heart failure and a reduced ejection fraction: the EMPEROR-reduced trial, *Circulation* 143 (2021) 326–336, <https://doi.org/10.1161/CIRCULATIONAHA.120.051783>.
- [9] A.B. Gevaert, R. Kataria, F. Zannad, A.J. Sauer, K. Damman, K. Sharma, S.J. Shah, H.G.C. Van Spall, Heart failure with preserved ejection fraction: recent concepts in diagnosis, mechanisms and management, *Heart (Br. Card. Soc.)* 108 (2022) 1342–1350, <https://doi.org/10.1136/heartjnl-2021-319605>.
- [10] P.A. Howard, Treating heart failure with preserved ejection fraction: a challenge for clinicians, *Hosp. Pharm.* 50 (2015) 454–459, <https://doi.org/10.1310/hpj5006-454>.
- [11] S.D. Anker, J. Butler, G. Filippatos, J.P. Ferreira, E. Bocchi, M. Böhm, H. P. Brunner-La Rocca, D.J. Choi, V. Chopra, E. Chuquiure-Valenzuela, N. Giannetti, J.E. Gomez-Mesa, S. Janssens, J.L. Januzzi, J.R. Gonzalez-Juanatey, B. Merkely, S.J. Nicholls, S.V. Perrone, I.L. Piña, P. Ponikowski, M. Senni, D. Sim, J. Spinar, I. Squire, S. Taddei, H. Tsutsui, S. Verma, D. Vinereanu, J. Zhang, P. Carson, C.S.P. Lam, N. Marx, C. Zeller, N. Sattar, W. Jamal, S. Schneid, J. M. Schnee, M. Brueckmann, S.J. Pocock, F. Zannad, M. Packer, EMPEROR-Preserved Trial Investigators, Empagliflozin in heart failure with a preserved ejection fraction, *N. Engl. J. Med.* 385 (2021) 1451–1461, <https://doi.org/10.1056/NEJMoa2107038>.
- [12] S.D. Solomon, R.A. de Boer, D. DeMets, A.F. Hernandez, S.E. Inzucchi, M. N. Kosiborod, C.S.P. Lam, F. Martinez, S.J. Shah, D. Lindholm, U. Wilderang, F. Öhrn, B. Claggett, A.M. Langkilde, M. Petersson, J.J.V. McMurray, Dapagliflozin in heart failure with preserved and mildly reduced ejection fraction: rationale and design of the DELIVER trial, *Eur. J. Heart Fail.* 23 (2021) 1217–1225, <https://doi.org/10.1002/ehfj.2249>.
- [13] Writing Committee Members, & ACC/AHA Joint Committee Members, 2022 AHA/ACC/HFSA guideline for the management of heart failure, *J. Card. Fail.* 28 (2022) e1–e167, <https://doi.org/10.1016/j.cardfail.2022.02.010>.
- [14] G.D. Plitt, J.T. Spring, M.J. Moulton, D.K. Agrawal, Mechanisms, diagnosis, and treatment of heart failure with preserved ejection fraction and diastolic dysfunction, *Expert Rev. Cardiovasc. Ther.* 16 (2018) 579–589, <https://doi.org/10.1080/14779072.2018.1497485>.
- [15] Z. Cai, C. Wu, Y. Xu, J. Cai, M. Zhao, L. Zu, The NO-cGMP-PKG Axis in HFpEF: from pathological mechanisms to potential therapies, *Aging Dis.* 14 (2023) 46–62, <https://doi.org/10.14336/AD.2022.0523>.
- [16] V. Mulkareddy, S.B. Racette, A.R. Coggan, L.R. Peterson, Dietary nitrate's effects on exercise performance in heart failure with reduced ejection fraction (HFREF), *Biochim. Et. Biophys. Acta Mol. Basis Dis.* 1865 (2019) 735–740, <https://doi.org/10.1016/j.bbadis.2018.09.026>.
- [17] S. Moncada, Nitric oxide: discovery and impact on clinical medicine, *J. R. Soc. Med.* 92 (1999) 164–169, <https://doi.org/10.1177/014107689909200402>.
- [18] C. Farah, L.Y.M. Michel, J.L. Balligand, Nitric oxide signalling in cardiovascular health and disease, *Nat. Rev. Cardiol.* 15 (2018) 292–316, <https://doi.org/10.1038/nrcardio.2017.224>.
- [19] R.M. Blanton, cGMP signaling and modulation in heart failure, *J. Cardiovasc. Pharm.* 75 (2020) 385–398, <https://doi.org/10.1097/FJC.0000000000000749>.
- [20] S.J. Greene, M. Gheorghiadu, B.A. Borlaug, B. Pieske, M. Vaduganathan, J.C. Jr Burnett, L. Roessig, J.P. Stasch, S.D. Solomon, W.J. Paulus, J. Butler, The cGMP signaling pathway as a therapeutic target in heart failure with preserved ejection fraction, *J. Am. Heart Assoc.* 2 (2013), e000536, <https://doi.org/10.1161/JAHA.113.000536>.
- [21] R.C. Kukreja, F.N. Salloum, A. Das, Cyclic guanosine monophosphate signaling and phosphodiesterase-5 inhibitors in cardioprotection, *J. Am. Coll. Cardiol.* 59 (2012) 1921–7, <https://doi.org/10.1016/j.jacc.2011.09.086>.
- [22] B. Dunkerly-Eyring, D.A. Kass, Myocardial phosphodiesterases and their role in cGMP regulation, *J. Cardiovasc. Pharm.* 75 (2020) 483–493, <https://doi.org/10.1097/FJC.0000000000000773>.
- [23] U. Förstermann, W.C. Sessa, Nitric oxide synthases: regulation and function, *Eur. Heart J.* 33 (2012) 829–837, <https://doi.org/10.1093/eurheartj/ehr304>.
- [24] S.J. Burke, B.L. Updegraff, R.M. Bellich, M.R. Goff, D. Lu, S.C. Jr Minkin, M. D. Karlstad, J.J. Collier, Regulation of iNOS gene transcription by IL-1 β and IFN- γ requires a coactivator exchange mechanism, *Mol. Endocrinol.* 27 (2013) 1724–1742, <https://doi.org/10.1210/me.2013-1159>.
- [25] V. Garcia, W.C. Sessa, Endothelial NOS: perspective and recent developments, *Br. J. Pharm.* 176 (2019) 189–196, <https://doi.org/10.1111/bph.14522>.
- [26] A. Pautz, H. Li, H. Kleinert, Regulation of NOS expression in vascular diseases, *Front Biosci. (Landmark Ed.)* 26 (2021) 85–101, <https://doi.org/10.52586/4926>.
- [27] S.S. Soskić, B.D. Dobutović, E.M. Sudar, M.M. Obradović, D.M. Nikolić, J. D. Djordjevic, D.J. Radak, D.P. Mikhailidis, E.R. Isenović, Regulation of inducible nitric oxide synthase (iNOS) and its potential role in insulin resistance, diabetes and heart failure, *Open Cardiovasc. Med. J.* 5 (2011) 153–163, <https://doi.org/10.2174/1874192401105010153>.
- [28] J. Maiuolo, F. Oppedisano, C. Carresi, M. Gliozzi, V. Musolino, R. Macrì, F. Scarano, A. Coppolletta, A. Cardamone, F. Bosco, R. Mollace, C. Muscoli, E. Palma, V. Mollace, The generation of nitric oxide from aldehyde dehydrogenase-2: the role of dietary nitrates and their implication in cardiovascular disease management, *Int. J. Mol. Sci.* 23 (2022) 15454, <https://doi.org/10.3390/ijms232415454>.
- [29] R.M. Palmer, D.S. Ashton, S. Moncada, Vascular endothelial cells synthesize nitric oxide from L-arginine, *Nature* 333 (1988) 664–666, <https://doi.org/10.1038/333664a0>.
- [30] P.B. Massion, O. Feron, C. Dessy, J.L. Balligand, Nitric oxide and cardiac function: ten years after, and continuing, *Circ. Res.* 93 (2003) 388–398, <https://doi.org/10.1161/01.RES.0000088351.58510.21>.
- [31] T. Wallerath, I. Gath, W.E. Aulitzky, J.S. Pollock, H. Kleinert, U. Förstermann, Identification of the NO synthase isoforms expressed in human neutrophil granulocytes, megakaryocytes and platelets, *Thromb. Haemost.* 77 (1997) 163–167.
- [32] J.L. Balligand, O. Feron, C. Dessy, eNOS activation by physical forces: from short-term regulation of contraction to chronic remodeling of cardiovascular tissues, *Physiol. Rev.* 89 (2009) 481–534, <https://doi.org/10.1152/physrev.00042.2007>.
- [33] E. Anter, K. Chen, O.M. Shapira, R.H. Karas, J.F. Jr Keane, p38 mitogen-activated protein kinase activates eNOS in endothelial cells by an estrogen receptor alpha-dependent pathway in response to black tea polyphenols, *Circ. Res.* 96 (10) (2005) 1072–1078, <https://doi.org/10.1161/01.RES.0000168807.63013.56>.
- [34] Y. Chan, J.E. Fish, C. D'Abreo, S. Lin, G.B. Robb, A.M. Teichert, F. Karantzoulis-Fegaras, A. Keightley, B.M. Steer, P.A. Marsden, The cell-specific expression of endothelial nitric-oxide synthase: a role for DNA methylation, *J. Biol. Chem.* 279 (2004) 35087–35100, <https://doi.org/10.1074/jbc.M405063200>.
- [35] J.Z. Qin, S.J. Wang, C. Xia, microRNAs regulate nitric oxide release from endothelial cells by targeting NOS3, *J. Thromb. Thrombolysis* 46 (2018) 275–282, <https://doi.org/10.1007/s11239-018-1684-4>.
- [36] V. Garcia, W.C. Sessa, Endothelial NOS: perspective and recent developments, *Br. J. Pharm.* 176 (2019) 189–196, <https://doi.org/10.1111/bph.14522>.
- [37] O. Feron, L. Belhassen, L. Kobzik, T.W. Smith, R.A. Kelly, T. Michel, Endothelial nitric oxide synthase targeting to caveolae. Specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells, *J. Biol. Chem.* 271 (1996) 22810–22814, <https://doi.org/10.1074/jbc.271.37.22810>.
- [38] G. García-Cardena, P. Oh, J. Liu, J.E. Schnitzer, W.C. Sessa, Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: implications for nitric oxide signaling, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 6448–6453, <https://doi.org/10.1073/pnas.93.13.6448>.
- [39] S. Dimmeler, I. Fleming, B. Fisslthaler, C. Hermann, R. Busse, A.M. Zeiher, Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation, *Nature* 399 (1999) 601–605, <https://doi.org/10.1038/21224>.
- [40] I. Fleming, Molecular mechanisms underlying the activation of eNOS, *Pflug. Arch.* 459 (2010) 793–806, <https://doi.org/10.1007/s00424-009-0767-7>.
- [41] D. Fulton, J.E. Church, L. Ruan, C. Li, S.G. Sood, B.E. Kemp, I.G. Jennings, R. C. Venema, Src kinase activates endothelial nitric-oxide synthase by phosphorylating Tyr-83, *J. Biol. Chem.* 280 (2005) 35943–35952, <https://doi.org/10.1074/jbc.M504606200>.
- [42] E.H. Heiss, V.M. Dirsch, Regulation of eNOS enzyme activity by posttranslational modification, *Curr. Pharm. Des.* 20 (2014) 3503–3513, <https://doi.org/10.2174/13816128113196660745>.
- [43] P.A. Erwin, A.J. Lin, D.E. Golan, T. Michel, Receptor-regulated dynamic S-nitrosylation of endothelial nitric-oxide synthase in vascular endothelial cells, *J. Biol. Chem.* 280 (2005) 19888–19894, <https://doi.org/10.1074/jbc.M413058200>.
- [44] D.D. Duan, C.Y. Kwan, A molecular switch of "yin and yang": S-glutathionylation of eNOS turns off NO synthesis and turns on superoxide generation, *Acta Pharm. Sin.* 32 (2011) 415–416, <https://doi.org/10.1038/aps.2011.21>.
- [45] F. Aktan, iNOS-mediated nitric oxide production and its regulation, *Life Sci.* 75 (2004) 639–653, <https://doi.org/10.1016/j.lfs.2003.10.042>.
- [46] J.L. Balligand, D. Ungureanu-Longrois, W.W. Simmons, D. Pimental, T. A. Malinski, M. Kapturczak, Z. Taha, C.J. Lowenstein, A.J. Davidoff, R.A. Kelly, Cytokine-inducible nitric oxide synthase (iNOS) expression in cardiac myocytes. Characterization and regulation of iNOS expression and detection of iNOS activity in single cardiac myocytes in vitro, *J. Biol. Chem.* 269 (1994) 27580–27588.

- [47] M. Gliozzi, M. Scicchitano, F. Bosco, V. Musolino, C. Carresi, F. Scarano, J. Maiuolo, S. Nucera, A. Maretta, S. Paone, R. Mollace, S. Ruga, M.C. Zito, R. Macri, F. Oppedisano, E. Palma, D. Salvemini, C. Muscoli, V. Mollace, Modulation of nitric oxide synthases by oxidized LDLs: Role in vascular inflammation and atherosclerosis development, *Int. J. Mol. Sci.* 20 (2019) 3294, <https://doi.org/10.3390/ijms20133294>.
- [48] P.J. Kolodziejki, A. Musial, J.S. Koo, N.T. Eissa, Ubiquitination of inducible nitric oxide synthase is required for its degradation, *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 12315–12320, <https://doi.org/10.1073/pnas.192345199>.
- [49] P. Zhang, X. Xu, X. Hu, E.D. van Deel, G. Zhu, Y. Chen, Inducible nitric oxide synthase deficiency protects the heart from systolic overload-induced ventricular hypertrophy and congestive heart failure, *Circ. Res.* 100 (2007) 1089–1098, <https://doi.org/10.1161/01.RES.0000264081.78659.45>.
- [50] J. Maiuolo, F. Oppedisano, C. Carresi, M. Gliozzi, V. Musolino, R. Macri, F. Scarano, A. Coppoletta, A. Cardamone, F. Bosco, R. Mollace, C. Muscoli, E. Palma, V. Mollace, The generation of nitric oxide from aldehyde dehydrogenase-2: the role of dietary nitrates and their implication in cardiovascular disease management, *Int. J. Mol. Sci.* 23 (2022) 15454, <https://doi.org/10.3390/ijms232415454>.
- [51] J.O. Lundberg, M. Govoni, Inorganic nitrate is a possible source for systemic generation of nitric oxide, *Free Radic. Biol. Med.* 37 (2004) 395–400, <https://doi.org/10.1016/j.freeradbiomed.2004.04.027>.
- [52] K. Cosby, K.S. Partovi, J.H. Crawford, R.P. Patel, C.D. Reiter, S. Martyr, B.K. Yang, M.A. Wacławski, G. Zalos, X. Xu, K.T. Huang, H. Shields, D.B. Kim-Shapiro, A. N. Schechter, R.O. 3rd Cannon, M.T. Gladwin, Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation, *Nat. Med.* 9 (2003) 1498–1505, <https://doi.org/10.1038/nm954>.
- [53] A.J. Webb, A.B. Millsom, K.S. Rathod, W.L. Chu, S. Qureshi, M.J. Lovell, F. M. Lecomte, D. Perrett, C. Raimondo, E. Khoshbin, Z. Ahmed, R. Uppal, N. Benjamin, A.J. Hobbs, A. Ahluwalia, Mechanisms underlying erythrocyte and endothelial nitrite reduction to nitric oxide in hypoxia: role for xanthine oxidoreductase and endothelial nitric oxide synthase, *Circ. Res.* 103 (2008) 957–964, <https://doi.org/10.1161/CIRCRESAHA.108.175810>.
- [54] P.R. Castello, P.S. David, T. McClure, Z. Crook, R.O. Poyton, Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes, *Cell Metab.* 3 (2006) 277–287, <https://doi.org/10.1016/j.cmet.2006.02.011>.
- [55] S. Velmurugan, V. Kapil, S.M. Ghosh, S. Davies, A. McKnight, Z. Aboud, R. S. Khambata, A.J. Webb, A. Poole, A. Ahluwalia, Antiplatelet effects of dietary nitrate in healthy volunteers: involvement of cGMP and influence of sex, *Free Radic. Biol. Med.* 65 (2013) 1521–1532, <https://doi.org/10.1016/j.freeradbiomed.2013.06.031>.
- [56] V. Kapil, R.S. Khambata, A. Robertson, M.J. Caulfield, A. Ahluwalia, Dietary nitrate provides sustained blood pressure lowering in hypertensive patients: a randomized, phase 2, double-blind, placebo-controlled study, *Hypertension* 65 (2) (2015) 320–327, <https://doi.org/10.1161/HYPERTENSIONAHA.114.04675>.
- [57] A. Webb, R. Bond, P. McLean, R. Uppal, N. Benjamin, A. Ahluwalia, Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia-reperfusion damage, *Proc. Natl. Acad. Sci. U. S. A.* 102 (2004) 13683–13688, <https://doi.org/10.1073/pnas.0402927101>.
- [58] P. Tripathy, N.S. Patel, A. Webb, K. Rathod, F.M. Lecomte, E. Mazzon, S. Cuzzocrea, M.M. Yaqoob, A. Ahluwalia, C. Thiemermann, Nitrite-derived nitric oxide protects the rat kidney against ischemia/reperfusion injury in vivo: role for xanthine oxidoreductase, *J. Am. Soc. Nephrol.* 18 (2007) 570–580, <https://doi.org/10.1681/ASN.2006050450>.
- [59] T.E. Ingram, A.G. Fraser, R.A. Bleasdale, E.A. Ellins, A.D. Margulescu, J.P. Halcox, P.E. James, Low-dose sodium nitrite attenuates myocardial ischemia and vascular ischemia-reperfusion injury in human models, *J. Am. Coll. Cardiol.* 61 (2013) 2534–2541, <https://doi.org/10.1016/j.jacc.2013.03.050>.
- [60] U.B. Hendgen-Cotta, P. Luedike, M. Totzke, M. Kropp, A. Schicho, P. Stock, C. Rammos, M. Niessen, C. Heiss, J.O. Lundberg, E. Weitzberg, M. Kelm, T. Rassaf, Dietary nitrate supplementation improves revascularization in chronic ischemia, *Circulation* 126 (2012) 1983–1992, <https://doi.org/10.1161/CIRCULATIONAHA.112.12912>.
- [61] C. Jädert, J. Petersson, S. Massena, D. Ahl, L. Grapensparr, L. Holm, J. O. Lundberg, M. Phillipson, Decreased leukocyte recruitment by inorganic nitrate and nitrite in microvascular inflammation and NSAID-induced intestinal injury, *Free Radic. Biol. Med.* 52 (2012) 683–692, <https://doi.org/10.1016/j.freeradbiomed.2011.11.018>.
- [62] R.L. Engler, M.D. Dahlgren, D.D. Morris, M.A. Peterson, G.W. Schmid-Schönbein, Role of leukocytes in response to acute myocardial ischemia and reflow in dogs, *Am. J. Physiol.* 251 (1986) H314–H323, <https://doi.org/10.1152/ajpheart.1986.251.2.H314>.
- [63] N.E. Houstis, A.S. Eisman, P.P. Pappagianopoulos, L. Wooster, C.S. Bailey, P. D. Wagner, G.D. Lewis, Exercise intolerance in heart failure with preserved ejection fraction: diagnosing and ranking its causes using personalized O₂ pathway analysis, *Circulation* 137 (2018) 148–161, <https://doi.org/10.1161/CIRCULATIONAHA.117.029058>.
- [64] S. Bhushan, K. Kondo, D.J. Polhemus, H. Otsuka, C.K. Nicholson, Y.X. Tao, H. Huang, V.V. Georgiopoulou, T. Murohara, J.W. Calvert, J. Butler, D.J. Lefler, Nitrite therapy improves left ventricular function during heart failure via restoration of nitric oxide-mediated cytoprotective signaling, *Circ. Res.* 114 (2014) 1281–1291, <https://doi.org/10.1161/CIRCRESAHA.114.301475>.
- [65] B.A. Borlaug, K.E. Koepf, V. Melenovsky, Sodium nitrite improves exercise hemodynamics and ventricular performance in heart failure with preserved ejection fraction, *J. Am. Coll. Cardiol.* 66 (2015) 1672–1682, <https://doi.org/10.1016/j.jacc.2015.07.067>.
- [66] E. Catry, L.B. Bindels, A. Tailleux, S. Lestavel, A.M. Neyrinck, J.F. Goossens, I. Lobyshyeva, H. Plovier, A. Essaghir, J.B. Demoulin, C. Bouzin, B.D. Pachikian, P. D. Cani, B. Staels, C. Dessy, N.M. Delzenne, Targeting the gut microbiota with inulin-type fructans: preclinical demonstration of a novel approach in the management of endothelial dysfunction, *Gut* 67 (2018) 271–283, <https://doi.org/10.1136/gutjnl-2016-313316>.
- [67] M. Russwurm, C. Russwurm, D. Koesling, E. Mergia, NO/cGMP: the past, the present, and the future, *Methods Mol. Biol.* 1020 (2013) 1–16, https://doi.org/10.1007/978-1-62703-459-3_1.
- [68] N. Shahani, A. Sawa, Protein S-nitrosylation: role for nitric oxide signaling in neuronal death, *Biochim Biophys. Acta* 1820 (2012) 736–742, <https://doi.org/10.1016/j.bbagen.2011.07.010>.
- [69] A.M. Shah, P.A. MacCarthy, Paracrine and autocrine effects of nitric oxide on myocardial function, *Pharm. Ther.* 86 (2000) 49–86, [https://doi.org/10.1016/S0163-7258\(99\)00072-8](https://doi.org/10.1016/S0163-7258(99)00072-8).
- [70] A.C. Straub, A.W. Lohman, M. Billaud, S.R. Johnstone, S.T. Dwyer, M.Y. Lee, P. S. Bortz, A.K. Best, L. Columbus, B. Gaston, B.E. Isakson, Endothelial cell expression of haemoglobin α regulates nitric oxide signalling, *Nature* 491 (2012) 473–477, <https://doi.org/10.1038/nature11626>.
- [71] A.L. Kleschyov, The NO-heme signaling hypothesis, *Free Radic. Biol. Med.* 112 (2017) 544–552, <https://doi.org/10.1016/j.freeradbiomed.2017.08.025>.
- [72] J.A. Wales, C.Y. Chen, L. Breci, A. Weichsel, S.G. Bernier, J.E. 2nd Sheppeck, R. Saling, T. Nakai, P.A. Renhowe, J. Jung, W.R. Montfort, Discovery of stimulator binding to a conserved pocket in the heme domain of soluble guanylyl cyclase, *J. Biol. Chem.* 293 (2018) 1850–1864, <https://doi.org/10.1074/jbc.RA117.000457>.
- [73] T.M. Lincoln, T.L. Cornwell, Intracellular cyclic GMP receptor proteins, *FASEB J.* 7 (1993) 328–338, <https://doi.org/10.1096/fasebj.7.2.7680013>.
- [74] D.A. Kass, E. Takimoto, T. Nagayama, H.C. Champion, Phosphodiesterase regulation of nitric oxide signaling, *Cardiovasc. Res.* 75 (2007) 303–314, <https://doi.org/10.1016/j.cardiores.2007.02.031>.
- [75] M. Zaccolo, M.A. Movsesian, cAMP and cGMP signaling cross-talk: role of phosphodiesterases and implications for cardiac pathophysiology, *Circ. Res.* 100 (2007) 1569–1578, <https://doi.org/10.1161/CIRCRESAHA.106.144501>.
- [76] J. Hammond, J.L. Balligand, Nitric oxide synthase and cyclic GMP signaling in cardiac myocytes: from contractility to remodeling, *J. Mol. Cell Cardiol.* 52 (2012) 330–340, <https://doi.org/10.1016/j.yjmcc.2011.07.029>.
- [77] S. Weber, M. Zeller, K. Guan, F. Wunder, M. Wagner, A. El-Armouche, PDE2 at the crossway between cAMP and cGMP signalling in the heart, *Cell Signal* 38 (2017) 76–84, <https://doi.org/10.1016/j.cellsig.2017.06.020>.
- [78] A. Zoccarato, N.C. Surdo, J.M. Arosen, L.A. Fields, L. Mancuso, G. Dodoni, A. Stangherlin, C. Livie, H. Jiang, Y.Y. Sin, F. Gesellchen, A. Terrin, G.S. Baillie, S. A. Nicklin, D. Graham, N. Szabo-Fresnais, J. Krall, F. Vandeput, M. Movsesian, L. Furlan, V. Corsetti, G. Hamilton, K. Lefkimiatis, I. Sjaastad, M. Zaccolo, Cardiac hypertrophy is inhibited by a local pool of cAMP regulated by phosphodiesterase 2, *Circ. Res.* 117 (2015) 707–719, <https://doi.org/10.1161/CIRCRESAHA.114.305892>.
- [79] M. Guazzi, M. Vicenzi, R. Arena, M.D. Guazzi, PDE5 inhibition with sildenafil improves left ventricular diastolic function, cardiac geometry, and clinical status in patients with stable systolic heart failure: results of a 1-year, prospective, randomized, placebo-controlled study, *Circ. Heart Fail.* 4 (2011) 8–17, <https://doi.org/10.1161/CIRCHEARTFAILURE.110.946694>.
- [80] E. Takimoto, H.C. Champion, M. Li, D. Belardi, S. Ren, E.R. Rodriguez, D. Bedja, K.L. Gabrielson, Y. Wang, D.A. Kass, Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy, *Nat. Med.* 11 (2005) 214–222, <https://doi.org/10.1038/nm1175>.
- [81] I.G. Sharina, E. Martin, The role of reactive oxygen and nitrogen species in the expression and splicing of nitric oxide receptor, *Antioxid. Redox Signal* 26 (2017) 122–136, <https://doi.org/10.1089/ars.2016.6687>.
- [82] D.J. Stuehr, S. Misra, Y. Dai, A. Ghosh, Maturation, inactivation, and recovery mechanisms of soluble guanylyl cyclase, *J. Biol. Chem.* 296 (2021), 100336, <https://doi.org/10.1016/j.jbc.2021.100336>.
- [83] H. Sellak, C.S. Choi, N.B. Dey, T.M. Lincoln, Transcriptional and post-transcriptional regulation of cGMP-dependent protein kinase (PKG-I): pathophysiological significance, *Cardiovasc. Res.* 97 (2013) 200–207, <https://doi.org/10.1093/cvr/cvs327>.
- [84] A. Fajmut, **Molecular Mechanisms and Targets of Cyclic Guanosine Monophosphate (cGMP) in Vascular Smooth Muscles. Muscle Cell and Tissue-Novel Molecular Targets and Current Advances, IntechOpen, 2021.**
- [85] J.A. Carvajal, A.M. Germain, J.P. Huidobro-Toro, C.P. Weiner, Molecular mechanism of cGMP-mediated smooth muscle relaxation, *J. Cell Physiol.* 184 (2000) 409–420, [https://doi.org/10.1002/1097-4652\(200009\)184:3<409::AID-JCP16>3.0.CO;2-K](https://doi.org/10.1002/1097-4652(200009)184:3<409::AID-JCP16>3.0.CO;2-K).
- [86] T. Seko, M. Ito, Y. Kureishi, R. Okamoto, N. Moriki, K. Onishi, N. Isaka, D. J. Hartshorne, T. Nakano, Activation of RhoA and inhibition of myosin phosphatase as important components in hypertension in vascular smooth muscle, *Circ. Res.* 92 (2003) 411–418, <https://doi.org/10.1161/01.RES.0000059987.90200.44>.
- [87] H.K. Surks, N. Mochizuki, Y. Kasai, S.P. Georgescu, K.M. Tang, M. Ito, T. M. Lincoln, M.E. Mendelsohn, Regulation of myosin phosphatase by a specific interaction with cGMP-dependent protein kinase I α , *Science* 286 (1999) 1583–1587, <https://doi.org/10.1126/science.286.5444.1583>.

- [88] X.Q. Hu, L. Zhang, Function and regulation of large conductance Ca(2+)-activated K⁺ channel in vascular smooth muscle cells, *Drug Discov. Today* 17 (2012) 974–987, <https://doi.org/10.1016/j.drudis.2012.04.002>.
- [89] P. Karczewski, M. Kelm, M. Hartmann, J. Schrader, Role of phospholamban in NO/EDRF-induced relaxation in rat aorta, *Life Sci.* 51 (1992) 1205–1210, [https://doi.org/10.1016/0024-3205\(92\)90357-u](https://doi.org/10.1016/0024-3205(92)90357-u).
- [90] J. Layland, J.M. Li, A.M. Shah, Role of cyclic GMP-dependent protein kinase in the contractile response to exogenous nitric oxide in rat cardiac myocytes, *J. Physiol.* 540 (2002) 457–467, <https://doi.org/10.1113/jphysiol.2001.014126>.
- [91] R. Thoenen, S. Giovanni, S. Govindan, R.L. Lee, G.R. Wang, T.D. Calamaras, E. Takimoto, D.A. Kass, S. Sadayappan, R.M. Blanton, Molecular screen identifies cardiac myosin-binding protein-C as a protein kinase G- α substrate, *Circ. Heart Fail.* 8 (2015) 1115–1122, <https://doi.org/10.1161/CIRCHEARTFAILURE.115.002308>.
- [92] M.K. Thomas, S.H. Francis, J.D. Corbin, Substrate- and kinase-directed regulation of phosphorylation of a cGMP-binding phosphodiesterase by cGMP, *J. Biol. Chem.* 265 (1990) 14971–14978.
- [93] R. Jäger, F. Schwede, H.G. Genieser, D. Koesling, M. Russwurm, Activation of PDE2 and PDE5 by specific GAF ligands: delayed activation of PDE5, *Br. J. Pharm.* 161 (2010) 1645–1660, <https://doi.org/10.1111/j.1476-5381.2010.00977.x>.
- [94] S.D. Rybalkin, I.G. Rybalkina, R. Feil, F. Hofmann, J.A. Beavo, Regulation of cGMP-specific phosphodiesterase (PDE5) phosphorylation in smooth muscle cells, *J. Biol. Chem.* 277 (2002) 3310–3317, <https://doi.org/10.1074/jbc.M106562200>.
- [95] F. Mullershausen, M. Russwurm, W.J. Thompson, L. Liu, D. Koesling, A. Friebe, Rapid nitric oxide-induced desensitization of the cGMP response is caused by increased activity of phosphodiesterase type 5 paralleled by phosphorylation of the enzyme, *J. Cell Biol.* 155 (2001) 271–278, <https://doi.org/10.1083/jcb.200107001>.
- [96] S.D. Rybalkin, C. Yan, K.E. Bornfeldt, J.A. Beavo, Cyclic GMP phosphodiesterases and regulation of smooth muscle function, *Circ. Res.* 93 (2003) 280–291, <https://doi.org/10.1161/01.RES.0000087541.15600.2B>.
- [97] J.R. Burgoyne, M. Madhani, F. Cuervo, R.L. Charles, J.P. Brennan, E. Schröder, D. D. Browning, P. Eaton, Cysteine redox sensor in PKG α enables oxidant-induced activation, *Science* 317 (2007) 1393–1397, <https://doi.org/10.1126/science.1144318>.
- [98] O. Prisyazhna, O. Rudyk, P. Eaton, Single atom substitution in mouse protein kinase G eliminates oxidant sensing to cause hypertension, *Nat. Med.* 18 (2012) 286–290, <https://doi.org/10.1038/nm.2603>.
- [99] D.X. Zhang, L. Borbouse, D. Gebremedhin, S.A. Mendoza, N.S. Zinkevich, R. Li, D. D. Guterman, H₂O₂-induced dilation in human coronary arterioles: role of protein kinase G dimerization and large-conductance Ca²⁺-activated K⁺ channel activation, *Circ. Res.* 110 (2012) 471–480, <https://doi.org/10.1161/CIRCRESAHA.111.258871>.
- [100] T. Nakamura, G. Zhu, M.J. Ranek, K. Kokkonen-Simon, M. Zhang, G.E. Kim, K. Tsujita, D.A. Kass, Prevention of PKG-1 α oxidation suppresses antihypertrophic/antifibrotic effects from PDE5 inhibition but not sGC stimulation, *Circ. Heart Fail.* 11 (2018), e004740, <https://doi.org/10.1161/CIRCHEARTFAILURE.117.004740>.
- [101] V. Fernando, X. Zheng, Y. Walia, V. Sharma, J. Letson, S. Furuta, S-nitrosylation: an emerging paradigm of redox signaling, *Antioxid. (Basel)* 8 (2019) 404, <https://doi.org/10.3390/antiox8090404>.
- [102] J. Sun, E. Murphy, Protein S-nitrosylation and cardioprotection, *Circ. Res.* 106 (2010) 285–296, <https://doi.org/10.1161/CIRCRESAHA.109.209452>.
- [103] J. Sun, E. Picht, K.S. Ginsburg, D.M. Bers, C. Steenbergen, E. Murphy, Hypercontractile female hearts exhibit increased S-nitrosylation of the L-type Ca²⁺ channel α 1 subunit and reduced ischemia/reperfusion injury, *Circ. Res.* 98 (2006) 403–411, <https://doi.org/10.1161/01.RES.0000202707.79018.0a>.
- [104] Z. Hu, B. Zhang, L.J.Y. Lim, W.Z.K. Loh, D. Yu, B.W.Q. Tan, M.C. Liang, Z. Huang, C.H. Leo, H. Huang, T.W. Soong, S-nitrosylation-mediated reduction of CaV1.2 surface expression and open probability underlies attenuated vasoconstriction induced by nitric oxide, *Hypertension* 79 (2022) 2854–2866, <https://doi.org/10.1161/HYPERTENSIONAHA.122.19103>.
- [105] J. Jia, A. Arif, F. Terenzi, B. Willard, E.F. Plow, S.L. Hazen, P.L. Fox, Target-selective protein S-nitrosylation by sequence motif recognition, *Cell* 159 (2014) 623–634, <https://doi.org/10.1016/j.cell.2014.09.032>.
- [106] L.A. Barouch, R.W. Harrison, M.W. Skaf, G.O. Rosas, T.P. Cappola, Z.A. Kobeissi, I.A. Hobai, C.A. Lemmon, A.L. Burnett, B. O'Rourke, E.R. Rodriguez, P.L. Huang, J.A. Lima, D.E. Berkowitz, J.M. Hare, Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms, *Nature* 416 (2002) 337–339, <https://doi.org/10.1038/416337a>.
- [107] A.M. Bellinger, S. Reiken, C. Carlson, M. Mongillo, X. Liu, L. Rothman, S. Matecki, A. Lacampagne, A.R. Marks, Hypernitrosylated ryanodine receptor calcium release channels are leaky in dystrophic muscle, *Nat. Med.* 15 (2009) 325–330, <https://doi.org/10.1038/nm.1916>.
- [108] D.R. Gonzalez, F. Beigi, A.V. Treuer, J.M. Hare, Deficient ryanodine receptor S-nitrosylation increases sarcoplasmic reticulum calcium leak and arrhythmogenesis in cardiomyocytes, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 20612–20617, <https://doi.org/10.1073/pnas.0706796104>.
- [109] N. Sayed, P. Baskaran, X. Ma, F. van den Akker, A. Beuve, Desensitization of soluble guanylyl cyclase, the NO receptor, by S-nitrosylation, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 12312–12317, <https://doi.org/10.1073/pnas.0703944104>.
- [110] N. Sayed, D.D. Kim, X. Fioramonti, T. Iwashita, W.N. Durán, A. Beuve, Nitroglycerin-induced S-nitrosylation and desensitization of soluble guanylyl cyclase contribute to nitrate tolerance, *Circ. Res.* 103 (2008) 606–614, <https://doi.org/10.1161/CIRCRESAHA.108.175133>.
- [111] Z. Cai, Q. Lu, Y. Ding, Q. Wang, L. Xiao, P. Song, M.H. Zou, Endothelial nitric oxide synthase-derived nitric oxide prevents dihydrofolate reductase degradation via promoting S-nitrosylation, *Arterioscler. Thromb. Vasc. Biol.* 35 (2015) 2366–2373, <https://doi.org/10.1161/ATVBHA.115.305796>.
- [112] K.E. Hatzistergos, E.C. Paulino, R.A. Dulce, L.M. Takeuchi, M.A. Bellio, S. Kulandavelu, Y. Cao, W. Balkan, R.M. Kanashiro-Takeuchi, J.M. Hare, S-nitrosoglutathione reductase deficiency enhances the proliferative expansion of adult heart progenitors and myocytes post myocardial infarction, *J. Am. Heart Assoc.* 4 (2015), e001974, <https://doi.org/10.1161/JAHA.115.001974>.
- [113] A.G. Salerno, A.C.B.A. Wanschel, R.A. Dulce, K.E. Hatzistergos, W. Balkan, J.M. Hare, S-nitrosoglutathione reductase (GSNOR) deficiency accelerates cardiomyocyte differentiation of induced pluripotent stem cells, *J. Cardiovasc. Aging* 1 (2021) 13, <https://doi.org/10.20517/jca.2021.19>.
- [114] T. Irie, P.Y. Sips, S. Kai, K. Kida, K. Ikeda, S. Hirai, K. Moazzami, P. Jiramongkolchai, D.B. Bloch, P.T. Doulias, A.A. Armondas, M. Kaneki, H. Ischiropoulos, E. Kranias, K.D. Bloch, J.S. Stamler, F. Ichinose, S-nitrosylation of calcium-handling proteins in cardiac adrenergic signaling and hypertrophy, *Circ. Res.* 117 (2015) 793–803, <https://doi.org/10.1161/CIRCRESAHA.115.307157>.
- [115] F. Beigi, D.R. Gonzalez, K.M. Minhas, Q.A. Sun, M.W. Foster, S.A. Khan, A. V. Treuer, R.A. Dulce, R.W. Harrison, R.M. Saraiva, C. Premer, I.H. Schulman, J. S. Stamler, J.M. Hare, Dynamic denitrosylation via S-nitrosoglutathione reductase regulates cardiovascular function, *Proc. Natl. Acad. Sci. U. S. A.* 109 (2012) 4314–4319, <https://doi.org/10.1073/pnas.1113319109>.
- [116] C. Szabo, Hydrogen sulfide, an enhancer of vascular nitric oxide signaling: mechanisms and implications, *Am. J. Physiol. Cell Physiol.* 312 (1) (2017) C3–C15, <https://doi.org/10.1152/ajpcell.00282.2016>.
- [117] B. Lv, S. Chen, C. Tang, H. Jin, J. Du, Y. Huang, Hydrogen sulfide and vascular regulation - an update, *J. Adv. Res.* 27 (2020) 85–97, <https://doi.org/10.1016/j.jare.2020.05.007>.
- [118] J. Beltowski, Synthesis, metabolism, and signaling mechanisms of hydrogen sulfide: an overview, *Methods Mol. Biol.* 2007 (2019) 1–8, https://doi.org/10.1007/978-1-4939-9528-8_1.
- [119] B.L. Predmore, D. Julian, A.J. Cardounel, Hydrogen sulfide increases nitric oxide production from endothelial cells by an akt-dependent mechanism, *Front. Physiol.* 2 (2011) 104, <https://doi.org/10.3389/fphys.2011.00104>.
- [120] S. Pardue, G.K. Kolluru, X. Shen, S.E. Lewis, C.B. Saffle, E.E. Kelley, C.G. Kevil, Hydrogen sulfide stimulates xanthine oxidoreductase conversion to nitrite reductase and formation of NO, *Redox Biol.* 34 (2020), 101447, <https://doi.org/10.1016/j.redox.2020.101447>.
- [121] Z. Zhou, E. Martin, I. Sharina, I. Esposito, C. Szabo, M. Bucci, G. Cirino, A. Papapetropoulos, Regulation of soluble guanylyl cyclase redox state by hydrogen sulfide, *Pharm. Res.* 111 (2016) 556–562, <https://doi.org/10.1016/j.phrs.2016.06.029>.
- [122] S.I. Bibli, G. Yang, Z. Zhou, R. Wang, S. Topouzis, A. Papapetropoulos, Role of cGMP in hydrogen sulfide signaling, *Nitric Oxide* 46 (2015) 7–13, <https://doi.org/10.1016/j.niox.2014.12.004>.
- [123] P. Panagiotis, G. Yang, A. Asimakopoulou, T. Topouzis, R. Wang, C. Szabo, A. Papapetropoulos, Selectivity of hydrogen sulfide toward cyclic nucleotide phosphodiesterases, *Nitric Oxide* 47 (2015) S39.
- [124] D. Stubbert, O. Prisyazhna, O. Rudyk, J. Scotcher, J.R. Burgoyne, P. Eaton, Protein kinase G α oxidation paradoxically underlies blood pressure lowering by the reductant hydrogen sulfide, *Hypertension* 64 (2014) 1344–1351, <https://doi.org/10.1161/HYPERTENSIONAHA.114.04281>.
- [125] D. Wu, Y. Gu, D. Zhu, Cardioprotective effects of hydrogen sulfide in attenuating myocardial ischemia reperfusion injury (Review), *Mol. Med. Rep.* 24 (2021), 875, <https://doi.org/10.3892/mmr.2021.12515>.
- [126] R. Zamora, Y. Vodovotz, T.R. Billiar, Inducible nitric oxide synthase and inflammatory diseases, *Mol. Med.* 5 (2000) 347–373.
- [127] L. Minghetti, Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases, *J. Neuropathol. Exp. Neurol.* 63 (2004) 901–910, <https://doi.org/10.1093/jnen/63.9.901>.
- [128] E. Ricciotti, G.A. FitzGerald, Prostaglandins and inflammation, *Arterioscler. Thromb. Vasc. Biol.* 31 (2011) 986–1000, <https://doi.org/10.1161/ATVBHA.110.207449>.
- [129] V. Mollace, C. Muscoli, E. Masini, S. Cuzzocrea, D. Salvemini, Modulation of prostaglandin biosynthesis by nitric oxide and nitric oxide donors, *Pharm. Rev.* 57 (2005) 217–252, <https://doi.org/10.1124/pr.57.2.1>.
- [130] A. Sorokin, Nitric oxide synthase and cyclooxygenase pathways: a complex interplay in cellular signaling, *Curr. Med. Chem.* 23 (2016) 2559–2578, <https://doi.org/10.2174/0929867323666160729105312>.
- [131] D. Salvemini, T.P. Misko, J.L. Masferrer, K. Seibert, M.G. Currie, P. Needleman, Nitric oxide activates cyclooxygenase enzymes, *Proc. Natl. Acad. Sci. U. S. A.* 90 (1993) 7240–7244, <https://doi.org/10.1073/pnas.90.15.7240>.
- [132] D. Salvemini, M.G. Currie, V. Mollace, Nitric oxide-mediated cyclooxygenase activation. A key event in the antiplatelet effects of nitrovasodilators, *J. Clin. Invest.* 97 (1996) 2562–2568, <https://doi.org/10.1172/JCI118704>.
- [133] S. Cuzzocrea, D. Salvemini, Molecular mechanisms involved in the reciprocal regulation of cyclooxygenase and nitric oxide synthase enzymes, *Kidney Int.* 71 (2007) 290–297, <https://doi.org/10.1038/sj.ki.5002058>.
- [134] S.H. Lee, E. Soyoola, P. Channugam, S. Hart, W. Sun, H. Zhong, S. Liou, D. Simmons, D. Hwang, Selective expression of mitogen-inducible cyclooxygenase in macrophages stimulated with lipopolysaccharide, *J. Biol. Chem.* 267 (1992) 25934–25938.

- [135] H. Okamoto, O. Ito, R.J. Roman, A.G. Hudetz, Role of inducible nitric oxide synthase and cyclooxygenase-2 in endotoxin-induced cerebral hyperemia, *Stroke* 29 (1998) 1209–1218, <https://doi.org/10.1161/01.str.29.6.1209>.
- [136] R. Clancy, B. Varenika, W. Huang, L. Ballou, M. Attur, A.R. Amin, S.B. Abramson, Nitric oxide synthase/COX cross-talk: nitric oxide activates COX-1 but inhibits COX-2-derived prostaglandin production, *J. Immunol.* 165 (2000) 1582–1587, <https://doi.org/10.4049/jimmunol.165.3.1582>.
- [137] L. Chen, M.N. Salafranca, J.L. Mehta, Cyclooxygenase inhibition decreases nitric oxide synthase activity in human platelets, *Am. J. Physiol.* 273 (1997) H1854–H1859, <https://doi.org/10.1152/ajpheart.1997.273.4.H1854>.
- [138] A.L. Tsai, C. Wei, R.J. Kulmacz, Interaction between nitric oxide and prostaglandin H synthase, *Arch. Biochem. Biophys.* 313 (1994) 367–372, <https://doi.org/10.1006/abbi.1994.1400>.
- [139] R.W. Egan, J. Paxton, F.A. Jr Kuehl, Mechanism for irreversible self-deactivation of prostaglandin synthetase, *J. Biol. Chem.* 251 (1976) 7329–7335, [https://doi.org/10.1016/S0021-9258\(17\)32853-3](https://doi.org/10.1016/S0021-9258(17)32853-3).
- [140] D.P. Hajjar, H.M. Lander, S.F.A. Pearce, R. Upmacis, K.B. Pomerantz, Nitric oxide enhances prostaglandin-H synthase-1 activity by a heme-independent mechanism: evidence implicating nitrosothiols, *J. Am. Chem. Soc.* 117 (1995) 3340–3346, <https://doi.org/10.1021/ja00117a004>.
- [141] C. Szabó, H. Ischiropoulos, R. Radi, Peroxynitrite: biochemistry, pathophysiology and development of therapeutics, *Nat. Rev. Drug Discov.* 6 (2007) 662–680, <https://doi.org/10.1038/nrd2222>.
- [142] L.M. Landino, B.C. Crews, M.D. Timmons, J.D. Morrow, L.J. Marnett, Peroxynitrite, the coupling product of nitric oxide and superoxide, activates prostaglandin biosynthesis, *Proc. Natl. Acad. Sci. U. S. A.* 93 (26) (1996) 15069–15074, <https://doi.org/10.1073/pnas.93.26.15069>.
- [143] R.K. Upmacis, R.S. Deeb, D.P. Hajjar, Regulation of prostaglandin H2 synthase activity by nitrogen oxides, *Biochemistry* 38 (1999) 12505–12513, <https://doi.org/10.1021/bi983049e>.
- [144] D. Salvemini, S.F. Kim, V. Mollace, Reciprocal regulation of the nitric oxide and cyclooxygenase pathway in pathophysiology: relevance and clinical implications, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 304 (2013) R473–R487, <https://doi.org/10.1152/ajpregu.00355.2012>.
- [145] X. Yu, L. Ge, L. Niu, X. Lian, H. Ma, L. Pang, The dual role of inducible nitric oxide synthase in myocardial ischemia/reperfusion injury: friend or foe? *Oxid. Med. Cell Longev.* 2018 (2018), 8364848 <https://doi.org/10.1155/2018/8364848>.
- [146] X. Jiang, E. Shi, Y. Nakajima, S. Sato, COX-2 mediates morphine-induced delayed cardioprotection via an iNOS-dependent mechanism, *Life Sci.* 78 (2006) 2543–2549, <https://doi.org/10.1016/j.lfs.2005.10.032>.
- [147] M.C. de Waard, R. van Haperen, T. Soullié, D. Tempel, R. de Crom, D.J. Duncker, Beneficial effects of exercise training after myocardial infarction require full eNOS expression, *J. Mol. Cell Cardiol.* 48 (2010) 1041–1049, <https://doi.org/10.1016/j.yjmcc.2010.02.005>.
- [148] N. Tran, T. Garcia, M. Aniq, S. Ali, A. Ally, S.M. Nauli, Endothelial nitric oxide synthase (eNOS) and the cardiovascular system: in physiology and in disease states, *Am. J. Biomed. Sci. Res.* 15 (2022) 153–177.
- [149] R. Schulz, M. Kelm, G. Heusch, Nitric oxide in myocardial ischemia/reperfusion injury, *Cardiovasc. Res.* 61 (2004) 402–413, <https://doi.org/10.1016/j.cardiores.2003.09.019>.
- [150] M.B. West, G. Rokosh, D. Obal, M. Velayutham, Y.T. Xuan, B.G. Hill, R.J. Keith, J. Schrader, Y. Guo, D.J. Conklin, S.D. Prabhu, J.L. Zweier, R. Bolli, A. Bhatnagar, Cardiac myocyte-specific expression of inducible nitric oxide synthase protects against ischemia/reperfusion injury by preventing mitochondrial permeability transition, *Circulation* 118 (2008) 1970–1978, <https://doi.org/10.1161/CIRCULATIONAHA.108.791533>.
- [151] M.M. Cortese-Krott, T. Suvorava, F. Leo, S.K. Heuser, A. LoBue, J. Li, S. Becher, R. Schneckmann, T. Srivastava, R. Erkens, G. Wolff, J.P. Schmitt, M. Grandoch, J. O. Lundberg, J. Pernow, B.E. Isaksson, E. Weitzberg, M. Kelm, Red blood cell eNOS is cardioprotective in acute myocardial infarction, *Redox Biol.* 54 (2022), 102370, <https://doi.org/10.1016/j.redox.2022.102370>.
- [152] J. Strasen, O. Ritter, Role of nNOS in cardiac ischemia-reperfusion injury, *Trends Cardiovasc. Med.* 21 (2011) 58–63, <https://doi.org/10.1016/j.tcm.2012.03.001>.
- [153] B. Fisslthaler, A.E. Loot, A. Mohamed, R. Busse, I. Fleming, Inhibition of endothelial nitric oxide synthase activity by proline-rich tyrosine kinase 2 in response to fluid shear stress and insulin, *Circ. Res.* 102 (2008) 1520–1528, <https://doi.org/10.1161/CIRCRESAHA.108.172072>.
- [154] M. Siragusa, J. Thöle, S.I. Bibli, B. Luck, A.E. Loot, K. de Silva, I. Wittig, J. Heidler, H. Stingl, V. Randriamboavonjy, K. Kohlstedt, B. Brüne, A. Weigert, B. Fisslthaler, I. Fleming, Nitric oxide maintains endothelial redox homeostasis through PKM2 inhibition, *EMBO J.* 38 (2019), e100938, <https://doi.org/10.15252/emboj.2018100938>.
- [155] S.I. Bibli, Z. Zhou, S. Zukunft, B. Fisslthaler, I. Andreadou, C. Szabo, P. Brouckaert, I. Fleming, A. Papapetropoulos, Tyrosine phosphorylation of eNOS regulates myocardial survival after an ischemic insult: role of PYK2, *Cardiovasc. Res.* 113 (2017) 926–937, <https://doi.org/10.1093/cvr/cvx058>.
- [156] D.J. Hausenloy, H. Erik Bøtker, G. Condorelli, P. Ferdinandy, D. Garcia-Dorado, G. Heusch, S. Lecour, L.W. van Laake, R. Madonna, M. Ruiz-Meana, R. Schulz, J. P. Sluiter, D.M. Yellon, M. Ovize, Translating cardioprotection for patient benefit: position paper from the Working Group of Cellular Biology of the Heart of the European Society of Cardiology, *Cardiovasc. Res.* 98 (2013) 7–27, <https://doi.org/10.1093/cvr/cvt004>.
- [157] R. Caballero, M.G. de la Fuente, R. Gómez, A. Barana, I. Amorós, P. Dolz-Gaitón, L. Osuna, J. Almendral, F. Atienza, F. Fernández-Avilés, A. Pita, J. Rodríguez-Roda, A. Pinto, J. Tamargo, E. Delpón, In humans, chronic atrial fibrillation decreases the transient outward current and ultrarapid component of the delayed rectifier current differentially on each atria and increases the slow component of the delayed rectifier current in both, *J. Am. Coll. Cardiol.* 55 (2010) 2346–2354, <https://doi.org/10.1016/j.jacc.2010.02.028>.
- [158] N.D. Roe, J. Ren, Nitric oxide synthase uncoupling: a therapeutic target in cardiovascular diseases, *Vasc. Pharm.* 57 (2012) 168–172, <https://doi.org/10.1016/j.vph.2012.02.004>.
- [159] C.T. Taylor, S. Moncada, Nitric oxide, cytochrome C oxidase, and the cellular response to hypoxia, *Arterioscler. Thromb. Vasc. Biol.* 30 (2010) 643–647, <https://doi.org/10.1161/ATVBAHA.108.181628>.
- [160] M. Brunori, A. Giuffrè, E. Forte, D. Mastronicola, M.C. Barone, P. Sarti, Control of cytochrome c oxidase activity by nitric oxide, *Biochim Biophys. Acta* 1655 (2004) 365–371, <https://doi.org/10.1016/j.bbabi.2003.06.008>.
- [161] P.D. Sampath, V. Kannan, Mitigation of mitochondrial dysfunction and regulation of eNOS expression during experimental myocardial necrosis by alpha-mangostin, a xanthone derivative from *Garcinia mangostana*, *Drug Chem. Toxicol.* 32 (2009) 344–352, <https://doi.org/10.1080/01480540903159210>.
- [162] M. Peleli, C. Zollbrecht, M.F. Montenegro, M. Hezel, J. Zhong, E.G. Persson, R. Holmdahl, E. Weitzberg, J.O. Lundberg, M. Carlström, Enhanced XOR activity in eNOS-deficient mice: Effects on the nitrate-nitrite-NO pathway and ROS homeostasis, *Free Radic. Biol. Med.* 99 (2016) 472–484, <https://doi.org/10.1016/j.freeradbiomed.2016.09.004>.
- [163] P.V. Katakam, E.A. Wappler, P.S. Katz, I. Rutkai, A. Institoris, F. Domoki, T. Gáspár, S.M. Grovenburg, J.A. Snipes, D.W. Busija, Depolarization of mitochondria in endothelial cells promotes cerebral artery vasodilation by activation of nitric oxide synthase, *Arterioscler. Thromb. Vasc. Biol.* 33 (2013) 752–759, <https://doi.org/10.1161/ATVBAHA.112.300560>.
- [164] A. Fujita, Y. Kurachi, Molecular aspects of ATP-sensitive K⁺ channels in the cardiovascular system and K⁺ channel openers, *Pharm. Ther.* 85 (2000) 39–53, [https://doi.org/10.1016/s0163-7258\(99\)00050-9](https://doi.org/10.1016/s0163-7258(99)00050-9).
- [165] Y. Wang, N. Ahmad, M. Kudo, M. Ashraf, Contribution of Akt and endothelial nitric oxide synthase to diazoxide-induced late preconditioning, *Am. J. Physiol. Heart Circ. Physiol.* 287 (2004) H1125–H1131, <https://doi.org/10.1152/ajpheart.00183.2004>.
- [166] Y. Wang, M. Kudo, M. Xu, A. Ayub, M. Ashraf, Mitochondrial K(ATP) channel as an end effector of cardioprotection during late preconditioning: triggering role of nitric oxide, *J. Mol. Cell Cardiol.* 33 (2001) 2037–2046, <https://doi.org/10.1006/jmcc.2001.1468>.
- [167] J.K. Bendall, T. Damy, P. Ratajczak, X. Loyer, V. Monceau, I. Marty, P. Milliez, E. Robidel, F. Marotte, J.L. Samuel, C. Heymes, Role of myocardial neuronal nitric oxide synthase-derived nitric oxide in beta-adrenergic hyporesponsiveness after myocardial infarction-induced heart failure in rat, *Circulation* 110 (2004) 2368–2375, <https://doi.org/10.1161/01.CIR.0000145160.04084.AC>.
- [168] E.N. Dedkova, L.A. Blatter, Characteristics and function of cardiac mitochondrial nitric oxide synthase, *J. Physiol.* 587 (2009) 851–872, <https://doi.org/10.1113/jphysiol.2008.165423>.
- [169] S.A. Khan, M.W. Skaf, R.W. Harrison, K. Lee, K.M. Minhas, A. Kumar, M. Fradley, A.A. Shoukas, D.E. Berkowitz, J.M. Hare, Nitric oxide regulation of myocardial contractility and calcium cycling: independent impact of neuronal and endothelial nitric oxide synthases, *Circ. Res.* 92 (2003) 1322–1329, <https://doi.org/10.1161/01.RES.0000078171.52542.9E>.
- [170] J. Ramachandran, R.D. Peluffo, Threshold levels of extracellular l-arginine that trigger NOS-mediated ROS/RNS production in cardiac ventricular myocytes, *Am. J. Physiol. Cell Physiol.* 312 (2017) C144–C154, <https://doi.org/10.1152/ajpcell.00150.2016>.
- [171] S. Matsushima, J. Sadoshima, Yin and Yang of NADPH oxidases in myocardial ischemia-reperfusion, *Antioxid. (Basel)* 11 (2022) 1069, <https://doi.org/10.3390/antiox11061069>.
- [172] S. Wedgwood, P.T. Schumacker, R.H. Steinhorn, Hypoxia and Hyperoxia: Effects on the Newborn Pulmonary Circulation. *The Newborn Lung*, 2nd ed., Elsevier, 2012, pp. 91–109.
- [173] M.S. Alkhatib, M.J. Crabtree, Recoupling the cardiac nitric oxide synthases: tetrahydrobiopterin synthesis and recycling, *Curr. Heart Fail Rep.* 9 (2012) 200–210, <https://doi.org/10.1007/s11897-012-0097-5>.
- [174] A. Sen, A. Singh, A. Roy, S. Mohanty, N. Naik, M. Kalaivani, L. Ramakrishnan, Role of endothelial colony forming cells (ECFCs) Tetrahydrobiopterin (BH4) in determining ECFCs functionality in coronary artery disease (CAD) patients, *Sci. Rep.* 12 (2022), 3076, <https://doi.org/10.1038/s41598-022-06758-8>.
- [175] Y. Feng, Y. Feng, L. Gu, P. Liu, J. Cao, S. Zhang, The critical role of tetrahydrobiopterin (BH4) metabolism in modulating radiosensitivity: BH4/NOS axis as an angel or a devil, *Front Oncol.* 11 (2021), 720632, <https://doi.org/10.3389/fonc.2021.720632>.
- [176] Y. Nishijima, A. Sridhar, I. Bonilla, M. Velayutham, M. Khan, R. Terentyeva, C. Li, P. Kuppusamy, T.S. Elton, D. Terentyev, S. Györke, J.L. Zweier, A.J. Cardounel, C. A. Carnes, Tetrahydrobiopterin depletion and NOS2 uncoupling contribute to heart failure-induced alterations in atrial electrophysiology, *Cardiovasc. Res.* 91 (2011) 71–79, <https://doi.org/10.1093/cvr/cvr087>.
- [177] H.K. Kim, J. Han, Tetrahydrobiopterin in energy metabolism and metabolic diseases, *Pharm. Res.* 157 (2020), 104827, <https://doi.org/10.1016/j.phrs.2020.104827>.
- [178] H. Akaiha, J. Hata, R. Tanji, R. Honda-Takinami, K. Matsuoka, Y. Sato, M. Kataoka, S. Ogawa, Y. Kojima, Tetrahydrobiopterin prevents chronic ischemia-related lower urinary tract dysfunction through the maintenance of nitric oxide bioavailability, *Sci. Rep.* 10 (2020), 19844, <https://doi.org/10.1038/s41598-020-76948-9>.
- [179] C.P. Tiefenbacher, T. Bleeke, C. Vahl, K. Amann, A. Vogt, W. Kübler, Endothelial dysfunction of coronary resistance arteries is improved by tetrahydrobiopterin in

- atherosclerosis, *Circulation* 102 (2000) 2172–2179, <https://doi.org/10.1161/01.cir.102.18.2172>.
- [180] Y. Liao, M. Asakura, S. Takashima, A. Ogai, Y. Asano, Y. Shintani, T. Minamino, H. Asanuma, S. Sanada, J. Kim, S. Kitamura, H. Tomoike, M. Hori, M. Kitakaze, Celiprolol, a vasodilatory beta-blocker, inhibits pressure overload-induced cardiac hypertrophy and prevents the transition to heart failure via nitric oxide-dependent mechanisms in mice, *Circulation* 110 (2004) 692–699, <https://doi.org/10.1161/01.CIR.0000137831.08683.E1>.
- [181] V. Wilmes, S. Scheiper, W. Roehr, C. Niess, S. Kippenberger, K. Steinhorst, M. A. Verhoff, S. Kaufenstein, Increased inducible nitric oxide synthase (iNOS) expression in human myocardial infarction, *Int. J. Leg. Med.* 134 (2020) 575–581, <https://doi.org/10.1007/s00414-019-02051-y>.
- [182] M. Buelna-Chontal, W.R. García-Niño, A. Silva-Palacios, C. Enríquez-Cortina, C. Zazueta, Implications of oxidative and nitrosative post-translational modifications in therapeutic strategies against reperfusion damage, *Antioxid. (Basel)* (2021).
- [183] M.B. West, G. Rokosh, D. Obal, M. Velayutham, Y.T. Xuan, B.G. Hill, R.J. Keith, J. Schrader, Y. Guo, D.J. Conklin, S.D. Prabhu, J.L. Zweier, R. Bolli, A. Bhatnagar, Cardiac myocyte-specific expression of inducible nitric oxide synthase protects against ischemia/reperfusion injury by preventing mitochondrial permeability transition, *Circulation* 118 (2008) 1970–1978, <https://doi.org/10.1161/CIRCULATIONAHA.108.791533>.
- [184] J. Sun, Protein S-nitrosylation: a role of nitric oxide signaling in cardiac ischemic preconditioning, *Sheng Li Xue Bao* 59 (5) (2007) 544–552.
- [185] J. Sun, M. Morgan, R.F. Shen, C. Steenbergen, E. Murphy, Preconditioning results in S-nitrosylation of proteins involved in regulation of mitochondrial energetics and calcium transport, *Circ. Res.* 101 (2007) 1155–1163, <https://doi.org/10.1161/CIRCRESAHA.107.155879>.
- [186] O. Arriagada, A.V. Treuer, D.R. González, Cardioprotective Effects of S-Nitrosothiols in Ischemia-Reperfusion: Role for Mitochondria and Calcium Channels. *Free Radicals, Antioxidants and Diseases, Intechopen*, 2018.
- [187] R. Wang, M. Wang, S. He, G. Sun, X. Sun, Targeting calcium homeostasis in myocardial ischemia/reperfusion injury: an overview of regulatory mechanisms and therapeutic reagents, *Front Pharm.* 11 (2020), 872, <https://doi.org/10.3389/fphar.2020.00872>.
- [188] A. Galkin, S. Moncada, S-nitrosation of mitochondrial complex I depends on its structural conformation, *J. Biol. Chem.* 282 (2007) 37448–37453, <https://doi.org/10.1074/jbc.M707543200>.
- [189] C.A. Piantadosi, Regulation of mitochondrial processes by protein S-nitrosylation, *Biochim. Biophys. Acta* 1820 (2012) 712–721, <https://doi.org/10.1016/j.bbagen.2011.03.008>.
- [190] E. Clementi, G.C. Brown, M. Feelisch, S. Moncada, Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione, *Proc. Natl. Acad. Sci. U. S. A.* 95 (1998) 7631–7636, <https://doi.org/10.1073/pnas.95.13.7631>.
- [191] M. Kim, A. Stepanova, Z. Niatetskaya, S. Sosunov, S. Arndt, M.P. Murphy, A. Galkin, V.S. Ten, Attenuation of oxidative damage by targeting mitochondrial complex I in neonatal hypoxic-ischemic brain injury, *Free Radic. Biol. Med.* 124 (2018) 517–524, <https://doi.org/10.1016/j.freeradbiomed.2018.06.040>.
- [192] R.J. Wilson, J.C. Drake, D. Cui, B.M. Lewellen, C.C. Fisher, M. Zhang, D. F. Kashatus, L.A. Palmer, M.P. Murphy, Z. Yan, Mitochondrial protein S-nitrosation protects against ischemia reperfusion-induced denervation at neuromuscular junction in skeletal muscle, *Free Radic. Biol. Med.* 117 (2018) 180–190, <https://doi.org/10.1016/j.freeradbiomed.2018.02.006>.
- [193] E.T. Chouchani, C. Methner, S.M. Nadtochiy, A. Logan, V.R. Pell, S. Ding, A. M. James, H.M. Cochemé, J. Reinhold, K.S. Lilly, L. Partridge, I.M. Fearley, A. J. Robinson, R.C. Hartley, R.A. Smith, T. Krieg, P.S. Brookes, M.P. Murphy, Cardioprotection by S-nitrosation of a cysteine switch on mitochondrial complex I, *Nat. Med.* 19 (2013) 753–759, <https://doi.org/10.1038/nm.3212>.
- [194] M. Musicante, H.H. Kim, Y. Chen, F. Liao, S.K. Bhattacharya, L. Lu, Y. Sun, Regulation of endothelial nitric oxide synthase in cardiac remodeling, *Int. J. Cardiol.* 364 (2022) 96–101, <https://doi.org/10.1016/j.ijcard.2022.05.013>.
- [195] I.M. Bonilla, A. Sridhar, S. Györke, A.J. Cardounel, C.A. Carnes, Nitric oxide synthases and atrial fibrillation, *Front. Physiol.* 3 (2012) 105, <https://doi.org/10.3389/fphys.2012.00105>.
- [196] S.N. Reilly, X. Liu, R. Carnicer, A. Muszkiewicz, R. Jayaram, M.C. Carena, R. Wijesurendra, M. Stefanini, N.C. Surdo, O. Lomas, C. Ratnatunga, R. Sayeed, G. Krasopoulos, T. Rajakumar, A. Bueno-Orovio, S. Verheule, T.A. Fulga, B. Rodriguez, U. Schotten, B. Casadei, Up-regulation of miR-31 in human atrial fibrillation begets the arrhythmia by depleting dystrophin and neuronal nitric oxide synthase, *Sci. Transl. Med.* 8 (2016) 340ra74, <https://doi.org/10.1126/scitranslmed.aac4296>.
- [197] D.E. Burger, X. Lu, M. Lei, F.L. Xiang, L. Hammoud, M. Jiang, H. Wang, D. L. Jones, S.M. Sims, Q. Feng, Neuronal nitric oxide synthase protects against myocardial infarction-induced ventricular arrhythmia and mortality in mice, *Circulation* 120 (2009) 1345–1354, <https://doi.org/10.1161/CIRCULATIONAHA.108.846402>.
- [198] D. Dawson, C.A. Lygate, M.H. Zhang, K. Hulbert, S. Neubauer, B. Casadei, nNOS gene deletion exacerbates pathological left ventricular remodeling and functional deterioration after myocardial infarction, *Circulation* 112 (2005) 3729–3737, <https://doi.org/10.1161/CIRCULATIONAHA.105.539437>.
- [199] R. Carnicer, M.J. Crabtree, V. Sivakumaran, B. Casadei, D.A. Kass, Nitric oxide synthases in heart failure, *Antioxid. Redox Signal* 18 (2013) 1078–1099, <https://doi.org/10.1089/ars.2012.4824>.
- [200] V.A. Skeberdis, V. Gendviliene, D. Zablockaitė, R. Treinys, R. Macianskiene, A. Bogdelis, J. Jurevicius, R. Fischmeister, beta3-adrenergic receptor activation increases human atrial tissue contractility and stimulates the L-type Ca²⁺ current, *J. Clin. Invest* 118 (2008) 3219–3227, <https://doi.org/10.1172/JCI32519>.
- [201] X. Niu, V.L. Watts, O.H. Cingolani, V. Sivakumaran, J.S. Leyton-Mange, C.L. Ellis, K.L. Miller, K. Vandegaer, D. Bedja, K.L. Gabrielson, N. Paolucci, D.A. Kass, L. A. Barouch, Cardioprotective effect of beta-3 adrenergic receptor agonism: role of neuronal nitric oxide synthase, *J. Am. Coll. Cardiol.* 59 (2012) 1979–1987, <https://doi.org/10.1016/j.jacc.2011.12.046>.
- [202] P. Varghese, R.W. Harrison, R.A. Lofthouse, D. Georgakopoulos, D.E. Berkowitz, J.M. Hare, beta (3)-adrenoceptor deficiency blocks nitric oxide-dependent inhibition of myocardial contractility, *J. Clin. Invest* 106 (2000) 697–703, <https://doi.org/10.1172/JCI9323>.
- [203] M.G. Sciolli, G. Storti, F. D'Amico, R. Rodríguez Guzmán, F. Centofanti, E. Doldo, E.M. Céspedes Miranda, A. Orlandi, Oxidative stress and new pathogenetic mechanisms in endothelial dysfunction: potential diagnostic biomarkers and therapeutic targets, *J. Clin. Med* 9 (2020) 1995, <https://doi.org/10.3390/jcm9061995>.
- [204] A. Daiber, N. Xia, S. Steven, M. Oelze, A. Hanf, S. Kröller-Schön, T. Münzel, H. Li, New therapeutic implications of endothelial nitric oxide synthase (eNOS) function/dysfunction in cardiovascular disease, *Int. J. Mol. Sci.* 20 (2019) 187, <https://doi.org/10.3390/ijms20010187>.
- [205] L. Chatre, A. Ducat, F.T. Spradley, A.C. Palei, C. Chéreau, B. Couderc, K. C. Thomas, A.R. Wilson, L.M. Amaral, I. Gaillard, C. Méhats, I. Lagoutte, S. Jacques, F. Miralles, F. Batteux, J.P. Granger, M. Ricchetti, D. Vaiman, Increased NOS coupling by the metabolite tetrahydrobiopterin (BH4) reduces pre-eclampsia/IUGR consequences, *Redox Biol.* 55 (2022), 102406, <https://doi.org/10.1016/j.redox.2022.102406>.
- [206] J.P.M. Soares, D.A. Gonçalves, R.X. de Sousa, M.G. Mouro, E.M.S. Higa, L. P. Sperandio, C.M. Vitoriano, E.B.S. Rosa, F.O.D. Santos, G.N. de Queiroz, R.S. S. Yamaguchi, G. Pereira, M.Y. Icimoto, F.H.M. Melo, Disruption of redox homeostasis by alterations in nitric oxide synthase activity and tetrahydrobiopterin along with melanoma progression, *Int. J. Mol. Sci.* 23 (2022) 5979, <https://doi.org/10.3390/ijms23115979>.
- [207] M.J. Crabtree, A.L. Tatham, A.B. Hale, N.J. Alp, K.M. Channon, Critical role for tetrahydrobiopterin recycling by dihydrofolate reductase in regulation of endothelial nitric-oxide synthase coupling: relative importance of the de novo biopterin synthesis versus salvage pathways, *J. Biol. Chem.* 284 (2009) 28128–28136, <https://doi.org/10.1074/jbc.M109.041483>.
- [208] M.J. Crabtree, A.L. Tatham, Y. Al-Wakeel, N. Warrick, A.B. Hale, S. Cai, K. M. Channon, N.J. Alp, Quantitative regulation of intracellular endothelial nitric-oxide synthase (eNOS) coupling by both tetrahydrobiopterin-eNOS stoichiometry and biopterin redox status: insights from cells with ret-regulated GTP cyclohydrolase I expression, *J. Biol. Chem.* 284 (2009) 1136–1144, <https://doi.org/10.1074/jbc.M805403200>.
- [209] L.A. Reyes, J. Boslett, S. Varadharaj, F. De Pascali, C. Hemann, L.J. Druhan, G. Ambrosio, M. El-Mahdy, J.L. Zweier, Depletion of NADPH due to CD38 activation triggers endothelial dysfunction in the posts ischemic heart, *Proc. Natl. Acad. Sci. U. S. A.* 112 (2015) 11648–11653, <https://doi.org/10.1073/pnas.1505556112>.
- [210] J. Bailey, A. Shaw, R. Fischer, B.J. Ryan, B.M. Kessler, J. McCullagh, R. Wade-Martins, K.M. Channon, M.J. Crabtree, A novel role for endothelial tetrahydrobiopterin in mitochondrial redox balance, *Free Radic. Biol. Med.* 104 (2017) 214–225, <https://doi.org/10.1016/j.freeradbiomed.2017.01.012>.
- [211] U. Novoa, K. Soto, C. Valdés, J. Villaseñor, A.V. Treuer, D.R. González, Tetrahydrobiopterin (BH4) supplementation prevents the cardiorenal effects of diabetes in mice by reducing oxidative stress, inflammation and fibrosis, *Biomedicines* 10 (2022) 2479, <https://doi.org/10.3390/biomedicines10102479>.
- [212] T.S. Koskenkorva-Frank, G. Weiss, W.H. Koppenol, S. Burckhardt, The complex interplay of iron metabolism, reactive oxygen species, and reactive nitrogen species: insights into the potential of various iron therapies to induce oxidative and nitrosative stress, *Free Radic. Biol. Med.* 65 (2013) 1174–1194, <https://doi.org/10.1016/j.freeradbiomed.2013.09.001>.
- [213] J.L. Beard, Iron biology in immune function, muscle metabolism and neuronal functioning, *discussion* 580S, *J. Nutr.* 131 (2001) 568S–579S, <https://doi.org/10.1093/jn/131.2.568S>.
- [214] H. Atamna, J. Liu, B.N. Ames, Heme deficiency selectively interrupts assembly of mitochondrial complex IV in human fibroblasts: relevance to aging, *J. Biol. Chem.* 276 (2001) 48410–6, <https://doi.org/10.1074/jbc.M108362200>.
- [215] J.H. Yoo, H.Y. Maeng, Y.K. Sun, Y.A. Kim, D.W. Park, T.S. Park, S.T. Lee, J. R. Choi, Oxidative status in iron-deficiency anemia, *J. Clin. Lab Anal.* 23 (2009) 319–323, <https://doi.org/10.1002/jcla.20335>.
- [216] T. Grune, O. Sommerburg, W.G. Siems, Oxidative stress in anemia, *Clin. Nephrol.* 53 (2000) S18–S22.
- [217] J.W. Choi, S.H. Pai, S.K. Kim, M. Ito, C.S. Park, Y.N. Cha, Iron deficiency anemia increases nitric oxide production in healthy adolescents, *Ann. Hematol.* 81 (2002) 1–6, <https://doi.org/10.1007/s00277-001-0409-4>.
- [218] Z. Ni, S. Morcos, N.D. Vaziri, Up-regulation of renal and vascular nitric oxide synthase in iron-deficiency anemia, *Kidney Int.* 52 (1997) 195–201, <https://doi.org/10.1038/ki.1997.319>.
- [219] F. Dong, X. Zhang, B. Culver, H.G. Jr. Chew, R.O. Kelley, J. Ren, Dietary iron deficiency induces ventricular dilation, mitochondrial ultrastructural aberrations and cytochrome c release: involvement of nitric oxide synthase and protein tyrosine nitration, *Clin. Sci. (Lond.)* 109 (2005) 277–286, <https://doi.org/10.1042/CS20040278>.

- [220] E. Kurtoglu, A. Ugur, A.K. Baltaci, L. Undar, Effect of iron supplementation on oxidative stress and antioxidant status in iron-deficiency anemia, *Biol. Trace Elem. Res.* 96 (2003) 117–123, <https://doi.org/10.1385/BTER:96:1-3:117>.
- [221] T. Michel, R is for arginine: metabolism of arginine takes off again, in new directions, *Circulation* 128 (2013) 1400–1404, <https://doi.org/10.1161/CIRCULATIONAHA.113.005924>.
- [222] X. Liu, L. Hou, D. Xu, A. Chen, L. Yang, Y. Zhuang, Y. Xu, J.T. Fasset, Y. Chen, Effect of asymmetric dimethylarginine (ADMA) on heart failure development, *Nitric Oxide* 54 (2016) 73–81, <https://doi.org/10.1016/j.niox.2016.02.006>.
- [223] F. Palm, M.L. Onozato, Z. Luo, C.S. Wilcox, Dimethylarginine dimethylaminohydrolase (DDAH): expression, regulation, and function in the cardiovascular and renal systems, *Am. J. Physiol. Heart Circ. Physiol.* 293 (2007) H3227–H3245, <https://doi.org/10.1152/ajpheart.00998.2007>.
- [224] Y.L. Tain, C.N. Hsu, Y.L. Tain, C.N. Hsu, Toxic dimethylarginines: asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), *Toxins (Basel)* 9 (2017) 92, <https://doi.org/10.3390/toxins9030092>.
- [225] J. Singh, Y. Lee, J.A. Kellum, A new perspective on NO pathway in sepsis and ADMA lowering as a potential therapeutic approach, *Crit. Care* 26 (2022), 246, <https://doi.org/10.1186/s13054-022-04075-0>.
- [226] G. Bouras, S. Dettores, D. Tousoulis, G. Giannopoulos, G. Chatzis, D. Tsounis, M. W. Cleman, C. Stefanadis, Asymmetric dimethylarginine (ADMA): a promising biomarker for cardiovascular disease? *Curr. Top. Med. Chem.* 13 (2013) 180–200, <https://doi.org/10.2174/1568026611313020007>.
- [227] C.A. Chen, T.Y. Wang, S. Varadharaaj, L.A. Reyes, C. Hemann, M.A. Talukder, Y. R. Chen, L.J. Druhan, J.L. Zweier, S-glutathionylation uncouples eNOS and regulates its cellular and vascular function, *Nature* 468 (2010) 1115–1118, <https://doi.org/10.1038/nature09599>.
- [228] E. Kalinina, M. Novichkova, Glutathione in protein redox modulation through S-Glutathionylation and S-Nitrosylation, *Molecules* 26 (2021) 435.
- [229] J. Tian, M.S. Popal, R. Huang, M. Zhang, X. Zhao, M. Zhang, X. Song, Caveolin as a novel potential therapeutic target in cardiac and vascular diseases: a mini review, *Aging Dis.* 11 (2020) 378–389, <https://doi.org/10.14338/AD.2019.09603>.
- [230] B. Nilius, G. Droogmans, Ion channels and their functional role in vascular endothelium, *Physiol. Rev.* 81 (2001) 1415–1459, <https://doi.org/10.1152/physrev.2001.81.4.1415>.
- [231] M.H. Lee, S.J. Chen, C.M. Tsao, C.C. Wu, Perivascular adipose tissue inhibits endothelial function of rat aortas via caveolin-1, *PLoS One* 9 (2014), e99947, <https://doi.org/10.1371/journal.pone.0099947>.
- [232] L. Yue, J.T. Bian, I. Grizelj, A. Cavka, S.A. Phillips, A. Makino, T. Mazzone, Apolipoprotein E enhances endothelial-NO production by modulating caveolin 1 interaction with endothelial NO synthase, *Hypertension* 60 (2012) 1040–1046, <https://doi.org/10.1161/HYPERTENSIONAHA.112.196667>.
- [233] G. Jia, J.R. Sowers, Caveolin-1 in cardiovascular disease: a double-edged sword, *Diabetes* 64 (2015) 3645–3647, <https://doi.org/10.2337/dbi15-0005>.
- [234] V. Mollace, A. Tavernese, R. Mollace, The essential role of a “healthy” relationship between caveolin-1 and endothelial nitric oxide synthase in counteracting vascular inflammation and atherothrombosis, *Kardiol. Pol.* 78 (2020) 96–97, <https://doi.org/10.33963/KP.15203>.
- [235] V. Mollace, M. Gliozzi, The potential role of TLR4/caveolin-1/NOS pathway in oxLDL-modulation of autophagic/apoptotic responses in endothelial cells, *Int J. Cardiol.* 203 (2016) 457–458, <https://doi.org/10.1016/j.ijcard.2015.10.157>.
- [236] Z. Chen, I.C. Peng, X. Cui, Y.S. Li, S. Chien, J.Y. Shyy, Shear stress, SIRT1, and vascular homeostasis, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010) 10268–10273, <https://doi.org/10.1073/pnas.1003833107>.
- [237] H. Nagar, S.B. Jung, M.J. Ryu, S.J. Choi, S. Piao, H.J. Song, S.K. Kang, N. Shin, D. W. Kim, S.A. Jin, J.O. Jeong, K. Irani, B.H. Jeon, M. Shong, G.R. Kwon, C.S. Kim, CR6-interacting factor 1 deficiency impairs vascular function by inhibiting the Sirt1-endothelial nitric oxide synthase pathway, *Antioxid. Redox Signal* 27 (2017) 234–249, <https://doi.org/10.1089/ars.2016.6719>.
- [238] A. Beuve, Thiol-based redox modulation of soluble guanylyl cyclase, the nitric oxide receptor, *Antioxid. Redox Signal* 26 (2017) 137–149, <https://doi.org/10.1089/ars.2015.6591>.
- [239] J.P. Stasch, P.M. Schmidt, P.I. Nedvetsky, T.Y. Nedvetskaya, A.K. HS, S. Meurer, M. Deile, A. Taye, A. Knorr, H. Lapp, H. Müller, Y. Turgay, C. Rothkegel, A. Tersteegen, B. Kemp-Harper, W. Müller-Esterl, H.H. Schmidt, Targeting the heme-oxidized nitric oxide receptor for selective vasodilatation of diseased blood vessels, *J. Clin. Invest.* 116 (2006) 2552–2561, <https://doi.org/10.1172/JCI28371>.
- [240] S.M. Krishnan, J.R. Kraehling, F. Eitner, A. Bénardeau, P. Sandner, The impact of the nitric oxide (NO)/soluble guanylyl cyclase (sGC) signaling cascade on kidney health and disease: a preclinical perspective, *Int. J. Mol. Sci.* 19 (2018) 1712, <https://doi.org/10.3390/ijms19061712>.
- [241] M.M. Rahaman, A.T. Nguyen, M.P. Miller, S.A. Hahn, C. Sparacino-Watkins, S. Jobbagy, N.T. Carew, N. Cantu-Medellin, K.C. Wood, C.J. Baty, F.J. Schopfer, E. E. Kelley, M.T. Gladwin, E. Martin, A.C. Straub, Cytochrome b5 reductase 3 modulates soluble guanylate cyclase redox state and cGMP signaling, *Circ. Res.* 121 (2017) 137–148, <https://doi.org/10.1161/CIRCRESAHA.117.310705>.
- [242] B.G. Durgin, S.A. Hahn, H.M. Schmidt, M.P. Miller, N. Hafeez, I. Mathar, D. Freitag, P. Sandner, A.C. Straub, Loss of smooth muscle CYB5R3 amplifies angiotensin II-induced hypertension by increasing sGC heme oxidation, *JCI Insight* 4 (2019), e129183, <https://doi.org/10.1172/jci.insight.129183>.
- [243] B.G. Durgin, K.C. Wood, S.A. Hahn, B. McMahon, J.J. Baust, A.C. Straub, Smooth muscle cell CYB5R3 preserves cardiac and vascular function under chronic hypoxic stress, *J. Mol. Cell Cardiol.* 162 (2022) 72–80, <https://doi.org/10.1016/j.jmcc.2021.09.005>.
- [244] R. Thoonen, A. Cauwels, K. Decaluwe, S. Geschka, R.E. Tainsh, J. Delanghe, T. Hochepeid, L. De Cauwer, E. Rogge, S. Voet, P. Sips, R.H. Karas, K.D. Bloch, M. Vuylsteke, J.P. Stasch, J. Van de Voorde, E.S. Buys, P. Brouckaert, Cardiovascular and pharmacological implications of haem-deficient NO-unresponsive soluble guanylate cyclase knock-in mice, *Nat. Commun.* 6 (2015), 8482, <https://doi.org/10.1038/ncomms9482>.
- [245] I.L. Megson, M.R. Miller, NO and sGC-stimulating NO donors, *Handb. Exp. Pharm.* 191 (2009) 247–276, https://doi.org/10.1007/978-3-540-68964-5_12.
- [246] A. Daiber, T. Münzel, Organic nitrate therapy, nitrate tolerance, and nitrate-induced endothelial dysfunction: emphasis on redox biology and oxidative stress, *Antioxid. Redox Signal* 23 (2015) 899–942, <https://doi.org/10.1089/ars.2015.6376>.
- [247] J.E. Ferguson 3rd, C.C. 3rd Carson, Phosphodiesterase type 5 inhibitors as a treatment for erectile dysfunction: current information and new horizons, *Arab J. Urol.* 11 (2013) 222–229, <https://doi.org/10.1016/j.aju.2013.07.009>.
- [248] M.K. Derici, G. Sadi, B. Ceni, T. Güray, E. Demirel-Yilmaz, Differential expressions and functions of phosphodiesterase enzymes in different regions of the rat heart, *Eur. J. Pharm.* 844 (2019) 118–129, <https://doi.org/10.1016/j.ejphar.2018.12.002>.
- [249] S.G. Chrysant, Effectiveness and safety of phosphodiesterase 5 inhibitors in patients with cardiovascular disease and hypertension, *Curr. Hypertens. Rep.* 15 (2013) 475–483, <https://doi.org/10.1007/s11906-013-0377-9>.
- [250] E.J. Tsai, D.A. Kass, Cyclic GMP signaling in cardiovascular pathophysiology and therapeutics, *Pharm. Ther.* 122 (2009) 216–238, <https://doi.org/10.1016/j.pharmthera.2009.02.009>.
- [251] R. Ockaili, F. Salloum, J. Hawkins, R.C. Kukreja, Sildenafil (Viagra) induces powerful cardioprotective effect via opening of mitochondrial K(ATP) channels in rabbits, *Am. J. Physiol. Heart Circ. Physiol.* 283 (2002) H1263–H1269, <https://doi.org/10.1152/ajpheart.00324.2002>.
- [252] J. Han, N. Kim, H. Joo, E. Kim, Y.E. Earm, ATP-sensitive K(+) channel activation by nitric oxide and protein kinase G in rabbit ventricular myocytes, *Am. J. Physiol. Heart Circ. Physiol.* 283 (2002) H1545–H1554, <https://doi.org/10.1152/ajpheart.01052.2001>.
- [253] F.N. Salloum, A. Abbate, A. Das, J.E. Houser, C.A. Mudrick, I.Z. Qureshi, N. N. Hoke, S.K. Roy, W.R. Brown, S. Prabhakar, R.C. Kukreja, Sildenafil (Viagra) attenuates ischemic cardiomyopathy and improves left ventricular function in mice, *Am. J. Physiol. Heart Circ. Physiol.* 294 (2008) H1398–H1406, <https://doi.org/10.1152/ajpheart.91438.2007>.
- [254] V.Q. Chau, F.N. Salloum, N.N. Hoke, A. Abbate, R.C. Kukreja, Mitigation of the progression of heart failure with sildenafil involves inhibition of RhoA/Rho-kinase pathway, *Am. J. Physiol. Heart Circ. Physiol.* 300 (2011) H2272–H2279, <https://doi.org/10.1152/ajpheart.00654.2010>.
- [255] A. Das, F.N. Salloum, L. Xi, Y.J. Rao, R.C. Kukreja, ERK phosphorylation mediates sildenafil-induced myocardial protection against ischemia-reperfusion injury in mice, *Am. J. Physiol. Heart Circ. Physiol.* 296 (2009) H1236–H1243, <https://doi.org/10.1152/ajpheart.00100.2009>.
- [256] Z. Lu, X. Xu, X. Hu, S. Lee, J.H. Traverse, G. Zhu, J. Fasset, Y. Tao, P. Zhang, C. dos Remedios, M. Pritzker, J.L. Hall, D.J. Garry, Y. Chen, Oxidative stress regulates left ventricular PDE5 expression in the failing heart, *Circulation* 121 (2010) 1474–1483, <https://doi.org/10.1161/CIRCULATIONAHA.109.906818>.
- [257] M. Scicchitano, C. Carresi, S. Nucera, S. Ruga, J. Maiuolo, R. Macri, F. Scarano, F. Bosco, R. Mollace, A. Cardamone, A.R. Coppoletta, L. Guarnieri, M.C. Zito, I. Bava, L. Cariati, M. Greco, D.P. Foti, E. Palma, M. Gliozzi, V. Musolino, V. Mollace, Icarin protects H9c2 rat cardiomyoblasts from doxorubicin-induced cardiotoxicity: role of caveolin-1 upregulation and enhanced autophagic response, *Nutrients* 13 (2021) 4070, <https://doi.org/10.3390/nu13114070>.
- [258] N. Ahmad, Y. Wang, A.K. Ali, M. Ashraf, Long-acting phosphodiesterase-5 inhibitor, tadalafil, induces sustained cardioprotection against lethal ischemic injury, *Am. J. Physiol. Heart Circ. Physiol.* 297 (2009) H387–H391, <https://doi.org/10.1152/ajpheart.00169.2009>.
- [259] F.N. Salloum, V.Q. Chau, N.N. Hoke, A. Abbate, A. Varma, R.A. Ockaili, S. Toldo, R.C. Kukreja, Phosphodiesterase-5 inhibitor, tadalafil, protects against myocardial ischemia/reperfusion through protein-kinase g-dependent generation of hydrogen sulfide, *Circulation* 120 (2009) S31–6, <https://doi.org/10.1161/CIRCULATIONAHA.108.843979>.
- [260] B.R. Lindman, A. Zajarías, J.A. Madrazo, J. Shah, B.F. Gage, E. Novak, S. N. Johnson, M.M. Chakinala, T.A. Hohn, M. Saghir, D.L. Mann, Effects of phosphodiesterase type 5 inhibition on systemic and pulmonary hemodynamics and ventricular function in patients with severe symptomatic aortic stenosis, *Circulation* 125 (2012) 2353–2362, <https://doi.org/10.1161/CIRCULATIONAHA.111.081125>.
- [261] B.A. Borlaug, D.A. Kass, Ventricular-vascular interaction in heart failure, *Cardiol. Clin.* 29 (2011) 447–459, <https://doi.org/10.1016/j.ccl.2011.06.004>.
- [262] M. Zhang, E. Takimoto, S. Hsu, D.I. Lee, T. Nagayama, T. Danner, N. Koitabashi, A.S. Barth, D. Bedja, K.L. Gabrielson, Y. Wang, D.A. Kass, Myocardial remodeling is controlled by myocyte-targeted gene regulation of phosphodiesterase type 5, *J. Am. Coll. Cardiol.* 56 (2010) 2021–2030, <https://doi.org/10.1016/j.jacc.2010.08.612>.
- [263] M.J. Andersen, M. Ersbøll, A. Axelsson, F. Gustafsson, C. Hassager, L. Køber, B. A. Borlaug, S. Boesgaard, L.T. Skovgaard, J.E. Møller, Sildenafil and diastolic dysfunction after acute myocardial infarction in patients with preserved ejection fraction: the Sildenafil and Diastolic Dysfunction After Acute Myocardial Infarction (SIDAMI) trial, *Circulation* 127 (2013) 1200–8, <https://doi.org/10.1161/CIRCULATIONAHA.112.000056>.
- [264] M. Guazzi, M. Vicenzi, R. Arena, M.D. Guazzi, Pulmonary hypertension in heart failure with preserved ejection fraction: a target of phosphodiesterase-5 inhibition

- in a 1-year study, *Circulation* 124 (2011) 164–174, <https://doi.org/10.1161/CIRCULATIONAHA.110.983866>.
- [265] G.D. Lewis, R. Shah, K. Shahzad, J.M. Camuso, P.P. Pappagianopoulos, J. Hung, A. Tawakol, R.E. Gerszten, D.M. Systrom, K.D. Bloch, M.J. Semigran, Sildenafil improves exercise capacity and quality of life in patients with systolic heart failure and secondary pulmonary hypertension, *Circulation* 116 (2007) 1555–1562, <https://doi.org/10.1161/CIRCULATIONAHA.107.716373>.
- [266] M. Guazzi, M. Vicenzi, R. Arena, Phosphodiesterase 5 inhibition with sildenafil reverses exercise oscillatory breathing in chronic heart failure: a long-term cardiopulmonary exercise testing placebo-controlled study, *Eur. J. Heart Fail* 14 (2012) 82–90, <https://doi.org/10.1093/eurjhf/hfr147>.
- [267] M. Bussotti, P. Montorsi, M. Amato, A. Magini, D. Baldassarre, F. Tantarini, F. Veglia, P. Agostoni, Sildenafil improves the alveolar-capillary function in heart failure patients, *Int J. Cardiol.* 126 (2008) 68–72, <https://doi.org/10.1016/j.ijcard.2007.03.118>.
- [268] F. Macera, J.L. Vachiéry, Management of Pulmonary Hypertension in Left Heart Disease, *Methodist Debakey Cardiovasc J.* 17 (2021) 115–123, <https://doi.org/10.14797/RKQN5397>.
- [269] B.A. Borlaug, G.D. Lewis, S.E. McNulty, M.J. Semigran, M. LeWinter, H. Chen, G. Lin, A. Deswal, K.B. Margulies, M.M. Redfield, Effects of sildenafil on ventricular and vascular function in heart failure with preserved ejection fraction, *Circ. Heart Fail* 8 (2015) 533–541, <https://doi.org/10.1161/CIRCHEARTFAILURE.114.001915>.
- [270] D. Tobler, J. Bouchardy, E. Reto, D. Heg, C. Müller, A. Frenk, H. Gabriel, J. Schwitler, T. Rutz, R.R. Buechel, M. Willhelm, L. Trachsel, M. Freese, M. Greutmann, M. Schwerzmann, SERVE trial, Effect of phosphodiesterase-5 inhibition with Tadalafil on Systolic Right Ventricular size and function - A multi-center, double-blind, randomized, placebo-controlled clinical trial - SERVE trial - Rational and design, *Int J. Cardiol.* 243 (2017) 354–359, <https://doi.org/10.1016/j.ijcard.2017.05.079>.
- [271] A.H. In 't Veld, M.C. Van de Veerdonk, O.A. Spruijt, J. Tim Marcus, H. Jan Bogaard, A.V. Noordegraaf, Goal orientated strategy to preserve right ventricular ejection fraction trial (GOSPEL trial), in: *European Respiratory Journal*, 50, 2017, p. OA1501.
- [272] M.M. Hoepfer, H. Al-Hiti, R.L. Benza, S.A. Chang, P.A. Corris, J.S.R. Gibbs, E. Grünig, P. Jansa, J.R. Klinger, D. Langleben, V.V. McLaughlin, G.M.B. Meyer, J. Ota-Arakaki, A.J. Peacock, T. Pulido, S. Rosenkranz, C.D. Vizza, A. Vonk-Noordegraaf, R.J. White, M. Chang, F. Kleinjung, C. Meier, K. Parascchin, H. A. Ghofrani, G. Simonneau, REPLACE investigators, Switching to riociguat versus maintenance therapy with phosphodiesterase-5 inhibitors in patients with pulmonary arterial hypertension (REPLACE): a multicenter, open-label, randomised controlled trial, *Lancet Respir. Med* 9 (2021) 573–584, [https://doi.org/10.1016/S2213-2600\(20\)30532-4](https://doi.org/10.1016/S2213-2600(20)30532-4).
- [273] S. Breitenstein, L. Roessig, P. Sandner, K.S. Lewis, Novel sGC stimulators and sGC activators for the treatment of heart failure, *Handb. Exp. Pharm.* 243 (2017) 225–247, https://doi.org/10.1007/164_2016_100.
- [274] G. Boerrigter, L.C. Costello-Boerrigter, A. Cataliotti, H. Lapp, J.P. Stasch, J.C. Jr. Burnett, Targeting heme-oxidized soluble guanylate cyclase in experimental heart failure, *Hypertension* 49 (2007) 1128–1133, <https://doi.org/10.1161/HYPERTENSIONAHA.106.083832>.
- [275] P. Sandner, D.P. Zimmer, G.T. Milne, M. Follmann, A. Hobbs, J.P. Stasch, Correction to: soluble guanylate cyclase stimulators and activators, *Handb. Exp. Pharm.* 264 (2021) 425, https://doi.org/10.1007/164_2019_249.
- [276] R. Dumitrescu, N. Weissmann, H.A. Ghofrani, E. Dony, K. Beuerlein, H. Schmidt, J.P. Stasch, M.J. Gnoth, W. Seeger, F. Grimminger, R.T. Schermuly, Activation of soluble guanylate cyclase reverses experimental pulmonary hypertension and vascular remodeling, *Circulation* 113 (2006) 286–295, <https://doi.org/10.1161/CIRCULATIONAHA.105.581405>.
- [277] C. Mátyás, B.T. Németh, A. Oláh, L. Hidi, E. Birtalan, D. Kellermayer, M. Ruppert, S. Korkmaz-Icöz, G. Kókény, E.M. Horváth, G. Szabó, B. Merkely, T. Radovits, The soluble guanylate cyclase activator cinaciguat prevents cardiac dysfunction in a rat model of type-1 diabetes mellitus, *Cardiovasc Diabetol.* 14 (2015), 145, <https://doi.org/10.1186/s12933-015-0309-x>.
- [278] K.P. Potoka, K.C. Wood, J.J. Baust, M. Bueno, S.A. Hahn, R.R. Vanderpool, T. Bachman, G.M. Mallampalli, D.O. Osei-Hwedieh, V. Schrott, B. Sun, G. C. Bullock, E.M. Becker-Pelster, M. Wittwer, J. Stampfuss, I. Mathar, J.P. Stasch, H. Truebel, P. Sandner, A.L. Mora, A.C. Straub, M.T. Gladwin, Nitric oxide-independent soluble guanylate cyclase activation improves vascular function and cardiac remodeling in sickle cell disease, *Am. J. Respir. Cell Mol. Biol.* 58 (2018) 636–647, <https://doi.org/10.1165/rcmb.2017-0292OC>.
- [279] C. Methner, G. Buonincontri, C.H. Hu, A. Vujic, A. Kretschmer, S. Sawiak, A. Carpenter, J.P. Stasch, T. Krieger, Riociguat reduces infarct size and post-infarct heart failure in mouse hearts: insights from MRI/PET imaging, *PLoS One* 8 (2013), e83910, <https://doi.org/10.1371/journal.pone.0083910>.
- [280] L.J. Rubin, N. Galie, F. Grimminger, E. Grünig, M. Humbert, Z.C. Jing, A. Keogh, D. Langleben, A. Fritsch, F. Menezes, N. Davie, H.A. Ghofrani, Riociguat for the treatment of pulmonary arterial hypertension: a long-term extension study (PATENT-2), *Eur. Respir. J.* 45 (2015) 1303–1313, <https://doi.org/10.1183/09031936.00090614>.
- [281] N. Galie, K. Müller, A.V. Scalise, E. Grünig, PATENT PLUS: a blinded, randomised and extension study of riociguat plus sildenafil in pulmonary arterial hypertension, *Eur. Respir. J.* 45 (2015) 1314–1322, <https://doi.org/10.1183/09031936.00105914>.
- [282] R.L. Benza, H.A. Ghofrani, E. Grünig, M.M. Hoepfer, P. Jansa, Z.C. Jing, N.H. Kim, D. Langleben, G. Simonneau, C. Wang C, D. Busse, C. Meier, S. Ghio, Effect of riociguat on right ventricular function in patients with pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension, *J. Heart Lung Transpl.* 40 (10) (2021) 1172–1180, <https://doi.org/10.1016/j.healun.2021.06.020>.
- [283] A.M. Marra, M. Halank, N. Benjamin, E. Bossone, A. Cittadini, C.A. Eichstaedt, B. Egenlauf, S. Harutyunova, C. Fischer, H. Gall, H.A. Ghofrani, M.M. Hoepfer, T. J. Lange, K.M. Olsson, H. Klose, E. Grünig, Right ventricular size and function under riociguat in pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension (the RIVER study), *Respir. Res* 19 (2018), 258, <https://doi.org/10.1186/s12931-018-0957-y>.
- [284] D. Bonderman, I. Pretsch, R. Steringer-Mascherbauer, P. Jansa, S. Rosenkranz, C. Tufaro, A. Bojic, C.S.P. Lam, R. Frey, M. Ochan Kilama, S. Unger, L. Roessig, I. M. Lang, Acute hemodynamic effects of riociguat in patients with pulmonary hypertension associated with diastolic heart failure (DILATE-1): a randomized, double-blind, placebo-controlled, single-dose study, *Chest* 146 (2014) 1274–1285, <https://doi.org/10.1378/chest.14-0106>.
- [285] M. Gheorghade, S.J. Greene, J. Butler, G. Filippatos, C.S. Lam, A.P. Maggioni, P. Ponikowski, S.J. Shah, S.D. Solomon, E. Kraigher-Krainer, E.T. Samano, K. Müller, L. Roessig, B. Pieske, SOCRATES-REDUCED Investigators and Coordinators, Effect of vericiguat, a soluble guanylate cyclase stimulator, on natriuretic peptide levels in patients with worsening chronic heart failure and reduced ejection fraction: the SOCRATES-REDUCED randomized trial, *JAMA* 314 (2015) 2251–2262, <https://doi.org/10.1001/jama.2015.15734>.
- [286] B. Pieske, A.P. Maggioni, C.S.P. Lam, E. Pieske-Kraigher, G. Filippatos, J. Butler, P. Ponikowski, S.J. Shah, S.D. Solomon, A.V. Scalise, K. Mueller, L. Roessig, M. Gheorghade, Vericiguat in patients with worsening chronic heart failure and preserved ejection fraction: results of the SOLuble guanylate Cyclase stimulator in heart failure patients with PRESERVED EF (SOCRATES-PRESERVED) study, *Eur. Heart J.* 38 (2017) 1119–1127, <https://doi.org/10.1093/eurheartj/ehw593>.
- [287] P.W. Armstrong, B. Pieske, K.J. Anstrom, J. Ezekowitz, A.F. Hernandez, J. Butler, C.S.P. Lam, P. Ponikowski, A.A. Voors, G. Jia, S.E. McNulty, M.J. Patel, L. Roessig, J. Koglin, C.M. O'Connor, VICTORIA Study Group, Vericiguat in patients with heart failure and reduced ejection fraction, *N. Engl. J. Med* 382 (2020) 1883–1893, <https://doi.org/10.1056/NEJMoa1915928>.
- [288] D.A. Gonçalves, M.G. Jasiulionis, F.H.M. Melo, The role of the BH4 cofactor in nitric oxide synthase activity and cancer progression: two sides of the same coin, *Int J. Mol. Sci.* 22 (2021) 9546, <https://doi.org/10.3390/ijms22179546>.
- [289] A.L. Moens, E. Takimoto, C.G. Tocchetti, K. Chakir, D. Bedja, G. Cormaci, E. A. Ketner, M. Majumdar, K. Gabrielson, M.K. Halushka, J.B. Mitchell, S. Biswal, K. M. Channon, M.S. Wolin, N.J. Alp, N. Paolucci, H.C. Champion, D.A. Kass, Reversal of cardiac hypertrophy and fibrosis from pressure overload by tetrahydrobiopterin: efficacy of recoupling nitric oxide synthase as a therapeutic strategy, *Circulation* 117 (2008) 2626–2636, <https://doi.org/10.1161/CIRCULATIONAHA.107.737031>.
- [290] J.P. Khoo, L. Zhao, N.J. Alp, J.K. Bendall, T. Nicoli, K. Rockett, M.R. Wilkins, K. M. Channon, Pivotal role for endothelial tetrahydrobiopterin in pulmonary hypertension, *Circulation* 111 (2005) 2126–2133, <https://doi.org/10.1161/01.CIR.0000162470.26840.89>.
- [291] C. Dumitrescu, R. Biondi, Y. Xia, A.J. Cardounel, L.J. Druhan, G. Ambrosio, J. L. Zweier, Myocardial ischemia results in tetrahydrobiopterin (BH4) oxidation with impaired endothelial function ameliorated by BH4, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 15081–15086, <https://doi.org/10.1073/pnas.0702986104>.
- [292] S. Yamashiro, K. Noguchi, T. Matsuzaki, K. Miyagi, J. Nakasone, M. Sakanashi, K. Koja, M. Sakanashi, Beneficial effect of tetrahydrobiopterin on ischemia-reperfusion injury in isolated perfused rat hearts, *J. Thorac. Cardiovasc Surg.* 124 (2002) 775–784, <https://doi.org/10.1067/mtc.2002.124393>.
- [293] T. Heitzer, K. Krohn, S. Albers, T. Meinertz T, Tetrahydrobiopterin improves endothelium-dependent vasodilation by increasing nitric oxide activity in patients with Type II diabetes mellitus, *Diabetologia* 43 (2000) 1435–1438, <https://doi.org/10.1007/s001250051551>.
- [294] M. Porkert, S. Sher, U. Reddy, F. Cheema, C. Niessner, P. Kolm, D.P. Jones, C. Hooper, W.R. Taylor, D. Harrison, A.A. Quyyumi, Tetrahydrobiopterin: a novel antihypertensive therapy, *J. Hum. Hypertens.* 22 (2008) 401–407, <https://doi.org/10.1038/sj.jhh.1002329>.
- [295] K. Bunsawat, R.M. Broxterman, C.L. Jarrett, J.C. Craig, M.A. Francisco, J. K. Alpenglow, J.J. Iacovelli, C.L. Ma, J.J. Ryan, D.W. Wray, D.W. The impact of short-term tetrahydrobiopterin (BH4) supplementation on peripheral vascular function in heart failure with preserved ejection fraction (HFpEF), *FASEB J.* 36 (2022), <https://doi.org/10.1096/fasebj.2022.36.S1.R3751>.
- [296] C. Cunningham, T. Van Assche, C. Shirodaria, I. Kyllintreas, A.C. Lindsay, J.M. Lee, C. Antoniadis, M. Margaritis, R. Lee, R. Cerrato, M.J. Crabtree, J.M. Francis, R. Sayeed, C. Ratnatunga, R. Pillai, R.P. Choudhury, S. Neubauer, K.M. Channon, Systemic and vascular oxidation limits the efficacy of oral tetrahydrobiopterin treatment in patients with coronary artery disease, *Circulation* 125 (2012) 1356–1366, <https://doi.org/10.1161/CIRCULATIONAHA.111.038919>.
- [297] M.J. Crabtree, K.M. Channon, Synthesis and recycling of tetrahydrobiopterin in endothelial function and vascular disease, *Nitric Oxide* 25 (2011) 81–88, <https://doi.org/10.1016/j.niox.2011.04.004>.
- [298] M.E. Hyndman, S. Verma, R.J. Rosenfeld, T.J. Anderson, H.G. Parsons, Interaction of 5-methyltetrahydrofolate and tetrahydrobiopterin on endothelial function, *Am. J. Physiol. Heart Circ. Physiol.* 282 (2002) H2167–H2172, <https://doi.org/10.1152/ajpheart.00935.2001>.
- [299] S.J. Moat, S.N. Doshi, D. Lang, I.F. McDowell, M.J. Lewis, J. Goodfellow, Treatment of coronary heart disease with folic acid: is there a future? *Am. J. Physiol. Heart Circ. Physiol.* 287 (2004) H1–H7, <https://doi.org/10.1152/ajpheart.00952.2003>.

- [300] M. Zhang, J. Wen, X. Wang, C. Xiao, High dose folic acid improves endothelial function by increasing tetrahydrobiopterin and decreasing homocysteine levels, *Mol. Med Rep.* 10 (2014) 1609–1613, <https://doi.org/10.3892/mmr.2014.2332>.
- [301] K. Chalupsky, D. Kračun, I. Kanchev, K. Bertram, A. Görlach, Folic acid promotes recycling of tetrahydrobiopterin and protects against hypoxia-induced pulmonary hypertension by recoupling endothelial nitric oxide synthase, *Antioxid. Redox Signal* 23 (2015) 1076–1091, <https://doi.org/10.1089/ars.2015.6329>.
- [302] S.N. Doshi, I.F. McDowell, S.J. Moat, N. Payne, H.J. Durrant, M.J. Lewis, J. Goodfellow, Folic acid improves endothelial function in coronary artery disease via mechanisms largely independent of homocysteine lowering, *Circulation* 105 (2002) 22–26, <https://doi.org/10.1161/hc1012.101388>.
- [303] C. Shirodaria, C. Antoniadis, J. Lee, C.E. Jackson, M.D. Robson, J.M. Francis, S. J. Moat, C. Ratnatunga, R. Pillai, H. Refsum, S. Neubauer, K.M. Channon, Global improvement of vascular function and redox state with low-dose folic acid: implications for folate therapy in patients with coronary artery disease, *Circulation* 115 (2007) 2262–2270, <https://doi.org/10.1161/CIRCULATIONAHA.106.679084>.
- [304] C.M. Albert, N.R. Cook, J.M. Gaziano, E. Zaharris, J. MacFadyen, E. Danielson, J. E. Buring, J.E. Manson, Effect of folic acid and B vitamins on risk of cardiovascular events and total mortality among women at high risk for cardiovascular disease: a randomized trial, *JAMA* 299 (2008) 2027–2036, <https://doi.org/10.1001/jama.299.17.2027>.
- [305] Y. Li, T. Huang, Y. Zheng, T. Muka, J. Troup, F.B. Hu, Folic acid supplementation and the risk of cardiovascular diseases: a meta-analysis of randomized controlled trials, *J. Am. Heart Assoc.* 5 (2016), e003768, <https://doi.org/10.1161/JAHA.116.003768>.
- [306] X. Qin, L. Shen, R. Zhang, Y. Li, X. Wang, B. Wang, X. Jiang, H. Jiang, Y. Lei, F. Hou, J. Gu, Y. Huo, Effect of folic acid supplementation on cancer risk among adults with hypertension in China: A randomized clinical trial, *Int. J. Cancer* 141 (2017) 837–847, <https://doi.org/10.1002/ijc.30094>.
- [307] T.N. Wien, E. Pike, T. Wisloff, A. Staff, S. Smeland, M. Klemp, Cancer risk with folic acid supplements: a systematic review and meta-analysis, *BMJ Open* 2 (2012), e000653, <https://doi.org/10.1136/bmjopen-2011-000653>.
- [308] S. Ollai Araghi, J.C. Kieft-de Jong, S.C. van Dijk, K.M.A. Swart, H.W. van Laarhoven, N.M. van Schoor, L.C.P.G.M. de Groot, V. Lemmens, B.H. Stricker, A. G. Uitterlinden, N. van der Velde, Folic acid and vitamin B12 supplementation and the risk of cancer: long-term follow-up of the B vitamins for the prevention of osteoporotic fractures (B-PROOF) trial, *Cancer Epidemiol. Biomark. Prev.* 28 (2019) 275–282, <https://doi.org/10.1158/1055-9965.EPI-17-1198>.
- [309] C.S.P. Lam, W. Doehner, J. Comin-Colet, IRON CORE Group, Iron deficiency in chronic heart failure: case-based practical guidance, *ESC Heart Fail* 5 (2018) 64–77, <https://doi.org/10.1002/ehf2.12333>.
- [310] S. von Haehling, N. Ebner, R. Evertz, P. Ponikowski, S.D. Anker, Iron Deficiency in Heart Failure, *Overv.*, *JACC Heart Fail* 7 (2019) 36–46, <https://doi.org/10.1016/j.jchf.2018.07.015>.
- [311] S.D. Anker, J. Comin Colet, G. Filippatos, R. Willenheimer, K. Dickstein, H. Drexler, T.F. Lüscher, B. Bart, W. Banasiak, J. Niegowska, B.A. Kirwan, C. Mori, B. von Eisenhart Rothe, S.J. Pocock, P.A. Poole-Wilson, P. Ponikowski, FAIR-HF Trial Investigators, Ferric carboxymaltose in patients with heart failure and iron deficiency, *N. Engl. J. Med* 361 (2009) 2436–2448, <https://doi.org/10.1056/NEJMoa0908355>.
- [312] P. Ponikowski, D.J. van Veldhuisen, G. Comin-Colet, J. Ertl, M. Komajda, V. Mareev, T. McDonagh, A. Parkhomenko, L. Tavazzi, V. Lesvesque, C. Mori, B. Roubert, G. Filippatos, F. Ruschitzka, S.D. Anker, CONFIRM-HF Investigators, Beneficial effects of long-term intravenous iron therapy with ferric carboxymaltose in patients with symptomatic heart failure and iron deficiency, *Eur. Heart J.* 36 (2015) 657–668, <https://doi.org/10.1093/eurheartj/ehu385>.
- [313] A. Mollace, R. Macri, R. Mollace, A. Tavernese, M. Gliozzi, V. Musolino, C. Carresi, J. Maiuolo, M. Nicta, R. Caminiti, S. Paone, F. Barilla, M. Volterrani, V. Mollace, Effect of ferric carboxymaltose supplementation in patients with heart failure with preserved ejection fraction: role of attenuated oxidative stress and improved endothelial function, *Nutrients* 14 (2022) 5057, <https://doi.org/10.3390/nu14235057>.
- [314] N. Paolucci, M.I. Jackson, B.E. Lopez, K. Miranda, C.G. Tocchetti, D.A. Wink, A. J. Hobbs, J.M. Fukuto, The pharmacology of nitroxyl (HNO) and its therapeutic potential: not just the Janus face of NO, *Pharm. Ther.* 113 (2007) 442–458, <https://doi.org/10.1016/j.pharmthera.2006.11.002>.
- [315] M.D. Bartberger, W. Liu, E. Ford, K.M. Miranda, C. Switzer, J.M. Fukuto, P. J. Farmer, D.A. Wink, K.N. Houk, The reduction potential of nitric oxide (NO) and its importance to NO biochemistry, *Proc. Natl. Acad. Sci. U. S. A.* 99 (2002) 10958–10963, <https://doi.org/10.1073/pnas.162095599>.
- [316] B.V. Nagpure, J.S. Bian, Interaction of hydrogen sulfide with nitric oxide in the cardiovascular system, *Oxid. Med Cell Longev.* 2016 (2016), 6904327, <https://doi.org/10.1155/2016/6904327>.
- [317] C.G. Tocchetti, B.A. Stanley, C.I. Murray, V. Sivakumaran, S. Donzelli, D. Mancardi, P. Pagliaro, W.D. Gao, J. van Eyk, D.A. Kass, D.A. Wink, N. Paolucci, Playing with cardiac “redox switches”: the “HNO way” to modulate cardiac function, *Antioxid. Redox Signal* 14 (2011) 1687–1698, <https://doi.org/10.1089/ars.2010.3859>.
- [318] H.J. Sun, Z.Y. Wu, L. Cao, M.Y. Zhu, X.W. Nie, D.J. Huang, M.T. Sun, J.S. Bian, Role of nitroxyl (HNO) in cardiovascular system: from biochemistry to pharmacology, *Pharm. Res* 159 (2020), 104961, <https://doi.org/10.1016/j.phrs.2020.104961>.
- [319] M.J. Kohr, N. Kaludercic, C.G. Tocchetti, W. Dong Gao, D.A. Kass, P.M. Janssen, N. Paolucci, M.T. Ziolo, Nitroxyl enhances myocyte Ca²⁺ transients by exclusively targeting SR Ca²⁺-cycling, *Front Biosci. (Elite Ed.)* 2 (2010) 614–626, <https://doi.org/10.2741/e118>.
- [320] J.L. Favalaro, B.K. Kemp-Harper, The nitroxyl anion (HNO) is a potent dilator of rat coronary vasculature, *Cardiovasc Res* 73 (2007) 587–596, <https://doi.org/10.1016/j.cardiores.2006.11.018>.
- [321] J.C. Irvine, J.L. Favalaro, B.K. Kemp-Harper, NO- activates soluble guanylate cyclase and Kv channels to vasodilate resistance arteries, *Hypertension* 41 (2003) 1301–1307.
- [322] E.Q. Lin, J.C. Irvine, A.H. Cao, A.E. Alexander, J.E. Love, R. Patel, J.R. McMullen, D.M. Kaye, B.K. Kemp-Harper, R.H. Ritchie, Nitroxyl (HNO) stimulates soluble guanylyl cyclase to suppress cardiomyocyte hypertrophy and superoxide generation, *PLoS One* 7 (2012), e34892, <https://doi.org/10.1371/journal.pone.0034892>.
- [323] R.H. Ritchie, G.R. Drummond, C.G. Sobey, T.M. De Silva, B.K. Kemp-Harper, The opposing roles of NO and oxidative stress in cardiovascular disease, *Pharm. Res* 116 (2017) 57–69, <https://doi.org/10.1016/j.phrs.2016.12.017>.
- [324] H.N. Sabbah, C.G. Tocchetti, M. Wang, S. Daya, R.C. Gupta, R.S. Tunin, R. Mazhari, E. Takimoto, N. Paolucci, D. Cowart, W.S. Colucci, D.A. Kass, Nitroxyl (HNO): a novel approach for the acute treatment of heart failure, *Circ. Heart Fail* 6 (2013) 1250–1258, <https://doi.org/10.1161/CIRCHEARTFAILURE.113.000632>.
- [325] C. Tita, E.M. Gilbert, A.B. Van Bakel, J. Grzybowski, G.J. Haas, M. Jarrah, S. H. Dunlap, S.S. Gottlieb, M. Klapholz, P.C. Patel, R. Pfister, T. Seidler, K.B. Shah, T. Zielinski, R.P. Venuti, D. Cowart, S.Y. Foo, A. Vishnevsky, V. Mitrovic, A Phase 2a dose-escalation study of the safety, tolerability, pharmacokinetics and haemodynamic effects of BMS-986231 in hospitalized patients with heart failure with reduced ejection fraction, *Eur. J. Heart Fail* 19 (2017) 1321–1332, <https://doi.org/10.1002/ehfj.897>.
- [326] G.M. Felker, J.J.V. McMurray, J.G. Cleland, C.M. O'Connor, J.R. Teerlink, A. A. Voors, J. Belohlavek, M. Böhm, M. Borentain, H. Bueno, R.T. Cole, M. M. DeSouza, J.A. Ezekowitz, G. Filippatos, N.N. Lang, P.D. Kessler, F.A. Martinez, A. Mebazaa, M. Metra, A. Mosterd, P.S. Pang, P. Ponikowski, N. Sato, D. Seiffert, J. Ye, Effects of a novel nitroxyl donor in acute heart failure: the STAND-UP AHF study, *JACC Heart Fail* 9 (2021) 146–157, <https://doi.org/10.1016/j.jchf.2020.10.012>.
- [327] G.M. Felker, M. Borentain, J.G. Cleland, M.M. DeSouza, P.D. Kessler, C. M. O'Connor, D. Seiffert, J.R. Teerlink, A.A. Voors, J.J.V. McMurray, Rationale and design for the development of a novel nitroxyl donor in patients with acute heart failure, *Eur. J. Heart Fail* 21 (2019) 1022–1031, <https://doi.org/10.1002/ehfj.1504>.
- [328] R.M. Broxterman, D.T. La Salle, J. Zhao, V.R. Reese, R.S. Richardson, J.D. Trinity, Influence of dietary inorganic nitrate on blood pressure and vascular function in hypertension: prospective implications for adjunctive treatment, *J. Appl. Physiol.* 127 (2019) 1085–1094, <https://doi.org/10.1152/jappphysiol.00371.2019>.
- [329] Y. He, J. Liu, H. Cai, J. Zhang, J. Yi, Y. Niu, H. Xi, X. Peng, L. Guo, Effect of inorganic nitrate supplementation on blood pressure in older adults: a systematic review and meta-analysis, *Nitric Oxide* 114 (2021) 13–22, <https://doi.org/10.1016/j.niox.2021.04.006>.
- [330] K.S. Hughan, A. Levine, N. Helbling, S. Anthony, J.P. DeLany, M. Stefanovic-Racic, B.H. Goodpaster, M.T. Gladwin, Effects of oral sodium nitrite on blood pressure, insulin sensitivity, and intima-media arterial thickening in adults with hypertension and metabolic syndrome, *Hypertension* 76 (2020) 866–874, <https://doi.org/10.1161/HYPERTENSIONAHA.120.14930>.
- [331] J.O. Lundberg, M.T. Gladwin, A. Ahluwalia, N. Benjamin, N.S. Bryan, A. Butler, P. Cabrales, A. Fago, M. Feelisch, P.C. Ford, B.A. Freeman, M. Frenneaux, J. Friedman, M. Kelm, C.G. Kevil, D.B. Kim-Shapiro, A.V. Kozlov, J.R. Jr Lancaster, D.J. Lefer, K. McColl, K. McCurry, R.P. Patel, J. Pettersson, T. Rassaf, V. P. Reutov, G.B. Richter-Addo, A. Schechter, S. Shiva, K. Tsuchiya, E.E. van Faassen, A.J. Webb, B.S. Zuckerbraun, J.L. Zweier, E. Weitzberg, Nitrate and nitrite in biology, nutrition and therapeutics, *Nat. Chem. Biol.* 5 (2009) 865–869, <https://doi.org/10.1038/nchembio.260>.
- [332] N. Siddiqi, C. Neil, M. Bruce, G. MacLennan, S. Cotton, S. Papadopolou, M. Feelisch, N. Bunce, P.O. Lim, D. Hildick-Smith, J. Horowitz, M. Madhani, N. Boon, D. Dawson, J.C. Kaski, M. Frenneaux, NIAMI investigators, Intravenous sodium nitrite in acute ST-elevation myocardial infarction: a randomized controlled trial (NIAMI), *Eur. Heart J.* 35 (2014) 1255–1262, <https://doi.org/10.1093/eurheartj/ehu096>.
- [333] D.A. Jones, C. Pellaton, S. Velmurugan, K.S. Rathod, M. Andiapan, S. Antoniou, S. van Eijl, A.J. Webb, M.A. Westwood, M.K. Parmar, A. Mathur, A. Ahluwalia, Randomized phase 2 trial of intracoronary nitrite during acute myocardial infarction, *Circ. Res* 116 (2015) 437–447, <https://doi.org/10.1161/CIRCRESAHA.116.305082>.
- [334] D.A. Jones, R.S. Khambata, M. Andiapan, K.S. Rathod, A. Mathur, A. Ahluwalia, Intracoronary nitrite suppresses the inflammatory response following primary percutaneous coronary intervention, *Heart* 103 (2017) 508–516, <https://doi.org/10.1136/heartjnl-2016-309748>.
- [335] M.J. Haykowsky, E.J. Kouba, P.H. Brubaker, B.J. Nicklas, J. Eggebeen, D. W. Kitzman, Skeletal muscle composition and its relation to exercise intolerance in older patients with heart failure and preserved ejection fraction, *Am. J. Cardiol.* 113 (2014) 1211–1216, <https://doi.org/10.1016/j.amjcard.2013.12.031>.
- [336] M.J. Haykowsky, P.H. Brubaker, T.M. Morgan, S. Kritchevsky, J. Eggebeen, D. W. Kitzman, Impaired aerobic capacity and physical functional performance in older heart failure patients with preserved ejection fraction: role of lean body mass, *J. Gerontol. A Biol. Sci. Med. Sci.* 68 (2013) 968–975, <https://doi.org/10.1093/gerona/glt011>.

- [337] B.A. Borlaug, V. Melenovsky, K.E. Koepf, Inhaled sodium nitrite improves rest and exercise hemodynamics in heart failure with preserved ejection fraction, *Circ. Res* 119 (2016) 880–886, <https://doi.org/10.1161/CIRCRESAHA.116.309184>.
- [338] P. Zamani, D. Rawat, P. Shiva-Kumar, S. Geraci, R. Bhuvu, P. Konda, P.T. Doulias, H. Ischiropoulos, R.R. Townsend, K.B. Margulies, T.P. Cappola, D.C. Poole, J. A. Chirinos, Effect of inorganic nitrate on exercise capacity in heart failure with preserved ejection fraction, discussion 380, *Circulation* 131 (2015) 371–380, <https://doi.org/10.1161/CIRCULATIONAHA.114.012957>.
- [339] Y.N.V. Reddy, M.J. Andersen, M. Obokata, K.E. Koepf, G.C. Kane, V. Melenovsky, T.P. Olson, B.A. Borlaug, Arterial stiffening with exercise in patients with heart failure and preserved ejection fraction, *J. Am. Coll. Cardiol.* 70 (2017) 136–148, <https://doi.org/10.1016/j.jacc.2017.05.029>.
- [340] B.A. Borlaug, K.J. Anstrom, G.D. Lewis, S.J. Shah, J.A. Levine, G.A. Koepf, M. M. Givertz, G.M. Felker, M.M. LeWinter, D.L. Mann, K.B. Margulies, A.L. Smith, W.H.W. Tang, D.J. Whellan, H.H. Chen, V.G. Davila-Roman, S. McNulty, P. Desvigne-Nickens, A.F. Hernandez, E. Braunwald, M.M. Redfield, National Heart, Lung, and Blood Institute Heart Failure Clinical Research Network, Effect of inorganic nitrite vs placebo on exercise capacity among patients with heart failure with preserved ejection fraction: the INDIE-HfPEF randomized clinical trial, *JAMA* 320 (2018) 1764–1773, <https://doi.org/10.1001/jama.2018.14852>.
- [341] F. Londono-Hoyos, P. Zamani, M. Beraun, I. Vasim, P. Segers, J.A. Chirinos, Effect of organic and inorganic nitrates on cerebrovascular pulsatile power transmission in patients with heart failure and preserved ejection fraction, *Physiol. Meas.* 39 (2018), 044001, <https://doi.org/10.1088/1361-6579/aab2ef>.
- [342] H.A. Shaltout, J. Eggebeen, A.P. Marsh, P.H. Brubaker, P.J. Laurienti, J. H. Burdette, S. Basu, A. Morgan, P.C. Dos Santos, J.L. Norris, T.M. Morgan, G. D. Miller, W.J. Rejeski, A.T. Hawfield, D.I. Diz, J.T. Becton, D.B. Kim-Shapiro, D. W. Kitzman, Effects of supervised exercise and dietary nitrate in older adults with controlled hypertension and/or heart failure with preserved ejection fraction, *Nitric Oxide* 69 (2017) 78–90, <https://doi.org/10.1016/j.niox.2017.05.005>.
- [343] F. Lv, J. Zhang, Y. Tao, Efficacy and safety of inorganic nitrate/nitrite supplementary therapy in heart failure with preserved ejection fraction, *Front Cardiovasc Med* 10 (2023), 1054666, <https://doi.org/10.3389/fcvm.2023.1054666>.
- [344] M.F. Montenegro, M.L. Sundqvist, F.J. Larsen, Z. Zhuge, M. Carlström, E. Weitzberg, J.O. Lundberg, Blood pressure-lowering effect of orally ingested nitrite is abolished by a proton pump inhibitor, *Hypertension* 69 (2017) 23–31, <https://doi.org/10.1161/HYPERTENSIONAHA.116.08081>.
- [345] K.S. Hughan, S.G. Wendell, M. Delmastro-Greenwood, N. Helbling, C. Corey, L. Bellavia, G. Potti, G. Grimes, B. Goodpaster, D.B. Kim-Shapiro, S. Shiva, B. A. Freeman, M.T. Gladwin, Conjugated linoleic acid modulates clinical responses to oral nitrite and nitrate, *Hypertension* 70 (2017) 634–644, <https://doi.org/10.1161/HYPERTENSIONAHA.117.09016>.
- [346] T.J. McCabe, D. Fulton, L.J. Roman, W.C. Sessa, Enhanced electron flux and reduced calmodulin dissociation may explain "calcium-independent" eNOS activation by phosphorylation, *J. Biol. Chem.* 275 (2000) 6123–6128, <https://doi.org/10.1074/jbc.275.9.6123>.
- [347] Z. Altaany, G. Yang, R. Wang, Crosstalk between hydrogen sulfide and nitric oxide in endothelial cells, *J. Cell Mol. Med* 17 (2013) 879–888, <https://doi.org/10.1111/jcmm.12077>.
- [348] S.C. Bir, G.K. Kolluru, P. McCarthy, X. Shen, S. Pardue, C.B. Pattillo, C.G. Kevil, Hydrogen sulfide stimulates ischemic vascular remodeling through nitric oxide synthase and nitrite reduction activity regulating hypoxia-inducible factor-1 α and vascular endothelial growth factor-dependent angiogenesis, *J. Am. Heart Assoc.* 1 (2012), e004093, <https://doi.org/10.1161/JAHA.112.004093>.
- [349] M. Bucchi, A. Papapetropoulos, V. Vellecco, Z. Zhou, A. Pyriochou, C. Roussos, F. Roviezzo, V. Brancaleone, G. Cirino, Hydrogen sulfide is an endogenous inhibitor of phosphodiesterase activity, *Arterioscler. Thromb. Vasc. Biol.* 30 (2010) 1998–2004, <https://doi.org/10.1161/ATVBAHA.110.209783>.
- [350] S.H. Francis, J.L. Busch, J.D. Corbin, D. Sibley, cGMP-dependent protein kinases and cGMP phosphodiesterases in nitric oxide and cGMP action, *Pharm. Rev.* 62 (2010) 525–563, <https://doi.org/10.1124/pr.110.020907>.
- [351] C. Coletta, A. Papapetropoulos, K. Erdelyi, G. Olah, K. Módi, P. Panopoulos, A. Asimakopoulou, D. Gerö, I. Sharina, E. Martin, C. Szabo, Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation, *Proc. Natl. Acad. Sci. U. S. A.* 109 (2012) 9161–9166, <https://doi.org/10.1073/pnas.1202916109>.
- [352] B.L. Predmore, K. Kondo, S. Bhushan, M.A. Zlatopolsky, A.L. King, J.P. Aragon, D. B. Grinsfelder, M.E. Condit, D.J. Lefer, The polysulfide diallyl trisulfide protects the ischemic myocardium by preservation of endogenous hydrogen sulfide and increasing nitric oxide bioavailability, *Am. J. Physiol. Heart Circ. Physiol.* 302 (2012) H2410–8, <https://doi.org/10.1152/ajpheart.00044.2012>.
- [353] J.W. Elrod, J.W. Calvert, J. Morrison, J.E. Doeller, D.W. Kraus, L. Tao, X. Jiao, R. Scalia, L. Kiss, C. Szabo, H. Kimura, C.W. Chow, D.J. Lefer, Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function, *Proc. Natl. Acad. Sci. USA* 104 (2007) 15560–5, <https://doi.org/10.1073/pnas.0705891104>.
- [354] D.J. Polhemus, Z. Li, C.B. Pattillo, G. Sr Gojon, G. Jr Gojon, T. Giordano, H. Krum, A novel hydrogen sulfide prodrug, SG1002, promotes hydrogen sulfide and nitric oxide bioavailability in heart failure patients, *Cardiovasc Ther.* 33 (2015) 216–226, <https://doi.org/10.1111/1755-5922.12128>.
- [355] M.L. de Koning, P. van Dorp, S. Assa, M.H. Hartman, M. Voskuil, R.L. Anthonio, D. Veen, G. Pundziute-Do Prado, T. Leiner, H. van Goor, P. van der Meer, D.J. van Veldhuisen, R. Nijveldt, E. Lipsic, P. van der Harst, Rationale and design of the groningen intervention study for the preservation of cardiac function with sodium thiosulfate after st-segment elevation myocardial infarction (GIPS-IV) trial, *Am. Heart J.* 243 (2022) 167–176, <https://doi.org/10.1016/j.ahj.2021.08.012>.
- [356] <https://www.escardio.org/Congresses-Events/ESC-TV/ESC-Expert-In-sights-at-ACC22#gips-iv>.
- [357] A.L. Moens, R. Yang, V.L. Watts, L.A. Barouch, Beta 3-adrenoreceptor regulation of nitric oxide in the cardiovascular system, *J. Mol. Cell Cardiol.* 48 (2010) 1088–1095, <https://doi.org/10.1016/j.yjmcc.2010.02.011>.
- [358] S. Moniotte, L. Kobzik, O. Feron, J.N. Trochu, C. Gauthier, J.L. Balligand, Upregulation of beta (3)-adrenoreceptors and altered contractile response to inotropic amines in human failing myocardium, *Circulation* 103 (2001) 1649–1655, <https://doi.org/10.1161/01.cir.103.12.1649>.
- [359] N. Hermida, L. Michel, H. Esfahani, E. Dubois-Deruy, J. Hammond, C. Bouzin, A. Markl, H. Colin, A.V. Steenbergen, C. De Meester, C. Beauloye, S. Hornman, X. Yin, M. Mayr, J.L. Balligand, Cardiac myocyte β 3-adrenergic receptors prevent myocardial fibrosis by modulating oxidant stress-dependent paracrine signaling, *Eur. Heart J.* 39 (10) (2018) 888–898, <https://doi.org/10.1093/eurheartj/ehx366>.
- [360] C. Gauthier, V. Leblais, L. Kobzik, J.N. Trochu, N. Khandoudi, A. Bril, J. L. Balligand, H. Le Mare, The negative inotropic effect of beta3-adrenoreceptor stimulation is mediated by activation of a nitric oxide synthase pathway in human ventricle, *J. Clin. Invest* 102 (1998) 1377–1384, <https://doi.org/10.1172/JCI2191>.
- [361] G. Tarone, J.L. Balligand, J. Bauersachs, A. Clerck, L. De Windt, S. Heymans, D. Hilfiker-Kleiner, D. E. Hirsch, G. Iaccarino, R. Knöll, A.F. Leite-Moreira, A. P. Lourenço, M. Mayr, T. Thum, Tocchetti paper from the Working Group on Myocardial Function of the European Society of Cardiology, *Eur. J. Heart Fail* 16 (2014) 494–508, <https://doi.org/10.1002/ehf.62>.
- [362] H. Bundgaard, A. Axelsson, J. Hartvig Thomsen, M. Sørgaard, K.F. Kofoed, R. Hasselbalch, N.A. Fry, N. Valeur, S. Boesgaard, F. Gustafsson, L. Køber, K. Iversen, H.H. Rasmussen, The first-in-man randomized trial of a beta3 adrenoreceptor agonist in chronic heart failure: the BEAT-HF trial, *Eur. J. Heart Fail* 19 (2017) 566–575, <https://doi.org/10.1002/ehf.714>.
- [363] C. Belge, J. Hammond, E. Dubois-Deruy, B. Manoury, J. Hamelet, C. Beauloye, A. Markl, A.C. Pouleur, L. Bertrand, H. Esfahani, K. Jnaoui, K.R. Götz, V. O. Nikolaev, A. Vanderper, P. Herijgers, I. Lobsysheva, G. Iaccarino, D. Hilfiker-Kleiner, G. Tavernier, D. Langin, C. Dessy, J.L. Balligand, Enhanced expression of β 3-adrenoreceptors in cardiac myocytes attenuates neurohormone-induced hypertrophic remodeling through nitric oxide synthase, *Circulation* 129 (2014) 451–462, <https://doi.org/10.1161/CIRCULATIONAHA.113.004940>.
- [364] A.C. Pouleur, S. Anker, D. Brito, O. Brosteanu, D. Hasenclaver, B. Casadei, F. Edelmann, G. Filippatos, D. Gruson, I. Ikonomidis, R. Lhomel, M. Mahmood, S. Neubauer, A. Persu, B.L. Gerber, S. Piechnik, B. Pieske, E. Pieske-Kraigher, F. Pinto, P. Ponikowski, M. Senni, J.N. Trochu, N. Van Overstraeten, R. Wachter, J.L. Balligand, Rationale and design of a multicenter, randomized, placebo-controlled trial of mirabegron, a Beta3-adrenergic receptor agonist on left ventricular mass and diastolic function in patients with structural heart disease Beta3-left ventricular hypertrophy (Beta3-LVH), *ESC Heart Fail* 5 (2018) 830–841, <https://doi.org/10.1002/ehf2.12306>.
- [365] <https://www.escardio.org/The-ESC/Press-Office/Press-releases/mirabegron-is-safe-but-fails-to-prevent-remodelling-in-patients-with-mild-heart>.
- [366] H. Bundgaard, A. Axelsson Raja, K. Iversen, N. Valeur, N. Tønder, M. Schou, A. H. Christensen, N.E. Bruun, H. Sjøholm, M. Ghanizada, N.A.S. Fry, E.J. Hamilton, S. Boesgaard, M.B. Møller, E. Wolsk, K. Rossing, L. Køber, H.H. Rasmussen, C. R. Vissing, Hemodynamic effects of cyclic guanosine monophosphate-dependent signaling through β 3 adrenoreceptor stimulation in patients with advanced heart failure: a randomized invasive clinical trial, *Circ. Heart Fail* 15 (2022), e009120, <https://doi.org/10.1161/CIRCHEARTFAILURE.121.009120>.
- [367] N.A.S. Fry, C.C. Liu, A. Garcia, E.J. Hamilton, K. Karimi Galougahi, Y.J. Kim, D. W. Whalley, H. Bundgaard, H.H. Rasmussen, Targeting cardiac myocyte Na $^{+}$ -K $^{+}$ pump function with β 3 adrenoreceptor agonist in rabbit model of severe congestive heart failure, *Circ. Heart Fail* 13 (2020), e006753, <https://doi.org/10.1161/CIRCHEARTFAILURE.119.006753>.
- [368] R. D'Orta, R. Schipani, A. Leonardini, A. Natalicchio, S. Perrini, A. Cignarelli, L. Laviola, F. Giorgino, The role of oxidative stress in cardiac disease: from physiological response to injury factor, *Oxid. Med Cell Longev.* 14 (2020), 5732956, <https://doi.org/10.1155/2020/5732956>.
- [369] Z. Ungvári, S.A. Gupte, F.A. Recchia, S. Bátkai, P. Pacher, Role of oxidative-nitrosative stress and downstream pathways in various forms of cardiomyopathy and heart failure, *Curr. Vasc. Pharm.* 3 (2005) 221–229, <https://doi.org/10.2174/1570161054368607>.
- [370] X. Chang, T. Zhang, W. Zhang, Z. Zhao, J. Sun, Natural drugs as a treatment strategy for cardiovascular disease through the regulation of oxidative stress, *Oxid. Med Cell Longev.* 27 (2020), 5430407, <https://doi.org/10.1155/2020/5430407>.
- [371] S.L. Chow, B. Bozkurt, W.L. Baker, B.E. Bleske, K. Breathett, G.C. Fonarow, B. Greenberg, P. Khazanie, J. Leclerc, A.A. Morris, N. Reza, C.W. Yancy, American Heart Association Clinical Pharmacology Committee and Heart Failure and Transplantation Committee of the Council on Clinical Cardiology; Council on Epidemiology and Prevention; and Council on Cardiovascular and Stroke Nursing, Complementary and alternative medicines in the management of heart failure: a scientific statement from the American Heart Association, *Circulation* 147 (2023) e4–e30, <https://doi.org/10.1161/CIR.0000000000001110>.
- [372] M. Oswal, R. Varghese, T. Zagade, C. Dhattrak, R. Sharma, D. Kumar, Dietary supplements and medicinal plants in urolithiasis: diet, prevention, and cure, *J. Pharm. Pharmacol.* 75 (6) (2023) 719–745, <https://doi.org/10.1093/jpp/rgac092>.

- [373] D. Karati, R. Varghese, K.R. Mahadik, R. Sharma, D. Kumar, Plant bioactives in the treatment of inflammation of skeletal muscles: a molecular perspective, *Evid. Based Complement Altern. Med* 2022 (2022), 4295802, <https://doi.org/10.1155/2022/4295802>.
- [374] J.A. Enriqueza, G. Lenazb, Coenzyme Q and the respiratory chain: coenzyme Q pool and mitochondrial supercomplexes, *Mol. Syndromol.* 5 (2014) 119–140, <https://doi.org/10.1159/000363364>.
- [375] C. Schmelzer, F. Döring, Micronutrient special issue: coenzyme Q(10) requirements for DNA damage prevention, *Mutat. Res.* 733 (2012) 61–68, <https://doi.org/10.1016/j.mrfmmm.2011.09.004>.
- [376] V. Mugoni, R. Postel, V. Catanzaro, E. De Luca, E. Turco, G. Digilio, L. Silengo, M. P. Murphy, C. Medana, D.Y., et al., Ubiad1 is an antioxidant enzyme that regulates eNOS activity by CoQ10 synthesis, *Cell* 152 (2013) 504–518, <https://doi.org/10.1016/j.cell.2013.01.013>.
- [377] A. Sharma, G.C. Fonarow, J. Butler, J.A. Ezekowitz, G.M. Felker, Coenzyme Q10 and heart failure: a state-of-the-art review, *Circ. Heart Fail* 9 (2016), e002639, <https://doi.org/10.1161/CIRCHEARTFAILURE.115.002639>.
- [378] S.A. Mortensen, F. Rosenfeldt, A. Kumar, P. Dolliner, K.J. Filipiak, D. Pella, A. Alehagen, G. Steurer, G.P. Littarru, Q-SYMBIO Study Investigators, The effect of coenzyme Q10 on morbidity and mortality in chronic heart failure: results from Q-SYMBIO: A randomized double-blind trial, *JACC Heart Fail* 2 (2014) 641–649, <https://doi.org/10.1016/j.jchf.2014.06.008>.
- [379] T. Al Saadi, Y. Assaf, M. Farwati, K. Turkmani, A. Al-Mouakeh, B. Shebli, M. Khoja, A. Essali, M.E. Madmani, Coenzyme Q10 for heart failure, *Cochrane Database Syst. Rev.* 2 (2021) CD008684, <https://doi.org/10.1002/14651858.CD008684.pub3>.
- [380] V. Mollace, G.M.C. Rosano, S.D. Anker, A.J.S. Coats, P. Seferovic, R. Mollace, A. Tavernese, M. Gliozi, V. Musolino, C. Carresi, J. Maiuolo, R. Macri, F. Bosco, M. Chiochi, F. Romeo, M. Metra, M. Volterrani, Pathophysiological basis for nutraceutical supplementation in heart failure: a comprehensive review, *Nutrients* 13 (2021) 257, <https://doi.org/10.3390/nu13010257>.
- [381] A. Bharani, A. Ganguly, K.D. Bhargava, Salutary effect of Terminalia Arjuna in patients with severe refractory heart failure, *Int J. Cardiol.* 49 (1995) 191–199, [https://doi.org/10.1016/0167-5273\(95\)02320-v](https://doi.org/10.1016/0167-5273(95)02320-v).
- [382] J.G. Zhang, N. Yang, H. He, G.H. Wei, D.S. Gao, X.L. Wang, X.Z. Wang, G.Y. Song, Effect of Astragalus injection on plasma levels of apoptosis-related factors in aged patients with chronic heart failure (Chinese), *Chin. J. Integr. Med* 11 (2005) 187–190, <https://doi.org/10.1007/BF02836502>.
- [383] V. Stangl, M. Lorenz, K. Stangl, The role of tea and tea flavonoids in cardiovascular health, *Mol. Nutr. Food Res* 50 (2006) 218–228, <https://doi.org/10.1002/mnfr.200500118>.
- [384] M. Yamakuchi, C. Bao, M. Ferlito, C.J. Lowenstein, Epigallocatechin gallate inhibits endothelial exocytosis, *Biol. Chem.* 389 (2008) 935–941, <https://doi.org/10.1515/BC.2008.095>.
- [385] E. Anter, K. Chen, O.M. Shapira, R.H. Karas, J.F. Jr Keaney, p38 mitogen-activated protein kinase activates eNOS in endothelial cells by an estrogen receptor alpha-dependent pathway in response to black tea polyphenols, *Circ. Res* 96 (2005) 1072–1078, <https://doi.org/10.1161/01.RES.0000168807.63013.56>.
- [386] V. Stangl, H. Dreger, K. Stangl, M. Lorenz, Molecular targets of tea polyphenols in the cardiovascular system, *Cardiovasc Res* 73 (2007) 348–358, <https://doi.org/10.1016/j.cardiores.2006.08.022>.
- [387] N.K. Andrikopoulos, A.C. Kaliora, A.N. Assimpoulou, V.P. Papageorgiou, Inhibitory activity of minor polyphenolic and nonpolyphenolic constituents of olive oil against in vitro low-density lipoprotein oxidation, *J. Med Food* 5 (Spring; 5(1)) (2002) 1–7, <https://doi.org/10.1089/109662002753723160>.
- [388] I. Andreadou, E.K. Iliodromitis, E. Mikros, M. Constantinou, A. Agalias, P. Magiatis, A.L. Skaltsounis, E. Kamber, A. Tsantili-Kakoulidou, D. T. Kremastinos, The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits, *J. Nutr.* 136 (2006) 2213–2219, <https://doi.org/10.1093/jn/136.8.2213>.
- [389] M. Fitó, R. Estruch, J. Salas-Salvadó, M.A. Martínez Gonzalez, F. Arós, J. Vila, D. Corella, O. Díaz, G. Sáez, R. de la Torre, M.-T. Mitjavila, et al., Effect of the Mediterranean diet on heart failure biomarkers: a randomized sample from the PREDIMED trial, *Eur. J. Heart Fail.* 16 (2014) 543–550, <https://doi.org/10.1002/ejhf.61>.
- [390] R. Casas, E. Sacanella, M. Urpí-Sardà, G. Chiva-Blanch, E. Ros, M.-A. Martínez-González, M.-I. Covas, J. Salas-Salvadó, M. Fiol, F. Arós, R. Estruch, The effects of the mediterranean diet on biomarkers of vascular wall inflammation and plaque vulnerability in subjects with high risk for cardiovascular disease. A randomized trial, *PLoS One* 9 (2014), e100084, <https://doi.org/10.1371/journal.pone.0100084>.
- [391] J. Burns, T. Yokota, H. Ashihara, M.E.J. Lean, A. Crozier, Plant foods and herbal sources of resveratrol, *J. Agric. Food Chem.* 50 (2002) 3337–3340, <https://doi.org/10.1021/jf0112973>.
- [392] M. Thirunavukkarasu, S.V. Penumathsa, S. Koneru, B. Juhasz, L. Zhan, H. Otani, D. Bagchi, D.K. Das, N. Maulik, Resveratrol alleviates cardiac dysfunction in streptozotocin-induced diabetes: role of nitric oxide, thioredoxin, and heme oxygenase, *Free Radic. Biol. Med.* 43 (2007) 720–729, <https://doi.org/10.1016/j.freeradbiomed.2007.05.004>.
- [393] N. Fourny, C. Lan, E. Séréé, M. Bernard, M. Desrois, 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) 105, <https://doi.org/10.3390/nu11010105>.
- [394] N.I. Chekalina, Resveratrol has a positive effect on parameters of central hemodynamics and myocardial ischemia in patients with stable coronary heart disease *Wiad. Lek* 70 (2017) 286–291.
- [395] Y. Cai, Q. Xin, J. Lu, Y. Miao, Q. Lin, W. Cong, K. Chen, A new therapeutic candidate for cardiovascular diseases: berberine, *Front Pharmacol. Front Pharmacol.* 12 (2021), <https://doi.org/10.3389/fphar.2021.631100>.
- [396] S. Yang, D. Li, Z. Yu, Y. Li, M. Wu, Multi-pharmacology of berberine in atherosclerosis and metabolic diseases: potential contribution of gut microbiota, *Front Pharm.* 12 (2021), 709629, <https://doi.org/10.3389/fphar.2021.709629>.
- [397] H. Zhang, X. Wu, Y. Tao, G. Lu, Berberine attenuates sepsis-induced cardiac dysfunction by upregulating the Akt/eNOS pathway in mice, *Exp. Ther. Med* 23 (2022), 371, <https://doi.org/10.3892/etm.2022.11298>.
- [398] M. Abudureyimu, W. Yu, R.Y. Cao, Y. Zhang, H. Liu, H. Zheng, Berberine promotes cardiac function by upregulating PINK1/Parkin-mediated mitophagy in heart failure, *Front. Physiol.* 11 (2020), 565751, <https://doi.org/10.3389/fphys.2020.565751>.
- [399] X.H. Zeng, X.J. Zeng, Y.Y. Li, Efficacy and safety of berberine for congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy, *Am. J. Cardiol.* 92 (2003) 173–176, [https://doi.org/10.1016/s0002-9149\(03\)00533-2](https://doi.org/10.1016/s0002-9149(03)00533-2).
- [400] D. Weissman, C. Maack, Redox signaling in heart failure and therapeutic implications, *Free Radic. Biol. Med.* 171 (2021) 345–364, <https://doi.org/10.1016/j.freeradbiomed.2021.05.013>.
- [401] A. Van Der Pol, W.H. Van Gilst, A.A. Voors, P. Van Der Meer, Treating oxidative stress in heart failure: past, present and future, *Eur. J. Heart Fail.* 21 (2019) 425–435, <https://doi.org/10.1002/ejhf.1320>.
- [402] C. Carresi, V. Musolino, M. Gliozi, J. Maiuolo, R. Mollace, S. Nucera, A. Maretta, D. Sergi, S. Muscoli, S. Gratteri, et al., Anti-oxidant effect of bergamot polyphenolic fraction counteracts doxorubicin-induced cardiomyopathy: role of autophagy and ckitposCD45negCD31neg cardiac stem cell activation, *J. Mol. Cell. Cardiol.* 119 (2018) 10–18, <https://doi.org/10.1016/j.yjmcc.2018.04.007>.
- [403] V. Musolino, M. Gliozi, S. Nucera, C. Carresi, J. Maiuolo, R. Mollace, S. Paone, F. Bosco, F. Scarano, M. Scicchitano, et al., The effect of bergamot polyphenolic fraction on lipid transfer protein system and vascular oxidative stress in a rat model of hyperlipemia, *Lipids Health Dis.* 18 (2019), 115, <https://doi.org/10.1186/s12944-019-1061-0>.
- [404] V. Mollace, M. Scicchitano, S. Paone, F. Casale, C. Calandrucchio, M. Gliozi, V. Musolino, C. Carresi, J. Maiuolo, S. Nucera, et al., Hypoglycemic and hypolipemic effects of a new lecithin formulation of bergamot polyphenolic fraction: a double blind, randomized, placebo-controlled study, *Endocr. Metab. Immune Disord. Drug Targets* 19 (2019) 136–143, <https://doi.org/10.2174/1871530319666181203151513>.
- [405] I. Andreadou, E.K. Iliodromitis, A. Lazou, A. Görbe, Z. Giricz, R. Schulz, et al., Effect of hypercholesterolaemia on myocardial function, ischaemia-reperfusion injury and cardioprotection by preconditioning, postconditioning and remote conditioning, *Br. J. Pharmacol.* 174 (2017) 1555–1569, <https://doi.org/10.1111/bph.13704>.
- [406] R. Mollace, R. Macri, A. Tavernese, M. Gliozi, V. Musolino, C. Carresi, J. Maiuolo, M. Fini, M. Volterrani, V. Mollace, Comparative effect of bergamot polyphenolic fraction and red yeast rice extract in rats fed a hyperlipidemic diet: role of antioxidant properties and PCSK9 expression, *Nutrients* 14 (2022) 477, <https://doi.org/10.3390/nu14030477>.
- [407] R. Mollace, M. Gliozi, A. Tavernese, V. Musolino, C. Carresi, M. Scicchitano, E. Palma, S. Nucera, F. Bosco, F. Scarano, et al., Bergamot polyphenolic fraction supplementation improves metabolic balance, endothelial function and maximal oxygen uptake in athletes, *J. Sports Med Ther.* 3 (2018) 053–061, <https://doi.org/10.29328/journal.jsmt.1001027>.