Copper Futures
Ceruloplasmin and Heart Failure
Dian J. Cao, Joseph A. Hill

Oxidative stress is a major mechanism contributing to heart failure (HF) pathogenesis. On one hand, oxidative reactions are central to a wide range of signaling cascades, both physiological and pathological. Such redox signaling events participate in the governance of myocyte plasticity, ion homeostasis, excitation–contraction coupling, and metabolism. However, high levels of oxidative stress can damage proteins, membranes, and DNA. There is great interest in understanding mechanisms whereby oxidative events contribute to disease pathogenesis, because clinical trials of antioxidant therapies in HF have disappointed.

One mechanism whereby reactive oxygen species contribute to disease pathophysiology is via post-translational modification of specific proteins.1 One such modification is tyrosine nitration. Tyrosine nitration is a covalent coupling of protein tyrosine residues with nitric oxide (NO)–derived oxidants. Three major sources of NO-derived reactive species have been identified: (1) peroxynitrite anion, formed as the product of NO metabolism and superoxide radicals; (2) (myelo)peroxidase-catalyzed nitrogen dioxide radical (NO2−), a product of hydrogen peroxide and nitrite; and (3) nitrogen dioxide radical derived from nitric oxide in oxygenated buffers used in vitro experimentation.

Although protein nitration has been recognized for a long time, its functional role in vivo is poorly understood. A wide variety of proteins involved in cardiovascular physiology are targets of tyrosine nitration, and the functional outcome for the targeted protein once modified is diverse, ranging from inactivation, which is most common, to gain of function. Proteins in the plasma, arterial wall, mitochondria, and sarcoplasm, many of which are involved in atherogenesis and vascular function, can be targeted. Some evidence suggests that protein nitration at tyrosine residues may serve as a marker of atherosclerotic heart disease.1 Nitrification of tyrosine 294/295 in sarcoendoplasmic reticulum calcium ATPase has been linked with diminished activity.4 Tyrosine nitration inhibits prostacyclin synthase in endothelial cells, thereby promoting inflammation.5 Site-specific nitration of apolipoprotein A-I at tyrosine 166 is abundant in human atherosclerotic coronary artery but nearly undetectable in normal coronary arteries.6 Nitration at tyrosine 192 in apolipoprotein A-I by myeloperoxidase has been linked to transforming high-density lipoprotein into a more atherogenic molecule and loss of its protective function.7 In each case, the functional implications of these events in vivo remain unclear. Also, tyrosine nitration can be detected in the basal physiological state, suggesting roles in normal homeostasis.

Ceruloplasmin (“blue substance from plasma”) is a copper-containing circulating protein first isolated in 1948,8 deficiency of which underlies Wilson disease. Synthesized and secreted by hepatocytes, ceruloplasmin accounts for 95% of total copper in the circulation and is a member of the evolutionarily ancient family of multicopper oxidases. Enzymes in this family oxidize substrates by accepting electrons at the copper centers, which is followed by reducing oxygen into water. Studied now for >60 years, several functions have been attributed to ceruloplasmin, and new roles continue to be identified.9 Among them, ceruloplasmin is the major source of serum ferroxidase I activity. Ferroxidase I is a copper-dependent oxidase capable of donating an electron to reduce free radicals and other species and catalyzing the conversion of oxidizing ferrous iron (Fe2+) into less toxic ferric iron (Fe3+). Thus, ceruloplasmin contributes to both oxidative and reactive events,10 including oxidation of lipids and nitric oxide.9,11

In this issue of Circulation Research, Cabassi et al12 investigated a possible association between ceruloplasmin and progression of HF. In a carefully designed and conducted study, the authors enrolled 96 stable patients with chronic HF with a mean age of 76 years and with a moderate preponderance of HF with preserved (61%) as opposed to reduced (39%) ejection fraction (EF). The major cause of HF was ischemic (81%). Thirty-five age-matched controls were also enrolled.

Subjects were divided into tertiles based on serum ferroxidase I activity and followed for a 2-year period, tracking clinical end points of all-cause mortality and frequency and duration of hospitalization. The investigators measured levels of total serum nitrated protein, nitrated ceruloplasmin, and ferroxidase I activity. Furthermore, ex vivo and in vitro experiments using serum samples from control subjects or commercially available purified ceruloplasmin, respectively, were performed to test the notion that peroxynitrite, one of the most powerful nitro-oxidative species, suppresses ceruloplasmin ferroxidase activity.

Several interesting findings emerged. For one, both total circulating nitrated proteins and nitrated ceruloplasmin were increased in patients with HF compared with control subjects. In contrast, ferroxidase I activity was decreased in the...
HF group. In fact, patients in the lowest tertile of ferroxidase activity were marked by the most advanced HF, as defined by lower EF and higher B-type natriuretic peptide levels. Patients in the lowest tertile of ferroxidase activity also manifested the greatest mortality at 2 years: 64% (tertile I) versus 29% (tertile III).

The inverse correlation between serum ferroxidase I activity and all-cause mortality in patients with HF is novel and interesting. That said, it tracked with powerful markers of bad outcome, including depressed EF and elevated B-type natriuretic peptide levels; whether ferroxidase I activity will emerge as an independent prognostic factor is unknown.

Given its central role in nitroso-oxidative events, it is not surprising that ceruloplasmin itself is subject to reactive oxygen species modification. Ceruloplasmin has 6 tyrosine residues that can be affected by tyrosine nitration. In the study by Cabassi et al., exposure of ceruloplasmin to peroxynitrite triggered ceruloplasmin tyrosine nitration and reduced ferroxidase I activity. However, whether oxidative stress–mediated tyrosine nitration of ceruloplasmin plays a mechanistic role in HF progression is unknown. Whether ferroxidase I activity and nitrated ceruloplasmin could serve as surrogate markers of overall oxidative stress remains to be determined. However, it is intriguing to speculate that these measures could be used to monitor the effectiveness of HF therapy, or even further, to design and tailor targeted interventions.

Whereas this interesting study is suggestive of a novel role of ceruloplasmin/ferroxidase I, it important to note that tertile III, with the highest ferroxidase I activity, highest EF, and a preponderance of female subjects, harbored an over-representation of patients with HF with preserved EF. Are differences in the events reported here confounded by differences in the distribution of patients with HF with preserved EF and reduced EF? Supporting this notion is the authors’ finding that when B-type natriuretic peptide was incorporated in a multivariate model, the predictive value of ferroxidase I activity ceased to be statistically significant. Circulating B-type natriuretic peptide and ventricular EF are among the most powerful prognostic factors in HF, and both of these parameters were significantly different between tertiles I and III.

This study must be interpreted in light of a few caveats. As acknowledged by the authors, the selection criteria were appropriately strict, eliminating patients with conditions that could affect serum ceruloplasmin levels and ferroxidase activity, including diabetes mellitus, myocardial infarction within the past 20 weeks, chronic renal disease, thyroid disorders, and more. Generalizing these data beyond the cohort studied over the past 20 weeks, chronic renal disease, thyroid disorders, including diabetes mellitus, myocardial infarction within the past 20 weeks, chronic renal disease, thyroid disorders, and more. Generalizing these data beyond the cohort studied here would be required before envisioning significant real-world clinical impact.

The majority of the subjects had ischemic heart disease (81%). A recent study of 4177 patients undergoing elective coronary angiography reported increased incidence of major cardiovascular events (death, myocardial infarction, stroke) in subjects with higher ceruloplasmin levels. Intriguingly, a close association between protein nitration and coronary artery disease has been reported. Together, these reports raise the possibility that nitrated ceruloplasmin and impaired ferroxidase I activity may reflect global oxidative stress and serve simply as a barometer of overall atherosclerosis burden rather than a true reflection of the severity and progression of HF.

In an isolated heart model, ceruloplasmin was protective of ischemia/reperfusion injury by affording antioxidant activity. After myocardial infarction, ceruloplasmin levels increase transiently, consistent with an acute-phase response. Ceruloplasmin’s nitric oxide oxidase activity raises the possibility that its elevation may diminish nitric oxide bioavailability and promote endovascular dysfunction. The seemingly contradictory functional profile of ceruloplasmin—both pro-oxidant and antioxidant—will continue to complicate delineation of its physiological and pathophysiological roles.

Conclusions and Perspectives
HF, a syndrome in which the myocardium is unable to provide blood supply commensurate with the requirements of the body, continues to explode in incidence and prevalence. Work reported here by Cabassi et al. draws our attention to ceruloplasmin and its ferroxidase activity as potentially involved. Whether these are markers or mechanisms of HF pathogenesis remains to be determined. Either way, these findings expand the functionality of the already versatile ceruloplasmin molecule, raise the intriguing prospect of gauging redox stress in this syndrome, and point to a rise in (circulating) copper futures.

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References


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