Heart failure with preserved ejection fraction: Defining the function of ROS and NO

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Submitted 23 December 2014; accepted in final form 5 May 2015

Zuo L, Chuang C, Hemmelgarn BT, Best TM. Heart failure with preserved ejection fraction: Defining the function of ROS and NO. J Appl Physiol 119: 944–951, 2015. First published May 14, 2015; doi:10.1152/japplphysiol.01149.2014.—The understanding of complex molecular mechanisms underlying heart failure (HF) is constantly under revision. Recent research has paid much attention to understanding the growing number of patients that exhibit HF symptoms yet have an ejection fraction similar to a normal phenotype. Termed heart failure with preserved ejection fraction (HFpEF), this novel hypothesis traces its roots to a proinflammatory state initiated in part by the existence of comorbidities that create a favorable environment for the production of reactive oxygen species (ROS). Triggering a cascade that involves reduced nitric oxide (NO) availability, elevated ROS levels in the coronary endothelium eventually contribute to hypertrophy and increased resting tension in cardiomyocytes. Improved understanding of the molecular pathways associated with HFpEF has led to studies that concentrate on reducing ROS production in the heart, boosting NO availability, and increasing exercise capacity for HFpEF patients. This review will explore the latest research into the role of ROS and NO in the progression of HFpEF, as well as discuss the encouraging results of numerous therapeutic studies.

superoxide; nitric oxide synthase; ejection fraction; NADPH oxidases; exercise; reactive oxygen species; nitric oxide; heart failure with preserved ejection fraction

Heart failure (HF) is described as a clinical condition that involves fatigue, dyspnea during routine or reduced exertion, and fluid retention (71). Ejection fraction (EF) is a traditional indicator of heart function that measures the amount of blood pumped out of the left ventricle (LV). Low EFs can be regarded as indexes for impaired hearts. Interestingly, ~50% of patients exhibiting HF symptoms report an EF at rest that exceeds 50% of the normal fraction (20, 39). Thus, HF with preserved ejection fraction (HFpEF) is a disease that is increasingly prevalent and somewhat challenging to diagnose. Because HFpEF patients may suffer from multiple common comorbidities (e.g., diabetes mellitus, vasculopathy, hypertension, renal disease, metabolic syndrome, and atrial fibrillation) (2, 51), therapies for this disease have been difficult to develop (71).

Reactive oxygen species (ROS) form a group of biological molecules that are capable of modulating essential cell signaling pathways (26). These reactive molecules, such as superoxide (O2•−) and hydroxyl radicals, are generated from different sources in the body through the process of electron transfer. This involves the movement of electrons between molecules, in which one molecule is “reduced” when electrons are accepted and the other is “oxidized” when electrons are removed (26). Typically, ROS are produced when electrons leak from the electron transport chain (ETC) in the mitochondria and are accepted by molecular oxygen. Smaller amounts of ROS can also arise from peroxisomes, the endoplasmic reticulum, and the cellular membrane (70). Aside from internal production, radiation and tobacco smoke are also demonstrated sources of ROS (46, 62, 66). At basal levels, ROS can behave as signaling molecules (10, 14, 44). Nonetheless, ectopic accumulation of ROS can overwhelm the body’s antioxidant defense system. In
this event, antioxidants are not able to efficiently remove excessive ROS, allowing the reactive molecules to oxidize (remove electrons from) important components of the cell, such as lipids, proteins, and DNA (52).

Over the past decade, efforts have been made to identify the complex pathophysiology of HFpEF, particularly concentrating on endothelial abnormality due to its prevailing significance in multiple mechanisms (9). An innovative model put forth by Paulus and Tschöpe examined the potential involvement of ROS in HFpEF development (40). Indeed, several studies and reviews have illustrated the importance of ROS in cardiovascular diseases, with HFpEF conditions in particular (54, 60, 63). In HFpEF patients, ROS produced during comorbidity-induced endothelial inflammation may trigger a signaling cascade involving nitric oxide (NO) that ultimately increases interstitial fibrosis and activates cardiac remodeling. These actions contribute to the hallmarks of HFpEF: ventricular stiffness, impaired relaxation, and cardiac dysfunction (40). Still, limited human data in myocardial analysis and difficulties to fully represent experimental models of HFpEF remain a main challenge in the field (54). The hypothetical concept postulated by Paulus and Tschöpe deviates from the traditional paradigm of LV afterload excess and provides a fresh perspective for developing effective HFpEF treatments (9, 40). This review aims to critically analyze current advances in the understanding of HFpEF pathology, the role of ROS and NO in these pathways, and the outlook on promising therapies for HFpEF patients.

**Physiological Relevance of ROS/Reactive Nitrogen Species in the Heart**

**Cardiovascular ROS production in cardiac dysfunction.** NADPH oxidases (Nox) are a family of proteins that transfer electrons from NADH and NADPH to molecular oxygen to produce ROS, most notably $O_2^-$ and hydrogen peroxide ($H_2O_2$) (30, 34) as follows:

$$\text{NADPH} + 2H_2O_2 \rightarrow 2O_2^- + \text{NAD(P)}^+ + H^+$$

Nox proteins were first examined for their role in the immune system. To attack invading microbes, Nox can generate immense amounts of ROS that are able to kill pathogens via oxidative stress (OS) (45). Nox proteins can be found in the heart, as well as almost every other part of the body, and their ROS generation capabilities are used for much more than just cellular warfare (30, 34, 51). In particular, Nox2 and Nox4 are highly expressed in the heart and are responsive to stimuli such as proinflammatory cytokines (69).

It has long been known that ROS have the ability to oxidize proteins, DNA, and lipids within the cell (6). This oxidative damage occurs when concentrations of ROS rise above normal levels, either due to increased production or reduced antioxidant capacity. However, endogenous levels of ROS have now been shown to act as signaling molecules that can affect a large number of well-known signaling paradigms; mitogen-activated protein kinase/extracellular signal-regulated kinase, protein kinase B, and cyclic guanosine monophosphate/cGMP-dependent protein kinase (cGMP/PKG) are all transcriptional pathways that can be influenced by ROS oxidation (24, 61). Additionally, ROS molecules can alter histone complexes, thereby epigenetically regulating certain genes (21). In the heart, these pathways are paramount to normal cardiac form and function. If misregulated, these mechanisms may cause maladaptive ventricular remodeling leading to the development of multiple types of heart disease (50).

**NO and redox signaling in HFpEF development.** Nitric oxide synthase (NOS) is the protein responsible for the primary production of NO (58). NO is an essential molecule for myocardial function, one of which is tetrahydrobiopterin (BH$_4$) (56, 58). In environments of high ROS, BH$_4$ can become oxidized and therefore unrecognizable by NOS. In this case, NOS produces $O_2^-$ (56), which can both oxidize cellular components or transition into other oxidants such as peroxynitrite (ONOO$^-$) when reacting with NO (60) as follows:

$$O_2^- + NO \rightarrow ONOO^-$$

This interplay between ROS and NO is a topic of great interest in relation to HFpEF (50). Decreased NO bioavailability from NOS uncoupling causes a chain reaction that may ultimately lead to hypertrophy and impaired relaxation (Fig. 1) (40). Moreover, the $O_2^-$ produced by uncoupled NOS can induce OS in the endothelium and trigger additional maladaptive pathways, which likely contribute to ventricular remodeling and HFpEF (50).

**Molecular Mechanisms of HFpEF: Endothelium to Cardiomyocyte Communication**

**ROS induction and function in the endothelium of HFpEF patients.** A number of comorbidities have been correlated to HFpEF. Obesity, chronic obstructive pulmonary disease (COPD), diabetes mellitus, hypertension, and chronic kidney disease are all capable of producing a systemic proinflammatory state through various mechanisms (29, 36, 57). In addition, comorbid illnesses are recognized to attribute to the exacerbation and mortality of HFpEF (9). High levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) observed in HFpEF patients with these comorbidities substantiate the observed incurred systemic inflammation (Fig. 1) (8). In many instances, the presence of this systemic inflammatory state is indicative of only HFpEF, not heart failure with reduced ejection fraction (HFrEF) (19). Aside from ROS production by the coronary endothelial cells (40), one effect of elevated IL-6 and TNF-α levels is the overabundance of vascular cell adhesion molecule (VCAM) and E-selectin (Fig. 1). These proteins are capable of activating circulating monocytes and may induce their infiltration into the coronary microvascular endothelium. The augmented monocyte activity further generates inflammatory and fibrogenic mediators such as monocoye chemoattractant protein-1 (MCP-1) and transforming growth factor-β (TGF-β), respectively, potentially exacerbating inflammation and fibrosis (19).

Nox stimulation is a likely response to increased proinflammatory cytokines, with stimulated Nox generating ROS (12). Aside from indirectly inducing ROS production via increased cytokine production, certain comorbidities are able to stimulate
ROS production in the coronary endothelium directly (Fig. 1). For instance, mitochondrial energetics are notably influenced by glucose levels (55). Increased glucose levels in diabetes mellitus can increase mitochondrial fission, causing ROS leakage due to ETC dysfunction (55). Endothelial dysfunction has also been linked to old age. Ribosomal protein S6 kinase-a, elevated in aged rat aortas, is linked to increased endothelial NOS (eNOS) uncoupling (43).

Of all the cells in the heart, only 30% are cardiomyocytes (68). ROS generated in the endothelium function as signaling molecules for endothelium/cardiomyocyte interactions, which can occur in three separate ways (68). ROS molecules, particularly H2O2 and NO due to their relatively long half-lives, can diffuse out of the endothelium and directly enter cardiomyocytes. Also, ROS can influence factors that induce the buildup of extracellular matrix (ECM) and fibrosis. Stiffening of the heart via fibrosis may be detrimental and contribute to the development of HFpEF (65). Lastly, ROS stimulate the release of paracrine factors from the endothelium, such as NO and vascular endothelial growth factor (68). Emphasizing the importance of inflammation in HFpEF, Paulus and Tschöpe outlined the possibility that coronary microvascular endothelial dysfunction promoted by inflammation can induce ROS generation, NO depletion, and subsequent detrimental effects via endothelium/cardiomyocyte interactions (40).

Disruption of NO signaling in HFpEF. NO is an essential molecule for myocardial function, and its multifaceted involvement in the cardiomyocyte contractility remains an intriguing area of study. NO has been shown to stimulate PKG-mediated phosphorylation of troponin I, a thin-filament regulatory protein, leading to calcium desensitization in the myofilament and subsequent myocardial relaxation (35, 53). Acting as a relaxant...
molecule for myofibers in the heart, any disturbance in NO bioavailability may hold dangerous consequences for cardiac function (16). Indeed, the depleted NO in HFpEF cardiomyocytes lowers PKG activity, and in turn reduces phosphorylated titin (33, 54). The cytoskeletal protein titin is responsible for the elasticity of myocardial muscle and is considered to be a major modulator of myocardial stiffness (33). The limited NO availability reduces stimulation of soluble guanylate cyclase (sGC), an essential enzyme responsible for the production of cGMP. Thus, decreased sGC activity translates to lower levels of cGMP, which conceivably further downregulates the PKG pathway. PKG also attenuates L-type calcium current thereby inhibiting β-adrenergic inotropy (35, 53), and lowers the activity of the calcineurin-nuclear factor of activated T cells (NFAT) pathway potentially restraining hypertrophic genes that are regulated by NFAT (13). These help to illustrate how NOS uncoupling is potentially capable of inducing irreversible myocardial damage by disrupting downstream signaling pathways, such as the cGMP/PKG pathway that is in charge of preventing cardiohypertrophy and increasing relaxation (28, 54).

**High nitrosative/OS in HFpEF myocardium.** High nitrosative/OS has been implicated in HFpEF and is seen as a possibly unique component of the disease when compared with HFpEF and atherosclerosis (63). The prevalence of comorbidities associated with HFpEF (e.g., COPD, hypertension, diabetes, and obesity) often leads to increased ROS levels, particularly O2•− and ONOO−. Elevated levels of O2•− and ONOO− lead to nitrosative stress/OS that has been documented in HFpEF patients (69). A study by van Heerebeek et al. inferred elevated nitrosative/OS levels in HFpEF subjects by observing increased nitrotyrosine levels in myocardial tissue samples (63). Nitrotyrosine acts as a nitrosative/OS signal due to its generation from tyrosine nitration mediated by ONOO− or nitrogen dioxide, and this nitrosative/OS has serious implications for downstream modulators of hypertrophic cardiac remodeling (63). However, the minimal availability of myocardial samples from patients is a serious limiting factor in these types of studies, and small sample size has unfortunately caused many to doubt the significance of the findings.

**OS-Associated Cardiac Remodeling in HFpEF**

Although still limited when compared with other HF patient populations, better accessibility to myocardial tissue from HFpEF patients over the past decade has provided insights to the pathological alterations in HFpEF myocardium, both structurally and functionally. The modifications of interrelated structures and functionalities help elucidate the major characteristics of HFpEF: diastolic LV dysfunction and concentric LV remodeling (40). Such cardiovascular abnormalities, including fibrosis and myocardial stiffness, can lead to subsequent exercise intolerance and other symptoms such as edema and chronotropic incompetence in HFpEF (54).

OS-induced cardiac remodeling can generate deleterious effects that disturb the normal coronary flow reserve and diastolic filling (41). The progression of ECM protein deposition, mostly collagen type I accumulation, is also closely related to myocardial stiffness (40, 41). Westermann et al. observed excess collagen production in a biopsy of a HFpEF patient suffering from increased inflammation with TGF-β (65). Monocytes migrate to the inflamed endothelium via VCAM and E-selectin mediation. Accordingly, profibrotic TGF-β can be released from the endothelium, inducing the activation of fibroblasts and subsequent differentiation of fibroblasts to myofibroblasts (Fig. 1). Increased fibroblast and myofibroblast activities potentially lead to pronounced collagen production in HFpEF myocardium (40, 64). In addition, reduced NO bioavailability promotes fibroblast proliferation via upregulated micro-RNA-21 and suppressed cardiac fibroblast apoptosis, further suggesting the relationship between OS and interstitial fibrosis (11, 40). An investigation of senescent hearts provides a model for such a relationship since aging is often associated with increased inflammatory markers and OS, thus increasing the propensity for HF (7, 33). Higher levels of ROS in the aged heart conceivably contribute directly to the activation of TGF-β. In particular, myofibroblast transformation mediated by TGF-β requires NAD4-generated H2O2 (33). Moreover, ROS are capable of activating inflammatory and fibrogenic mediators directly, such as MCP-1 and TGF-β, respectively (7), which may also contribute to inflammatory and fibrogenic responses (19).

**Potential Treatment Options Targeting ROS in HFpEF**

Antioxidants to scavenge ROS in heart cells. The treatment approaches presented in this review will emphasize the hypothetical mechanism regarding the elevated OS and disrupted NO-cGMP-PKG pathway in HFpEF (Fig. 1). To date, research is gradually recognizing the likely importance of OS in HFpEF pathophysiology (40, 54). The potentially significant involvement of ROS-mediated cellular signaling in HFpEF development could provide a new approach to treating the disease with antioxidants. Studies have emerged that examine different antioxidants and their ability to relieve OS and restore antioxidant capacity (3). For instance, ubiquinol is of great interest in cardiovascular studies due to its high antioxidant potency (3). It is the fully reduced form of coenzyme Q10 (CoQ10), a key component of the ETC that exists in three redox states. Ubiquinol is capable of reducing oxidative damage and myocardial inflammation by removing reactive electrons from ROS, whereas the oxidized state, ubiquinone, is less effective (3). It is suggested that reduced formation of ubiquinol, either due to less efficient reduction of ubiquinone or less biosynthesis of CoQ10, is responsible for dysfunctional mitochondria and the resulting impaired myocardial energetics in HFpEF. Tsai et al. illustrated in human endothelial cells how ubiquinol could ameliorate excessive ROS production while limiting oxidative damage (59). Huynh et al. also demonstrated the capability of ubiquinol in abating O2•− generation (23). Moreover, treatment with CoQ10 enhances NO bioavailability in human endothelial cells by recovering eNOS expression (59), which is essential for proper endothelial function and protection of the vasculature (15). The only obstacle is that for CoQ10 to be effective, it needs to be retained in its fully reduced ubiquinol form. This requires enzymatic maintenance to catalyze the reduction (addition of electrons) of ubiquinone into ubiquinol, thereby ensuring the effectiveness of the treatment. Although the effect of ubiquinol supplementation in improving myocardial function is hopeful, additional clinical trials are needed to confirm its benefits in HFpEF patients (Table 1) (3).
Table 1. Potential therapeutics for heart failure with preserved ejection fraction: targeting reactive oxygen species and nitric oxide

<table>
<thead>
<tr>
<th>Category</th>
<th>Name</th>
<th>Effects</th>
<th>Ref No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant</td>
<td>Ubiquinol</td>
<td>Diminish oxidative damage and inflammation</td>
<td>3, 23, 59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Curtail excess ROS production</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drawback: must be retained in its reduced form</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trans-resveratrol</td>
<td>Enhance endogenous antioxidant expression</td>
<td>15</td>
</tr>
<tr>
<td>NO enhancer</td>
<td>Apocynin</td>
<td>Inhibit Nox function</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Ubiquinol</td>
<td>Restore eNOS expression</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Trans-resveratrol</td>
<td>Elevate eNOS levels via eNOS mRNA stabilization, BH₄ elevation, and inhibit Rho GTPase geranylgeranylation</td>
<td>1, 15</td>
</tr>
<tr>
<td>Exercise therapy</td>
<td>Folic acid</td>
<td>Reverse uncoupling of NOS</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Preconditioning</td>
<td>Enhance endogenous antioxidants</td>
<td>17, 25, 42, 67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induce cardiac mitochondrial adaptations</td>
<td>17, 25, 42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Combat myocardial injury</td>
<td>25</td>
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NO, nitric oxide; BH₄, tetrahydrobiopterin; eNOS, endothelial nitric oxide synthase; Nox, NADPH oxidase; ROS, reactive oxygen species; SOD, superoxide dismutase.

**Trans-resveratrol**, commonly found in red grapes and wine, is a phytoalexin that has been investigated as a treatment supplement for a wide variety of diseases. Natural compounds such as *trans*-resveratrol exert their main protective effects via enhancing the expression of endogenous antioxidants, including superoxide dismutase (SOD), catalase, and glutathione peroxidase, whereas its direct ROS-scavenging ability holds little therapeutic advantage (15). Additionally, eNOS uncoupling has been shown to be reduced in human endothelial cells with *trans*-resveratrol application, further stimulating NO production (43). These beneficial effects fit well within the HFpEF pathway as we have described; therefore, *trans*-resveratrol may be an attractive candidate as a natural treatment option for HFpEF patients (Table 1).

Although its antioxidant scavenging ability is not as robust as the others listed, apocynin is a treatment option that aims to stop ROS production at the source: by inhibiting Nox activity. As a well-documented inhibitor of Nox, apocynin has been used in many studies to examine the function of Nox, as well as the therapeutic benefits of Nox inhibition (Table 1). A study by Roe et al. showed how reduced maximal velocity, fractional shortening, and peak shortening caused by diabetic cardiomyopathy could be reversed with apocynin treatment. However, there was little or no effect on heart shape and size (49). In addition, apocynin demonstrates cardioprotective effects by reversing cardiac fibrosis and attenuating OS in an angiotensin II-induced hypertensive mouse model (31). Although there are some controversies surrounding its use as a Nox inhibitor, apocynin is generally accepted to function as an effective regulator of endothelial ROS production (22, 31). Therapies focused on apocynin for reducing OS in the heart are another promising treatment option.

Despite the benefits provided by numerous antioxidants described above, other studies have demonstrated that antioxidant therapy has little to no effect on cardiovascular function. Indeed, the Heart Outcomes Prevention Evaluation saw no effect for long-term vitamin supplementation (e.g., vitamins C and E) in cardiovascular disease relief (6). In addition, Omenn et al. reported no significant preventive effect for β-carotene during a 12-yr study regarding a population with high cardiovascular risks (38). It is likely that enormous amounts of antioxidants are required to influence cellular redox status, which could be a drawback for clinical application. To our best knowledge, antioxidant therapy remains controversial for prevention against cardiovascular diseases (37).

**Therapeutics targeting eNOS and NO bioavailability.** In addition to CoQ₁₀ and *trans*-resveratrol described as above, several compounds have demonstrated therapeutic effects for the prevention of eNOS uncoupling and the upregulation of eNOS expression (15). Excess O₂⁻ generated by uncoupled eNOS via BH₄ cofactor deficiency has been observed in various cardiovascular diseases, including HFpEF. As such, therapies that lead to enhanced NO bioavailability and reduced ROS in the endothelium hold potentially beneficial and protective opportunities (15, 47). As a commonly prescribed medication for cardiovascular disease, statins contain cholesterol-imparing properties that are shown to reduce both morbidity and mortality (4, 15). Similar to *trans*-resveratrol, statins enhance SOD activity and suppress Nox activity in treated subjects. This impedes O₂⁻ formation in the endothelium, a proposed mechanism that may induce the HFpEF paradigm. Also mimicking *trans*-resveratrol, eNOS expression benefits from statin treatment (15). By eNOS mRNA stabilization, BH₄ level elevation, and inhibition of Rho GTPase geranylgeranylation, statins are able to increase eNOS levels and NO bioavailability (Table 1) (1, 15). Interestingly, the findings of Boutilir et al. have suggested that the small increases of ROS following statin treatment can induce mitochondrial biogenesis via peroxisome proliferator-activated receptor-γ coactivator 1 (PGC-1) activation (4). The PGC-1 signaling pathway is a crucial regulator in cardiac metabolism that can effectively reestablish homeostasis under OS by improving antioxidant capacity and stabilizing eNOS (4). The beneficial effects of statins in experimental models seem promising; however, randomized controlled trials examining the efficacy of statins have shown mixed results, and further clinical investigation is necessary (32).

Roe et al. investigated the cardioprotective potential of folic acid due to its ability to recouple NOS (48). Insulin-resistant mice were fed either a high-starch or high-sucrose diet for 8 wk, followed by folic acid in drinking water or placebo for 4 wk. Increased O₂⁻ and ONOO⁻ levels were observed in the myocardium of mice provided with a sucrose diet, along with the decreased level of NO. Folic acid treatment reversed NO uncoupling, and the resulting increase in NO carried multiple beneficial effects that ultimately restored heart function (Table 1). It is reasonable therefore to consider the results of this study in the context of HFpEF given that NOS uncoupling is a plausible cause of cardiac dysfunction in both diabetes and HFpEF (48). By restoring endothelial NO levels, alleviating OS, and reversing endothelial dysfunction, these drugs provide

*J Appl Physiol* • doi:10.1152/japplphysiol.01149.2014 • www.jappl.org
potential protective effects against diseases associated with ROS-mediated pathology, including HFpEF.

**Exercise preconditioning.** Exercise may be considered the oldest therapeutic intervention to treat or prevent diseases in the history of mankind (17). Tai Chi Chuan, more commonly known as Tai Chi, is a Chinese martial art with ancient origins. A review by Kuramoto elaborated on Tai Chi’s ability to alleviate arthritic pain, reduce blood pressure, and improve aerobic capacity (27). Exercise not only promotes overall health, but many studies have also proposed the idea that exercise preconditioning protects organs that are readily damaged by OS or other stresses (17). Production of ROS and inflammatory cytokines are elevated in the heart during exercise, a process that mimics the pathophysiology of various cardiovascular-related diseases (17, 67), as well as the proposed novel paradigm of HFpEF (40). The so-called cardio-protection induced by exercise preconditioning is attributed to receptor-associated signaling cascades such as protein kinase C (PKC)-dependent mechanisms that lead to transcriptional modifications, including upregulation of endogenous antioxidants (e.g., SOD 2; MnSOD) and mitochondrial adaptations (e.g., mitochondrial potassium ATP channels) (17, 42). Yamashita et al. identified the activation of MnSOD upon the interaction with ROS, TNF-α, and IL-1β, whose levels were elevated after exercise (67). Accordingly, Frasier et al. showed enhanced glutathione replenishment in response to ROS via an exercise-induced redox-dependent alteration in glutathione reductase (18). Studies have indicated the effectiveness of exercise preconditioning in resisting myocardial injury through various mechanisms such as cardiac mitochondria adaptation and increased antioxidant capacity, as mentioned above (Table 1) (17, 25, 42). Therefore, prevention of HF development, including HFpEF, via exercise preconditioning may be a possible approach, although additional studies are required.

In conclusion, because of the increasing prevalence in the population, understanding HFpEF pathology at a molecular level has become paramount to developing and testing new therapies. Many studies have pointed to the role of ROS in the development of HFpEF. The proposed novel paradigm of HFpEF explains that the inflammatory cascade induced by comorbidities associated with HFpEF is a likely cause of excessive ROS production and subsequent high nitrosative/OS in the myocardium. ROS can lead to reduced NO bioavailability and NO/cGMP/PKG signaling impairment, leading to cardiac remodeling and dysfunction in patients with HFpEF. These cardiac modifications also contribute to exercise intolerance and reduced quality of life in HFpEF patients. Because of insufficient understanding of HFpEF, treatments typically rely on the management of comorbidities and symptoms. Therefore, therapies surrounding the hypothetical concept proposed by Paulus and Tschöpe, such as reducing ROS production, increasing NO bioavailability, and expanding exercise capacity, may be beneficial in treating HFpEF. Antioxidants such as ubiquinol and NO-boosting statins have been investigated for their ability to restore redox homeostasis in the diseased heart. Aside from pharmacotherapies, exercise preconditioning has similarly generated promising results in enhancing heart health. As more investigations advance the understanding of ROS and NO in the development of HFpEF, the future of HFpEF management looks promising.

**ACKNOWLEDGMENTS**

We thank Benjamin Pannell, Andrew Graef, and Tingyang Zhou for assistance during manuscript preparation.

**DISCLOSURES**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**AUTHOR CONTRIBUTIONS**

Author contributions: L.Z. conception and design of research; L.Z., C.-C.C., and B.T.H. prepared figures; L.Z., C.-C.C., B.T.H., and T.M.B. drafted manuscript; L.Z., C.-C.C., B.T.H., and T.M.B. edited and revised manuscript; L.Z., C.-C.C., B.T.H., and T.M.B. approved final version of manuscript.

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ROS and NO in HFrEF

Zuo L et al.


