

Oxidative stress and heart failure

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Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. *Am J Physiol Heart Circ Physiol* 301: H2181–H2190, 2011. First published September 23, 2011; doi:10.1152/ajpheart.00554.2011.—Oxidative stress, defined as an excess production of reactive oxygen species (ROS) relative to antioxidant defense, has been shown to play an important role in the pathophysiology of cardiac remodeling and heart failure (HF). It induces subtle changes in intracellular pathways, redox signaling, at lower levels, but causes cellular dysfunction and damage at higher levels. ROS are derived from several intracellular sources, including mitochondria, NAD(P)H oxidase, xanthine oxidase, and uncoupled nitric oxide synthase. The production of ROS is increased within the mitochondria from failing hearts, whereas normal antioxidant enzyme activities are preserved. Chronic increases in ROS production in the mitochondria lead to a catastrophic cycle of mitochondrial DNA (mtDNA) damage as well as functional decline, further ROS generation, and cellular injury. ROS directly impair contractile function by modifying proteins central to excitation-contraction coupling. Moreover, ROS activate a broad variety of hypertrophy signaling kinases and transcription factors and mediate apoptosis. They also stimulate cardiac fibroblast proliferation and activate the matrix metalloproteinases, leading to the extracellular matrix remodeling. These cellular events are involved in the development and progression of maladaptive myocardial remodeling and failure. Oxidative stress is also involved in the skeletal muscle dysfunction, which may be associated with exercise intolerance and insulin resistance in HF. Therefore, oxidative stress is involved in the pathophysiology of HF in the heart as well as in the skeletal muscle. A better understanding of these mechanisms may enable the development of novel and effective therapeutic strategies against HF.

heart failure; remodeling; oxidative stress; reactive oxygen species; mitochondria

HEART FAILURE (HF) is defined as a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood (18, 36). Cardiac manifestations of HF are fluid retention, which leads to pulmonary congestion and peripheral edema, as well as low output, which may limit exercise capacity (18, 36). HF is a leading cause of morbidity and mortality in industrialized countries (30, 31, 104, 110). It is also a growing public health problem, mainly because of aging of the population and the increase in the prevalence of HF in the elderly (109).

The major causes of HF are myocardial infarction (MI), hypertension, cardiomyopathy, and valvular heart disease (109). Following MI, the heart usually adapts through a pathophysiological process known as “cardiac remodeling,” which involves changes in the structure and function of cardiac myocytes as well as the extracellular matrix in the noninfarcted myocardium. These changes lead to substantial alterations in the shape and volume of the heart and progressive ventricular dilatation and impairment of pump function (24, 78). The mechanisms responsible for the development and progression of HF are the subject of intensive investigation. Alterations of

various signaling pathways, including the sympathetic nervous and renin-angiotensin-aldosterone systems have been shown to exert profound effects on the phenotype of the failing myocardium (67). In parallel to these basic findings, a number of clinical studies as well as registry data demonstrated the clinical benefits of medications targeting on these systems such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, aldosterone antagonists, and β -blockers on the clinical outcomes of HF patients (15a, 29, 75, 80, 81, 103, 105). Despite these extensive studies, the fundamental mechanisms responsible for the development and progression of HF have not yet been fully elucidated.

Over the past several decades, clinical and experimental studies have provided substantial evidence that oxidative stress, defined as an excess production of reactive oxygen species (ROS) relative to antioxidant defense, is enhanced in HF (9, 34, 35, 62). Excessive ROS cause cellular dysfunction, protein and lipid peroxidation, and DNA damage and can lead to irreversible cell damage and death, which have been implicated in a wide range of pathological cardiovascular conditions. The importance of oxidative stress is increasingly emerging with respect to a pathophysiological mechanism of cardiac remodeling responsible for the development and progression of HF (100). Specifically, ROS can directly impair contractile function by modifying proteins central to excitation-contraction coupling. Moreover, ROS activate a broad variety of hypertrophy signaling kinases and transcription factors and

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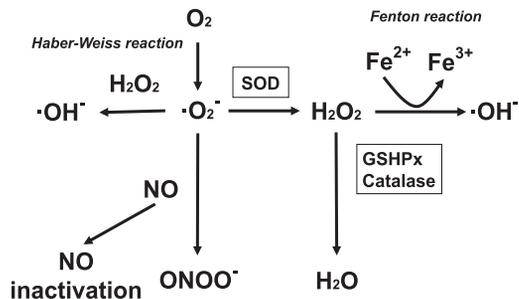


Fig. 1. Reactions underlying the generation and degradation of reactive oxygen species. A small amount of $O_2^{\cdot-}$ is normally produced as a byproduct of the use of molecular oxygen during mitochondrial oxidative phosphorylation. $O_2^{\cdot-}$ is inactivated by either nitric oxide (NO) or superoxide dismutase (SOD). A family of SOD enzymes rapidly converts $O_2^{\cdot-}$ to H_2O_2 , which is itself broken down by glutathione peroxidase (GSHPx) and catalase to water. Under pathological conditions, the single-electron reduction of H_2O_2 may lead to the formation of highly reactive OH radicals, either via the Fenton reaction in the presence of iron or via Haber-Weiss reaction by reacting with $O_2^{\cdot-}$. Furthermore, the reaction of $O_2^{\cdot-}$ with NO results in the inactivation of cytoprotective NO and the formation of peroxynitrite ($\cdot ONOO^-$).

mediate apoptosis. They also stimulate cardiac fibroblast proliferation and activate the matrix metalloproteinases (MMPs), leading to the extracellular matrix remodeling. These cellular events are involved in the development and progression of maladaptive myocardial remodeling and failure.

Generation of ROS and Antioxidants

The balance between ROS production and their removal by antioxidant systems is the “redox state.” Oxidative stress is defined as an excess production of ROS relative to the levels of antioxidants. ROS are oxygen-based chemical species with high reactivity. They include free radicals, such as superoxide ($O_2^{\cdot-}$) and hydroxyl radical ($\cdot OH$), and nonradicals capable of generating free radicals, such as hydrogen peroxide (H_2O_2) (Fig. 1). $O_2^{\cdot-}$ is a primary radical that could lead to the formation of other ROS, such as H_2O_2 and $\cdot OH$. $\cdot OH$ is also generated by the reduction of H_2O_2 in the presence of endogenous iron by means of the Fenton reaction. In addition, $\cdot OH$ could arise from electron exchange between $O_2^{\cdot-}$ and H_2O_2 via the Haber-Weiss reaction. Furthermore, when both $O_2^{\cdot-}$ with NO are synthesized within a few cell diameters, they will combine spontaneously to form peroxynitrite ($\cdot ONOO^-$) by a diffusion-limited reaction (74).

NO is necessary for normal cardiac physiology in the regulation of cardiac function, including coronary vasodilatation, inhibition of platelet and neutrophil adhesion and activation, and modulation of cardiac contractile function (100). NO also has a protective role against the ischemic and/or failing heart. This protective role is mediated by several mechanisms, including the stimulation of soluble guanylyl cyclase, which leads to a decrease of the concentration of intracellular Ca^{2+} , and the inhibition of oxidative stress. Therefore, $O_2^{\cdot-}$ can exert cytotoxic effects not only due directly to $O_2^{\cdot-}$ itself but are mediated by the inactivation of cytoprotective NO and the formation of highly reactive oxidant $\cdot ONOO^-$, which is produced following interaction of NO with $O_2^{\cdot-}$ (Fig. 1).

Diverse specific and nonspecific antioxidant defense systems exist to scavenge and degrade ROS to nontoxic molecules. Under physiological conditions, their toxic effects can be

prevented by such scavenging enzymes as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase, as well as by other nonenzymatic antioxidants (Fig. 1). GSHPx is a key antioxidant that catalyzes the reduction of H_2O_2 and hydroperoxides. It not only scavenges H_2O_2 but also prevents the formation of other more toxic radicals such as $\cdot OH$. GSHPx possesses a higher affinity for H_2O_2 than catalase. Furthermore, it is present in relatively high amounts within the heart, especially in the cytosolic and mitochondrial compartments (57). These lines of evidence imply the primary importance of GSHPx as a defense mechanism within the heart. Moreover, GSHPx is expected to exert greater protective effects against oxidative damage than SOD because greater dismutation of $O_2^{\cdot-}$ by SOD may result in an increase of H_2O_2 . In fact, the mice with GSHPx gene overexpression were more resistant to myocardial oxidative stress as well as remodeling and failure (65, 94).

When the production of ROS exceeds the capacity of antioxidant defense, oxidative stress has a harmful effect on the functional and structural integrity of biological tissue (Fig. 2). Specifically, in the heart, excess ROS can cause myocardial remodeling, including contractile dysfunction and structural alterations.

Oxidative stress has also been suggested as major mechanisms causing endothelial dysfunction not only in atherosclerosis but also in HF (56). Clinical studies suggested that endothelial dysfunction was independently associated with adverse long-term outcomes in patients with HF (47).

Increased ROS in the Failing Heart

A number of experimental and clinical studies have demonstrated the increased generation of ROS in HF (9, 34, 35, 62). The majority of experimental studies using various kinds of animal models of HF, including of our own, were performed in young animals with no coexisting risk factors such as hypertension. However, they have consistently provided substantial evidence that oxidative stress is increased in HF and contributes to its development and progression. Therefore, we consider that oxidative stress is increased not only in patients with

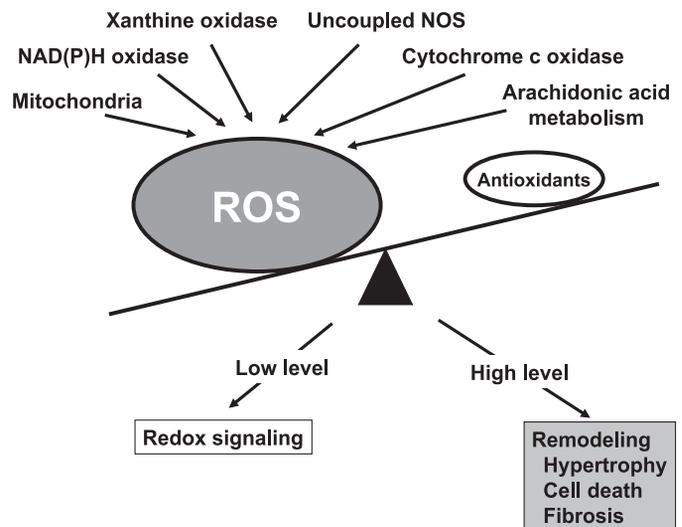


Fig. 2. Enzymatic sources of reactive oxygen species (ROS) and their pathophysiological role. NOS, nitric oxide synthase.

HF but in animal models even though they only mimic the part of clinical HF phenotypes seen in patients. In this review, our studies used mainly two types of animal models of HF: rapid pacing-induced HF in dogs and HF following MI (postinfarct HF) in mice. Both animals show similar structural and functional/hemodynamic characteristics to those in patients with HF. Belch et al. (9) reported that there was a significant negative correlation between malondialdehyde and left ventricular (LV) ejection fraction ($r = -0.35$). Mallat et al. (62) demonstrated that levels of lipid peroxides and 8-iso-prostaglandin $F_{2\alpha}$, the major biochemical markers of ROS generation, were elevated in the plasma and pericardial fluid of patients with HF and also positively correlated with its severity.

Electron spin resonance (ESR) spectroscopy combined with the nitroxide radical 4-hydroxy-2,2,6,6-tetramethyl-piperidine-*N*-oxyl provided a definitive and direct evidence for enhanced generation of ROS within the failing myocardium (38). The generation of $\bullet\text{OH}$ implies a pathophysiological significance of ROS in HF because $\bullet\text{OH}$ radicals are the predominant oxidant species causing cellular injury.

Oxidative stress results from an imbalance between ROS generation and antioxidant defense mechanisms. Therefore, impaired antioxidant defense mechanisms (SOD, catalase, and GSHPx) or reduced concentrations of endogenous antioxidants (vitamin E, ascorbic acid, and glutathione) can increase ROS levels. Previous studies by Hill and Singal (35) demonstrated that HF subsequent to MI was associated with an antioxidant deficit as well as increased oxidative stress. Furthermore, these changes correlated with the hemodynamic function, suggesting their role in the pathogenesis of cardiac dysfunction (35). In contrast, there was no decrease in the activities of the scavenging enzymes, including SOD and catalase. GSHPx activity was even increased in the heart obtained from pacing-induced HF (107). Our results indicated that oxidative stress in HF might be primarily due to the enhancement of ROS generation rather than to the decline in antioxidant defense within the heart.

Sources of ROS in the Failing Heart

The cellular sources of ROS generation within the heart include cardiac myocytes, endothelial cells, and neutrophils. Within cardiac myocytes, ROS can be produced by several sources, including mitochondria, NAD(P)H oxidase, xanthine oxidase, and uncoupled nitric oxide synthases (NOS) (Fig. 2).

Mitochondria produce ROS through a single electron transport to molecular oxygen in the respiratory chain (Fig. 3). Under physiological conditions, small quantities of ROS are formed during mitochondrial respiration, which, however, can be detoxified by the endogenous scavenging mechanisms. By using ESR spectroscopy with 5,5'-dimethyl-1-pyrroline-*N*-oxide as a spin trap, the inhibition of electron transport at the sites of complex I and complex III in the normal submitochondrial particles resulted in a significant production of $\text{O}_2^{\bullet-}$ (39). Mitochondria from the failing heart produced more $\text{O}_2^{\bullet-}$ than normal mitochondria in the presence of NADH, indicating that mitochondrial electron transport could be the predominant source of such $\text{O}_2^{\bullet-}$ production. Furthermore, the failing mitochondria were associated with a decrease in complex enzyme activity. Therefore, mitochondria are an important source of

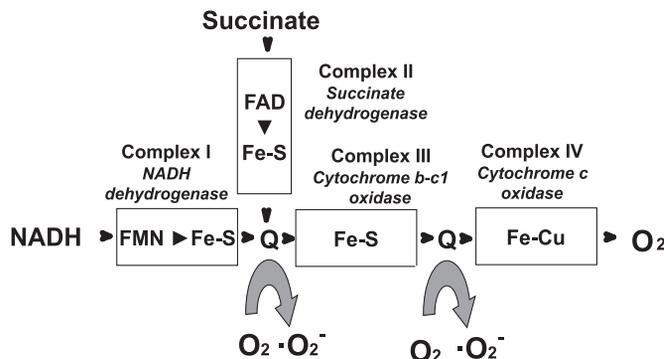


Fig. 3. Mitochondrial electron transport. Localized in the inner mitochondrial membrane, the mitochondrial electron transport chain is formed by a series of cytochrome-based enzymes (complex I: NADH dehydrogenase; complex III: cytochrome *b-c1* oxidase; complex IV: cytochrome oxidase and the smaller molecules coenzyme Q[Q]) that transfer the electrons to molecular oxygen. The transport starts with the transfer of e^- from NADH⁺ to the iron-sulfur (Fe-S) center of NADH dehydrogenase, which passes them to Q, complex III, cytochrome *c*, complex IV, and finally to molecular oxygen. FADH₂ donates its e^- directly to Q, and the transfer proceeds as above. During this process, the high free energy of the electrons is gradually extracted and converted into ATP. Physiologically, >98% of e^- are tightly coupled with the production of ATP, and only 1–2% “leak” to form $\text{O}_2^{\bullet-}$ and are scavenged by mitochondrial SOD. However, when the electron chain transfer is blocked at the level of complex I or III, e^- are inappropriately diverted by one electron reduction directly to O_2 , with the resulting formation of a large amount of $\text{O}_2^{\bullet-}$. NADH, nicotinamide adenine dinucleotide; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide.

ROS in failing hearts, indicating a pathophysiological link between mitochondrial dysfunction and oxidative stress (88). Within the mitochondria, most of the oxygen is reduced to water at the respiratory chain. Therefore, when oxygen availability is reduced in conditions such as ischemia or hypoxia, mitochondrial formation of ROS is increased, which can contribute to the induction of myocyte damage or MI (77).

ROS can be generated also via NAD(P)H oxidase and/or xanthine oxidase in the vascular endothelial cells as well as via NAD(P)H oxidase in activated leukocytes. Each member of the NAD(P)H oxidase family contains a catalytic unit termed Nox that forms a heterodimer with a lower-molecular-weight subunit called p22^{phox}; this heterodimeric cytochrome is the site of electron transfer from NAD(P)H to molecular O_2 , resulting in the formation of $\text{O}_2^{\bullet-}$. Five Nox isoforms (Nox1–5) have been identified, each encoded by separate genes and forming the basis of different NAD(P)H oxidases (54). Nox1 and Nox2 require the association of cytosolic regulatory subunits (p47^{phox}, p67^{phox}, p40^{phox}, and Rac) with the cytochrome to activate $\text{O}_2^{\bullet-}$ production. In contrast, Nox4 activation does not require these cytosolic subunits. Nox1 is highly expressed in vascular smooth muscle cells but not in cardiac myocytes or endothelial cells. In contrast, Nox2 is abundantly expressed in cardiac myocytes, endothelial cells, and fibroblasts. Nox4 is the most widely expressed isoform in endothelial cells, cardiac myocytes, and fibroblasts. Importantly, NADPH oxidase activity has been shown to be significantly increased by several stimuli that are relevant to the pathophysiology of HF, e.g., mechanical stretch, angiotensin II, endothelin-1, and tumor necrosis factor- α , acting both through posttranslational modification of oxidase regulatory subunits and transcriptional pathways (58). Bauersachs et al. (7) demonstrated increased vascular NAD(P)H oxidase activities and $\text{O}_2^{\bullet-}$ production in HF.

An increase in myocardial NAD(P)H oxidase activity has also been observed in human HF (33). By using mice lacking p47^{phox} (p47^{phox}^{-/-} mice), Doerries et al. (20) demonstrated that a deficiency of the NAD(P)H oxidase protected the heart from LV remodeling and dysfunction after MI. Doughan et al. (21) provided the direct evidence that angiotensin II could mediate mitochondrial dysfunction via the activation of NAD(P)H oxidases in vascular endothelial cells. Angiotensin II increased mitochondrial ROS production, which was associated with decreased endothelial NO[•] bioavailability. Therefore, among five Nox isoforms, Nox2 and Nox4 are the main isoforms in the diseased myocardium. Recent studies have demonstrated that Nox4, localized primarily within the mitochondria in cardiac myocytes, is responsible for enhanced ROS production and cardiac remodeling due to pressure overload and aging, thereby playing an important role in mediating cardiac dysfunction (2, 52). The role of Nox5 has not yet been clarified in HF.

Increased xanthine oxidase expression and activity were also reported in HF (11). Furthermore, LV contractile function and myocardial efficiency were improved by the treatment of HF animals with the xanthine oxidase inhibitor allopurinol (111). In addition, chronic treatment of animals following experimental MI with allopurinol significantly reduced adverse LV remodeling (68). These detrimental effects of xanthine oxidase might involve, at least in part, the inactivation of NO because it could reduce myocardial O₂ consumption and improve cardiac efficiency (50).

Uncoupled NOS can potentially lead to further ROS production via the oxidation of the essential NOS cofactor BH₄ (55). NOS3 [endothelial NOS (eNOS)] has been shown to be uncoupled and functionally important in cardiovascular pathological remodeling including HF (100). Under normal conditions, NOS3 consumes NADPH and generates NO and L-citrulline from L-arginine and O₂. When exposed to oxidative stress or when deprived of BH₄ or L-arginine, NOS3 becomes structurally unstable and generates ROS. It is unclear which cell type contributes mostly to ROS generated by NOS3 uncoupling. However, given that NOS3 is expressed in vascular endothelial cells and cardiac myocytes within the heart, these cells are well expected to be involved in this process. Uncoupled NOS3 has been shown to contribute to LV remodeling in response to chronic pressure overload in mice (99). Mice subjected to transverse thoracic aortic constriction had reduced BH₄ levels and uncoupling of eNOS in association with LV dilatation and contractile dysfunction, which could be partially inhibited by BH₄ treatment. In contrast, Ruetten et al. (85) reported that eNOS^{-/-} mice subjected to aortic constriction developed worse contractile function, greater hypertrophy, and more interstitial fibrosis. Similarly, Scherrer-Crosbie et al. (89) reported that post-MI LV remodeling was more extensive in eNOS^{-/-} mice. The reasons for these discrepant results remain unclear; however, it may be partly due to the opposing effects of NO and ROS derived from uncoupled NOS on cardiac hypertrophy and fibrosis.

Cytochrome *c* oxidase (COX), the terminal oxidase of the mitochondrial electron transport chain (complex IV), is composed of 13 subunits. The subunits COX I, II, and III are encoded by a single mitochondrial gene. COX I and II belong to the catalytic core, which is key for the assembly and the function of the complex. We have shown that the enzyme

activity of electron transport chain complex I, III, and IV all decreased in mice subjected to MI (37). Wu et al. (115) also demonstrated that COX III overexpression resulted in a decreased abundance of COX I and a decrease in COX activity, accompanied by increased apoptosis in HF following MI.

The contribution of leukocytes has been suggested in the generation of ROS based on the findings that plasma levels of myeloperoxidase (MPO) correlated with the severity of HF and were independent predictors of outcomes in these patients (101). Plasma MPO indicates MPO mass in plasma as a marker of heightened leukocyte activation rather than systemic inflammation.

Oxidative Stress and Mitochondrial DNA Damage

ROS can damage mitochondrial macromolecules either at or near the site of their formation. Therefore, in addition to the role of mitochondria as a source of ROS, the mitochondria themselves can be damaged by ROS. Mitochondria have their own genomic system, mitochondrial DNA (mtDNA), a closed-circular double-stranded DNA molecule of ~16.5 kb. mtDNA contains 2 promoters, the light-strand (LSP) and heavy-strand promoters from which transcripts are produced and then processed to yield the individual mRNAs encoding 13 subunits of the oxidative phosphorylation, including 7 subunits (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6) of rotenone-sensitive NADH-ubiquinone oxidoreductase (complex I), 1 subunit (cytochrome *b*) of ubiquinol-cytochrome *c* oxidoreductase (complex III), 3 subunits (COI, COII, and COIII) of COX (complex IV), and 2 subunits (ATPases 6 and 8) of complex V along with 22 tRNAs and 2 rRNA (12S and 16S) subunits (4, 92). Transcription from the LSP also produces RNA primer, which is necessary for initiating mtDNA replication (Fig. 4) (14). Mitochondrial function is controlled by the mtDNA as well as by factors that regulate mtDNA transcription and/or replication such as mitochondrial transcription factor A (Fig. 4) (45).

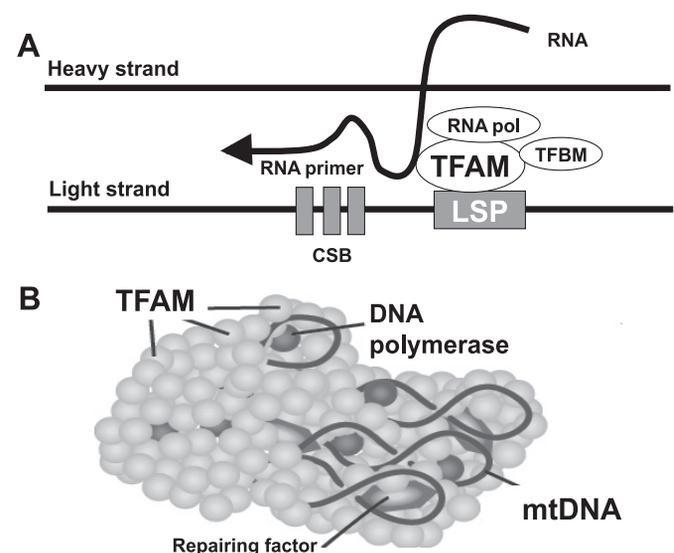


Fig. 4. Role of mitochondrial transcription factor A (TFAM) in mitochondrial DNA (mtDNA) replication (A) and maintenance (B). TFBM, mitochondrial transcription factor B; CSB, conserved sequence block; LSP, light-strand promoter.

The mtDNA could be a major target for ROS-mediated damage for several reasons. First, mitochondria do not have a complex chromatin organization consisting of histone proteins, which may serve as a protective barrier against ROS. Second, mtDNA has a limited repair activity against DNA damage. Third, a large part of $O_2^{\cdot -}$, formed inside the mitochondria, is unable to pass through the membranes and, hence, ROS damage occurs largely within the mitochondria. In fact, mtDNA accumulates significantly higher levels of the DNA oxidation product, 8-hydroxydeoxyguanosine, than nuclear DNA (26). As opposed to nuclear-encoded genes, mitochondrial-encoded gene expression is largely regulated by the copy number of mtDNA (113). Therefore, mitochondrial injury is reflected by mtDNA damage as well as by a decline in the mitochondrial RNA (mtRNA) transcripts, protein synthesis, and mitochondrial function (5).

Increased generation of ROS in the failing hearts was associated with mitochondrial damage and dysfunction, characterized by an increased lipid peroxidation in the mitochondria, a decreased mtDNA copy number, a decrease in the number of mtRNA transcripts, and a reduced oxidative capacity due to low complex enzyme activities (37). They thus can lead to a catastrophic cycle of mitochondrial functional decline, further ROS generation, and cellular injury. There is now a consensus view that the abnormalities in mtDNA replication/transcription as well as repair occur not only in a limited small subset of mitochondrial diseases but also in a more common form of HF phenotype such as post-MI and cardiomyopathy (40, 59, 64, 98, 108).

Oxidative Stress in Myocardial Remodeling

The tightly regulated production of relatively low levels of ROS is involved in modulating the activity of diverse intracellular molecules and signaling pathways, "redox signaling," with the potential to induce highly specific regulation in the cellular phenotype (Fig. 2) (22).

Alternatively, oxidative stress has direct effects on cellular structure and function and may activate integral signaling molecules in myocardial remodeling and failure (Fig. 5). Oxidative stress stimulates myocardial growth, matrix remodeling, and cellular dysfunction, which involve the activation of several downstream signaling pathways. First, ROS activate a broad variety of hypertrophy signaling kinases and transcription factors (86). ROS stimulate the tyrosine kinase Src, GTP-binding protein Ras, protein kinase C, mitogen-activated protein kinases (MAPK), and Jun-nuclear kinase (JNK). Low levels of H_2O_2 are associated with MAPK activation and protein synthesis, whereas higher levels stimulate MAPK, JNK, p38, and protein kinase B (Akt) kinases to induce apoptosis (53). Second, ROS induces apoptosis, another important contributor to remodeling and dysfunction, which is induced by ROS-mediated DNA and mitochondrial damage and activation of proapoptotic signaling kinases (12). Third, ROS cause DNA strand breaks, activating the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1). PARP-1 regulates the expression of a variety of inflammatory mediators, which facilitate the progression of cardiac remodeling. Fourth, ROS can activate MMPs, a family of proteolytic enzymes (97). MMPs are generally secreted in an inactive form and are activated posttranslationally by ROS from targeted interactions with critical cysteines in the propeptide autoinhibitory domain. ROS also stimulate transcription factors nuclear factor- κ B, Ets, and activator protein-1 to stimulate MMP expression. MMPs play a pivotal role in normal tissue remodeling processes, such as cell migration, invasion, proliferation, and apoptosis. MMP activity has been shown to be increased in the failing hearts (16, 97). Furthermore, an MMP inhibitor can limit LV dilatation after an experimental MI (84). We have shown significant improvement in survival after MI in MMP-2 knockout mice, which was mainly attributable to the inhibition of early cardiac rupture and the development of subsequent LV remodeling and failure (32). Because MMP can be activated by ROS, one

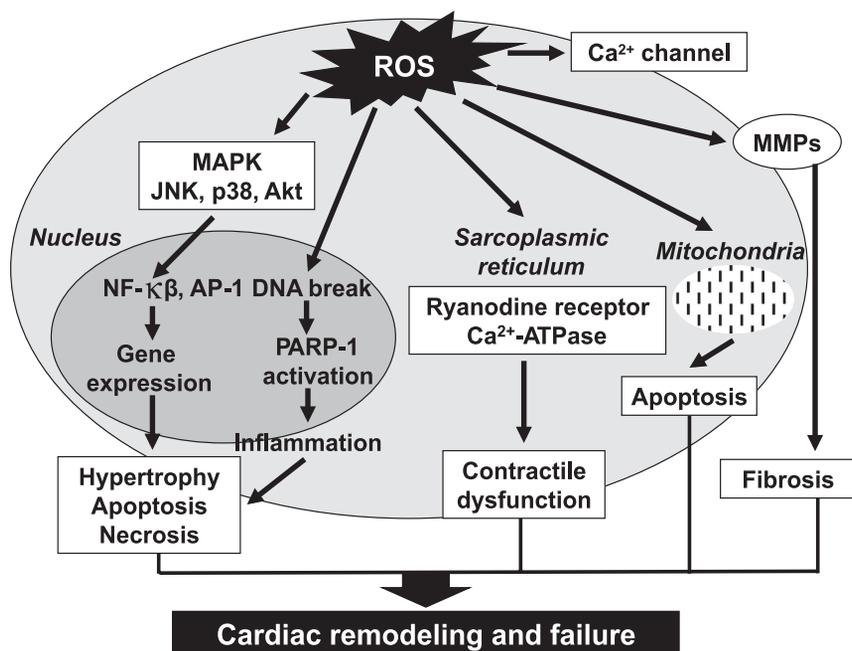


Fig. 5. Potential cellular and subcellular targets of oxidative stress relevant to heart failure (HF). MAPK, mitogen-activated protein kinases; JNK, Jun-nuclear kinase; PARP-1, poly(ADP-ribose) polymerase-1; MMPs, matrix metalloproteinases; AP-1, activator protein-1.

proposed mechanism of LV remodeling is the activation of MMPs secondary to increased ROS (82). Sustained MMP activation might influence the structural properties of the myocardium by providing an abnormal extracellular environment with which the myocytes interact. An $\bullet\text{OH}$ scavenger, dimethylthiourea, inhibited the activation of MMP-2 in association with the development of LV remodeling and failure after MI (51). These findings raise the possibility that enhanced oxidative stress can be a stimulus for myocardial MMP activation, which plays an important role in the development and progression of HF. Finally, ROS directly influence contractile function by modifying proteins involved in excitation-contraction coupling (117). This includes modification of critical thiol groups ($-\text{SH}$) groups on the ryanodine receptor to enhance its open probability, the suppression of L-type calcium channel, and oxidative interaction with Ca^{2+} ATPase in the sarcoplasmic reticulum to inhibit Ca^{2+} uptake.

Oxidative Stress in Aging, Hypertension, and Diabetes Mellitus

Oxidative stress is highly relevant to aging and the development of various aging-related cardiovascular diseases, including HF. However, the involvement of specific forms of ROS and each antioxidant and/or ROS-producing enzymes in the process of aging remain obscure. Neither overexpression nor heterozygous knockout of mitochondrial SOD affected lifespan in mice (42, 112). In contrast, in transgenic mice overexpressing catalase in the mitochondria, maximal lifespan was extended by 20%, and aging-associated cardiac pathology was significantly delayed (90).

There is also substantial evidence that ROS generation is increased in hypertension (102). Moreover, the concomitant increase in myocardial ROS production was accompanied by the transition from compensated hypertrophy to failure in Dahl salt-sensitive rats fed by high-salt diet (106).

Insulin resistance and diabetes mellitus have been well known to adversely affect the development and progression of HF (41, 46). Indeed, the prevalence of diabetes in patients with HF is higher than in subjects without HF (15, 79). Diabetes mellitus often leads to HF, even in the absence of any other risk factors such as coronary artery disease or hypertension, suggesting that diabetes itself causes a specific form of cardiomyopathic state (8). It causes myocardial structural remodeling characterized by myocyte hypertrophy, interstitial fibrosis, and apoptosis (23), which increases cardiac muscle stiffness and may contribute to diastolic dysfunction. Diastolic dysfunction has been regarded as a hemodynamic hallmark in diabetes and ultimately contributes to the development of HF (1, 69).

A growing body of evidence suggests that the production of ROS is increased in the diabetic heart (43). Specifically, ROS are generated within the mitochondria from the diabetic heart (44). ROS impair pro-survival signaling pathways such as Akt in diabetic hearts and activates proinflammatory and cell death pathways such as NF- κB and the nuclear enzyme PARP-1, which in turn regulate the expression of proinflammatory cytokines, cell adhesion molecules, and inducible NOS (83). Overexpression of GSHPx could attenuate diastolic dysfunction, myocyte hypertrophy, and interstitial fibrosis in diabetic heart (65). These findings are consistent with previous studies demonstrating that ROS are involved in the structural altera-

tions of the extracellular matrix collagens (72). Another important impact of diabetes mellitus in HF is the exacerbation of systolic dysfunction after MI. Previous clinical studies demonstrated that patients with diabetes had a worse outcome after MI than that without diabetes despite similar coronary patency and baseline LV function (27). Poor outcomes in patients with diabetes have been shown to be due to the progression of HF (3). Experimental studies demonstrated that hyperglycemia induced by streptozotocin exaggerates LV remodeling and failure after MI (93, 95). Similar to type 1 diabetes, LV remodeling and failure after MI were exacerbated also in high-fat diet-induced type 2 diabetes (66, 116).

Insulin resistance can occur as a consequence of HF (49, 76, 114). Patients with symptomatic dilated cardiomyopathy, excluding previously diagnosed type 2 diabetes, showed the abnormal response compared with healthy subjects by oral glucose tolerance tests (114) or the euglycemic-hyperinsulinemic clamp technique (49). Insulin resistance has been recognized also in several animal models of HF. Myocardial glucose uptake was decreased with the development of HF in a pacing-induced dog model (70, 71). Myocardial insulin resistance was due to the impairment of insulin signaling and associated with the decrease in ATP concentration. Liao et al. (60) demonstrated that the glucose tolerance was abnormal in mice with cardiac hypertrophy and HF due to pressure overload. Moreover, the control of postprandial hyperglycemia by α -glucosidase inhibitor could ameliorate cardiac hypertrophy and slow the progression to HF. These findings suggest that HF itself can cause insulin resistance, which may lead to the further exacerbation of HF.

Very little information has been available for the mechanisms responsible for the abnormalities in insulin signaling in the skeletal muscle from HF. Previous studies reported that serine phosphorylation of Akt was decreased in the skeletal

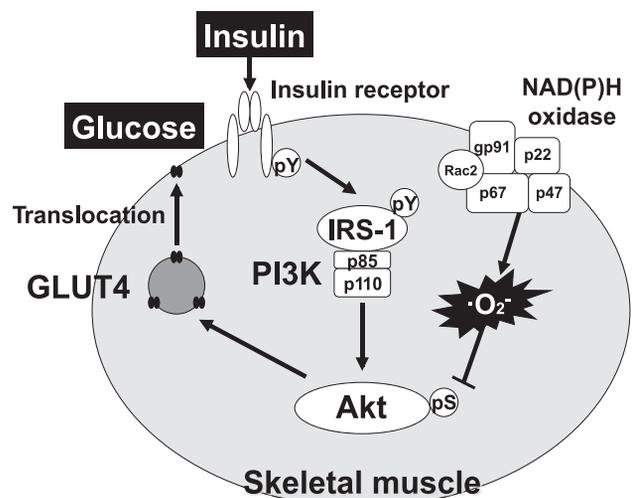


Fig. 6. Role of NAD(P)H oxidase-derived superoxide in the impairment of insulin signaling in the skeletal muscle. Insulin receptor, insulin receptor substrate-1 (IRS-1), phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), and translocation of glucose transporter-4 (GLUT4) to plasma membrane from cytosol are involved in the insulin signaling in the skeletal muscle. Serine phosphorylation of Akt and GLUT4 translocation is impaired in insulin-stimulated skeletal muscle isolated from HF, which was consistent with the attenuation of changes in blood glucose after insulin load. NAD(P)H oxidase-derived superoxide impairs serine phosphorylation of Akt and GLUT4 translocation in the skeletal muscle with HF.

muscle from a HF model of post-MI (91). Another report showed that serine phosphorylation of Akt and glucose transporter-4 (GLUT4) translocation was decreased in the myocardial tissue from a pacing-induced HF model (71). The similar impairment of insulin signaling was observed in both heart and skeletal muscle obtained from HF, indicating that systemic factors may be involved for this abnormality. We recently found that whole body insulin resistance was induced in a murine HF model of post-MI, which was accompanied by the impaired insulin signaling in the skeletal muscle, specifically the decreases in serine phosphorylation of Akt and GLUT4 translocation (Fig. 6) (73). Importantly, NAD(P)H oxidase inhibitor significantly ameliorated insulin resistance as well as the impaired insulin signaling in the skeletal muscle. ROS production via NAD(P)H oxidase leads to the impairment of insulin signaling and glucose uptake in the skeletal muscle also in type 2 diabetes (96).

Clinical Perspectives

There were clinical studies reported that examined the effects of various antioxidants on HF (87). The vitamin antioxidants α -tocopherol (vitamin E) and ascorbic acid (vitamin C) scavenge ROS and prevent free radical chain reactions and have been studied extensively in HF. α -Tocopherol levels were decreased, and dietary supplements of α -tocopherol exerted a therapeutic effect in animal models of HF (17). Short-term vitamin E supplementation reduced the levels of oxidative stress biomarkers also in patients with HF (25). However, no significant effects were proved on symptoms or clinical outcomes (48). Moreover, large-scale clinical trials reported that the long-term supplementation of vitamin E exerted no effects on primary prevention of cardiovascular events and was even associated with increased risk of developing HF (61, 63).

Xanthine oxidase inhibition with allopurinol is expected to be beneficial based on the findings that uric acid, the product of xanthine oxidoreductase, was increased in the failing human heart and was associated with poor outcomes (28). In fact, xanthine oxidase inhibition with allopurinol has been shown to improve endothelial as well as cardiac function in HF (13, 19). However, there were little effects of xanthine oxidase inhibition on clinical endpoints in HF patients except for modest improvement in symptoms in the subgroup of increased uric acid levels. Moreover, various drugs, including ACE inhibitors, β -blockers such as carvedilol, and statins, may directly or indirectly modulate oxidative stress in the cardiovascular system. However, further work will be needed to determine whether any of these drugs have beneficial therapeutic effects on human HF.

Oxidative stress markers such as plasma-oxidized low-density lipoproteins, malondialdehyde and MPO (an index of leukocyte activation), urinary biopyrrins (oxidative metabolites of bilirubin), and plasma and urine isoprostane levels are expected to provide important information regarding the pathogenesis of HF or the identification of subjects at risk for HF, the future risk stratification, the diagnosis, or monitoring therapy of HF as biomarkers (10).

Conclusion

To improve the prognosis of patients with HF, we need to develop therapeutic strategies based on a novel insight into the

pathophysiology of HF. The approach of regulating oxidative stress in the heart as well as in the skeletal muscle may contribute to establish the effective treatment strategies against HF. Therefore, therapeutic strategies to modulate this maladaptive response should become a target for future extensive investigation.

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DISCLOSURES

Conflict of interest: none declared

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