Congestive heart failure is a devastating clinical syndrome characterized by progressive decline in cardiac performance with chamber dilation and multiple abnormalities of molecular signaling that adversely affect cell function and survival.\(^1\) Nearly 6 million Americans experience congestive heart failure, and more than 250,000 individuals die annually.\(^2\) Despite major advances in medical management, a large number of patients with advanced heart failure (HF) are refractory to optimal medical therapy, and HF remains one of the leading causes of death worldwide.\(^2,3\) Importantly, left ventricular dyssynchrony resulting from an intraventricular conduction delay is present in \(\approx30\%)\) of HF patients and is considered an independent predictor of adverse cardiovascular outcomes in patients with HF.\(^3\) Dyssynchrony exacerbates HF, causing cardiac inefficiency, as well as pathological alterations at the organ, cellular, biochemical, and molecular levels.

In addition to optimal medical therapy, device therapy has gained acceptance to treat left ventricular dyssynchrony in selected patients. Importantly, cardiac resynchronization therapy (CRT) has emerged as a well-established therapy for advanced HF because it prolongs survival, improves symptoms, and increases exercise capacity in patients with HF (New York Heart Association class II/IV), left ventricular ejection fraction \(\leq35\%)\) and QRS duration of \(\approx120\) ms in electrocardiogram.\(^2,3\) Interestingly, the greatest improvements with CRT are reported in patients with a nonsymmetric cause of HF.\(^6\) Although one third of CRT recipients are nonresponders, CRT is safe and efficacious even in patients receiving optimal medical therapy.\(^4\)

Oxidative stress is an important pathogenic feature of many cardiovascular diseases, including coronary atherosclerosis, myocardial infarction, and HF.\(^5,7\) Chronic oxidative/nitrite stress can lead to oxidative cardiomyopathy due to altered intracellular \(Ca^{2+}\) homeostasis, mitochondrial damage, oxidative modification of essential cardiac contractile proteins, and direct cardiac toxicity of reactive oxygen species (ROS). Redox imbalance also reduces the bioavailability of nitric oxide (NO) in the cardiovascular system. Importantly, oxidative stress can alter the expression levels of different intrinsic antioxidant defense mechanisms. The excessive cardiac oxidative stress associated with ventricular remodeling can be amplified by other sources. Oxidative stress can act in a feed-forward fashion to boost superoxide production by xanthine oxidoreductase, NO synthase (NOS), and the mitochondrial respiratory chain.\(^7\) Furthermore, ROS/reactive nitrogen species, by oxidizing the cofactor tetrahydrobiopterin, can induce an “uncoupling” of NOS, switching NOS from its classical NO generation to superoxide generation.\(^8,9\) Importantly, redox-sensitive gene expression is involved in the development and progression of both early and late ventricular remodeling. Thus, tissue redox status might play an important regulatory role in the overall outcome of novel HF treatment.

The heart has the highest energy demand of any tissue and relies on mitochondrial metabolism to provide the chemical energy needed for beat-to-beat cardiac function.\(^10,11\) The high-energy molecule adenosine triphosphate (ATP) is required for cell viability and myocardial pump function. Mitochondrial metabolism produces ATP by oxidative phosphorylation that involves the coupling of oxygen reduction to phosphorylation of ADP into ATP. During the development of HF, the heart undergoes extensive metabolic remodeling, and the failing heart is characterized as an “engine out of fuel” because ATP is \(\approx30\%)\) lower than in normal myocardium and the rate of fall of ATP progresses because of the loss of the adenine nucleotide pool.\(^10\) Mitochondria from failing hearts produce more ROS than normal mitochondria in the presence of NADH, indicating a link between mitochondrial dysfunction and oxidative stress.\(^11\) Thus, in addition to being a source of ROS, mitochondria can be damaged by ROS.

Growing evidence suggests that the reversible oxidative modification of protein thiols plays a critical role in cellular redox regulation and signaling, whereas irreversible modifications lead to cellular injury and death (Figure 1).\(^12-14\) Because the sulphydryl group of cysteine is a redox-sensitive center in proteins, the oxidation of these protein thiols would be expected under oxidative stress, such as occurs during ischemia/reperfusion injury and heart failure. In cells, glutathione (GSH), a tripeptide containing glutamate, cysteine, and glycine, is the most abundant low-molecular-weight thiol. With an increase in oxidants under oxidative stress, disulfide bond formation between a protein thiol and GSH, ie, \(S\)-glutathionylation, is a potential product of oxidative reactions.\(^15\) Two major mechanisms of \(S\)-glutathionylation have been proposed.\(^16\) Thiol-disulfide exchange with the oxidized
disulfide of glutathione is believed to be the major mechanism for S-glutathionylation. In addition, as an alternative mechanism, ROS- and reactive nitrogen species–derived thiol radicals of protein thiols can, in turn, react with GSH to generate protein S-glutathionylation.17

In this issue of Circulation Research, Wang et al18 demonstrate that CRT can increase mitochondrial ATP synthase activity through the reversal of oxidative posttranslational modification (Ox-PTM) of its specific subunits, and they showed this to enhance ATP synthesis. The authors further showed that the formation of disulfide bonds and S-glutathionylation within ATP synthase complexes contributes to the dysfunction of ATP synthase from dysynchronous HF, and these modifications were reversed by CRT treatment. Here, it is conceptually important to understand whether CRT treatment enhances mitochondrial antioxidant defense systems or increases the cellular and mitochondrial reducing status, as reflected by the ratio of GSH to the oxidized disulfide of glutathione. ROS-induced protein thyl radicals can react with GSH or vicinal cysteine thiols, resulting in protein S-glutathionylation or disulfide bond formation, an efficient mechanism for protein thiol oxidative modification.17 If CRT treatment increases mitochondrial antioxidants, such as superoxide dismutase or catalase, the increase in antioxidants can prevent the formation of ROS-induced protein thiol radical intermediates, which will lead to S-glutathionylation or disulfide bond formation. Mitochondria have the highest GSH pool, and an increase in GSH correlates with the reversed process of protein thiol oxidative modification. Mitochondrial ATP synthase cannot independently produce ATP without a fully functional electron transport chain; therefore, it will be important to know how CRT modulates electron transport chain function.

Ox-PTM and its reversal have been shown to play a critical role in regulating many protein functions under disease conditions.14,19 In this study, CRT treatment induced S-nitrosation of ATP synthase. As such, the authors hypothesize that S-nitrosation might reverse the impaired function of ATP synthase and positively regulate its function, leading to improved ATP synthase function. Moreover, mass spectrometry revealed that Cys244 and Cys294 of ATPα and Cys103 of ATPγ are involved in these Ox-PTMs. Cys294 is actively involved in various oxidative modifications, including interdisulfide bond formation, S-glutathionylation, and S-nitrosation (Figure 2). Site-directed mutagenesis of these Cys residues confirmed that Cys244 and Cys294 are required for the functionality of ATP synthase and play a critical role in redox regulation of ATP production. These observations of S-nitrosation of ATP synthase lead to several interesting questions related to the causative or mechanistic relationship between mitochondrial remodeling after CRT and molecular changes. First, how does this process of S-nitrosation occur? What fraction of the ATPase is S-nitrosated? Does S-nitrosation under some conditions lead to S-glutathionylation, whereas under others, S-glutathionylation is converted to S-nitrosation?

An appealing mechanism that may trigger the alterations seen in HF and benefits of CRT therapy may be through redox-posttranslational modification of endothelial NOS in the heart. It has recently been shown that oxidative stress triggers S-glutathionylation, which causes endothelial NOS uncoupling, switching endothelial NOS from its classical NO synthase function to that of an NADPH-dependent oxidase generating superoxide.13 It is possible that increased oxidative stress in HF might result in endothelial NOS S-glutathionylation, uncoupling the enzyme and leading to a lack of NO production with further oxidative stress. This might contribute to the decrease in thiol nitrosylation and increase in S-glutathionylation seen on ATP-synthase and perhaps also other critical proteins as well. Thus, the critical modulations of thiol posttranslational modifications of ATP synthase with HF and their reversal with CRT are likely part of a larger process of redox protein modification and thus provide us with important insight into the role of redox stress on the molecular basis of HF and the efficacy of CRT to improve it.

Recent clinical studies have yielded insights into the molecular and cellular phenotype of dysynchronous HF. Vanderheyden et al20 showed that CRT responders are accompanied by favorable changes in established molecular markers of HF, including genes that regulate contractile function and pathological hypertrophy. Ukkonen et al21 reported that CRT improves myocardial efficiency without increasing global left ventricular oxidative metabolism. Although simply restoring electric synchrony with CRT greatly improves global cardiac function, energetics, and molecular and cellular phenotype, unfortunately, one third of patients treated with CRT, those with a previous history of myocardial infarction or coronary artery disease, did not respond clinically.22

Wang et al18 have shown a benefit of CRT in reversing mitochondrial Ox-PTM with improved ATP production. Although these findings expand the authors’ and others’ earlier reports,23,24 the major step forward in the present study is their effort to understand the molecular mechanisms of CRT.

![Figure 1. Oxidative modification of protein thiols. Under oxidative stress, protein thiols are subject to either reversible or irreversible oxidative modification. Reversible modifications, S-nitrosation, S-glutathionylation, disulfide bond, and cysteine sulfenic acid formation play important roles in cellular redox regulation. When cellular reducing status returns to normal, these are reversed. For irreversible modification, protein thiols are oxidized to sulfonic and sulfenic acid, leading to loss of protein function and protein degradation, which can trigger cellular apoptosis or necrosis. ROS indicates reactive oxygen species; RNS, reactive nitrogen species; Pr, protein.](http://circres.ahajournals.org/doi/10.1161/CIRCRESAHA.117.311021)
in metabolic remodeling of dyssynchronous HF based on Ox-PTM of the mitochondria. Although these studies demonstrate efficacy of CRT in a canine model of nonischemic HF, the tachypacing-induced dyssynchronous HF model does not mimic all features of human HF because the changes in myocardial structure occurring with tachypacing are dissimilar to clinical HF caused by chronic ischemia, myocardial infarction, or hypertensive heart disease. The different types of cardiomyopathy at different stages of progression with variable electric and structural remodeling could be critical in the overall outcome of CRT. Moreover, effects of HF medications were not investigated in this model. Although these issues remain to be addressed, Wang et al18 in their model have demonstrated a beneficial effect of CRT in ameliorating mitochondrial Ox-PTM for improved ATP production that is necessary for cardiac function. Future studies will be required to further elucidate the role and mechanism of Ox-PTM in HF and how this process can be modulated to enhance the efficacy of treatment and beneficial reverse remodeling in patients.

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Figure 2. Oxidative posttranslational modification of ATP synthase in dys- synchronous heart failure (DHF) and effect of cardiac resynchronization ther- apy (CRT). In DHF, Cys244 and Cys294 of the α-subunit are S-glutathionylated (2), or interdisulfide bonding occurs between Cys294 of α-subunits, as well as between Cys294 and Cys103 of the γ-subunit (3). S-Glutathionylation and interdisulfide formation of ATP synthase inhibit its ATP synthesis, leading to mito- chondrial energy starvation and dysfunc- tion. CRT increases S-nitrosation of ATP synthase at Cys103 of the γ-subunit and Cys294 of the α-subunit (4), along with ATPase activity. NO\textsuperscript{·} indicates nitroso- dium ion; GSH, glutathione; GSSG, oxi- dized GSH; RSNO, S-nitrosothiols.


**KEY WORDS:** cardiac resynchronization therapy ■ oxidative stress ■ mitochondrial ATP synthase ■ nitric oxide ■ S-glutathionylation ■ S-nitrosation
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