Published in final edited form as:

J Cardiovasc Pharmacol. 2014 October ; 64(4): 393–400. doi:10.1097/FJC.0000000000000125.

ZINC AND THE PROOXIDANT HEART FAILURE PHENOTYPE

Nephertiti Efeovbokhan, MD¹, Syamal K. Bhattacharya, PhD¹, Robert A. Ahokas, PhD², Yao Sun, MD, PhD¹, Ramareddy V. Guntaka, PhD³, Ivan C. Gerling, PhD⁴, and Karl T. Weber, MD¹

¹Division of Cardiovascular Diseases, University of Tennessee Health Science Center, Memphis, TN

²Department of Obstetrics & Gynecology, University of Tennessee Health Science Center, Memphis, TN

³Department of Microbiology, Immunology & Biochemistry, University of Tennessee Health Science Center, Memphis, TN

⁴Division of Endocrinology, University of Tennessee Health Science Center, Memphis, TN

Abstract

Neurohormonal activation with attendant aldosteronism contributes to the clinical appearance of congestive heart failure (CHF). Aldosteronism is intrinsically coupled to Zn^{2+} and Ca^{2+} dyshomeostasis, in which consequent hypozincemia compromises Zn^{2+} homeostasis and Zn^{2+} -based antioxidant defenses that contribute to the CHF prooxidant phenotype. Ionized hypocalcemia leads to secondary hyperparathyroidism with parathyroid hormone-mediated Ca^{2+} overloading of diverse cells, including cardiomyocytes. When mitochondrial Ca^{2+} overload exceeds a threshold, myocyte necrosis follows. The reciprocal regulation involving cytosolic free $[\text{Zn}^{2+}]_i$ as antioxidant and $[\text{Ca}^{2+}]_i$ as prooxidant that can be uncoupled in favor of Zn^{2+} -based antioxidant defenses. Increased $[\text{Zn}^{2+}]_i$ acts as a multifaceted antioxidant by: *i*) inhibiting Ca^{2+} entry via L-type channels and hence cardioprotectant from the Ca^{2+} -driven mitochondriocentric signal-transducer-effector pathway to nonischemic necrosis; *ii*) serving as catalytic regulator of Cu/Zn-superoxide dismutase; and *iii*) activating its cytosolic sensor, metal-responsive transcription factor that regulates the expression of relevant antioxidant defense genes. Albeit present in subnanomolar range, increased cytosolic free $[\text{Zn}^{2+}]_i$ enhances antioxidant capacity that confers cardioprotection. It can be achieved exogenously by ZnSO_4 supplementation or endogenously, using a β_3 receptor agonist, (e.g., nebivolol) that enhances NO generation to release inactive cytosolic Zn^{2+} bound to metallothionein. By recognizing the pathophysiologic relevance of Zn^{2+} dyshomeostasis in the prooxidant CHF phenotype and by exploiting the pharmacophysiologic potential of $[\text{Zn}^{2+}]_i$ as antioxidant, vulnerable cardiomyocytes under assault from neurohormonal activation can be protected and the myocardium spared from adverse structural remodeling.

Correspondence: Karl T. Weber, M.D., Division of Cardiovascular Diseases, University of Tennessee Health Science Center, 956 Court Ave., Suite A312, Memphis, TN 38163, Tel: (901) 448-5750, Fax: (901) 448-8084, KTWeber@uthsc.edu.

Authors have no conflicts of interest to disclose.

Keywords

cytosolic free $[Zn^{2+}]_i$; cardiomyocytes; antioxidant defenses; cardioprotection; congestive heart failure

Introduction

Zinc is an essential micronutrient with crucial biologic functions in both contractile and noncontractile cells. Zn^{2+} -based regulatory actions can be categorized as catalytic, structural and regulatory (1). For example, Zn^{2+} catalyzes the activity of matrix metalloproteinases and angiotensin-converting enzyme, metalloenzymes expressed by inflammatory cells and myofibroblasts at sites of tissue repair. Zn^{2+} finger proteins serve as coordination complexes essential for the control of transcriptional and translational responses in which diminished $[Zn^{2+}]_i$ leads to protein misfolding and impaired function. Cytosolic free $[Zn^{2+}]_i$ in cardiomyocytes regulates the expression of its intracellular sensor, metal-responsive transcription factor (MTF)1.

Hypozincemia of varying severity, based on normal control values (75–140 $\mu\text{g/dL}$), has been widely reported in patients with congestive heart failure (CHF) irrespective of its etiologic origins (2–5). The most relevant underlying pathophysiologic mechanism for hypozincemia is renin-angiotensin-aldosterone system (RAAS) activation with renin-dependent aldosteronism, where a marked increase in fecal and urinary Zn excretion occurs and which can be blocked by an aldosterone receptor antagonist (6). Other factors contributing to hypozincemia in CHF include urinary Zn^{2+} losses associated with angiotensin-converting enzyme (ACE) inhibitor treatment (7) and reduced dietary Zn^{2+} intake and/or impaired small intestine absorption of Zn^{2+} (8). Resultant Zn^{2+} deficiency contributes to the systemic illness of CHF and its prooxidant phenotype and where increasing cardiomyocyte $[Zn^{2+}]_i$ functions as a multifaceted antioxidant to confer cardioprotection.

$[Zn^{2+}]_i$ contributes to the regulation of antioxidant defense genes by activating MTF1. Upon its translocation to the nucleus, MTF1 upregulates: metallothionein (MT)1, a Zn^{2+} binding protein and oxygen radical scavenger; glutathione synthase; and Cu/Zn-superoxide dismutase (SOD), a metalloenzyme and antioxidant (9, 10). $[Zn^{2+}]_i$ also plays a critical role as an endogenous competitive inhibitor to Ca^{2+} entry via L-type Ca^{2+} channels. Other contributions of Zn^{2+} in maintaining cardiomyocyte redox equilibrium include its catalytic regulation of Cu/Zn-SOD and its pool of inactive cytosolic Zn^{2+} , bound to MT1 cysteine clusters, which upon its release by eNOS-mediated action of nitric oxide provides for $[Zn^{2+}]_i$ -based defenses (11–13).

This review focuses on the hitherto less well-recognized aspects of Zn^{2+} dyshomeostasis in the CHF prooxidant phenotype and where increasing $[Zn^{2+}]_i$ serves as antioxidant and cardioprotectant. The role of $[Zn^{2+}]_i$ as an antioxidant in preventing ischemia/reperfusion injury and diabetic cardiomyopathy has been reported elsewhere (14–16).

PATHOPHYSIOLOGY OF CHF

CHF: A Prooxidant Phenotype

Neurohormonal Activation—The symptoms and signs of the CHF syndrome have their origins rooted in neurohormonal activation, where endocrine properties of circulating effector hormones of the RAAS and adrenergic nervous (ANS) systems orchestrate an unwanted salt-avid state. Another deleterious outcome to such inappropriate hormonal activation is a systemic illness, in which persistent circulating hormones affect such diverse tissues as skeletal muscle, bone and peripheral blood mononuclear cells (i.e., lymphocytes and monocytes) to adversely influence their behavior. It is the concerted endocrine actions of RAAS and ANS hormones that account for this systemic illness, one of whose major features is a *prooxidant phenotype* (17) in which the rate of reactive oxygen species generation in diverse tissues overwhelms their rate of detoxification at these sites by endogenous antioxidant defenses that include crucial Zn^{2+} -dependent enzymes.

Secondary Hyperparathyroidism (SHPT)—A contemporaneous dyshomeostasis of Zn^{2+} , Ca^{2+} and Mg^{2+} is present on admission in patients hospitalized with decompensated biventricular failure having a dilated cardiomyopathy of ischemic or nonischemic origins (3–5, 18–20). This includes hypozincemia, ionized hypocalcemia and hypomagnesemia, together with abnormal elevations (>65 pg/mL) in plasma parathyroid hormone (PTH). SHPT accompanies the chronic aldosteronism of CHF, where PTH becomes a crucial mediator of pathologic remodeling. SHPT arises because of ionized hypocalcemia and hypomagnesemia, thus providing major stimuli to the parathyroids' elaborated secretion of PTH (see Figure 1).

SHPT is invoked to restore extracellular cation homeostasis through PTH-mediated resorption of bone Ca^{2+} , Mg^{2+} and Zn^{2+} (21), while nephron-derived $1,25(\text{OH})_2\text{D}_3$ promotes their absorption from gut and kidneys (see Figure 1). PTH with its receptor-ligand binding property paradoxically leads to increased L-type Ca^{2+} channel activity and intracellular Ca^{2+} overloading involving various tissues (22–24). In the case of cardiomyocytes, adverse consequences of excessive intracellular Ca^{2+} accumulation include both cytosolic and mitochondrial domains. There follows the induction of oxidative stress by these organelles and ultimate necrosis of parenchymal cells with consequent tissue repair. Lost parenchyma and the appearance of microscopic scarring further compromise ventricular diastolic and systolic dysfunctions (25–29).

Oxidative Stress and Cardiomyocyte Necrosis

The failing heart is not spared the onslaught of circulating hormones (25–30). The myocardium undergoes a hormone-regulated structural and biochemical remodeling with progressive decline in contractile and diastolic functions (31, 32). This includes the ongoing prooxidant-based loss of cardiomyocytes and consequent appearance of reparative fibrosis that follows each bout of “dirty cell death,” or necrosis, to account for widely scattered foci of scarring found in the right and left heart. This contrasts to “sterile cell death,” or apoptosis, which does not evoke inflammatory cell-mediated tissue repair and, therefore, does not result in myofibroblast-based fibrous tissue formation (33).

Several sources account for the generation of reactive oxygen species in cardiomyocytes that includes superoxide and H_2O_2 : membrane-bound NADPH oxidase; cytosolic-based xanthine oxidase; and subcellular organelles with the all-important role of subsarcolemmal mitochondria accounting for the altered redox state of the failing heart (9, 10, 34). Increased production of superoxide in the explanted failing heart, either ischemic or nonischemic origins, is coupled to increased activities of antioxidant defenses that include metalloenzymes: Cu/Zn- or Mn-based superoxide dismutases; and Se-dependent glutathione peroxidase (GPX) (35–37).

A Mitochondriocentric Pathway to Necrosis—As conceptually depicted in Figure 2, the early PTH-mediated Ca^{2+} overloading seen during wks 1–3 of aldosteronism is coupled to an induction of oxidative stress, expressed as H_2O_2 production, by subsarcolemmal mitochondria. Oxidative stress appears as the rate of reactive oxygen species (ROS) generation and overwhelms their rate of detoxification by the cumulative capacity of antioxidant defenses. Mitochondrial Ca^{2+} overloading is a determinant of irreversible cell injury in particular when it exceeds a threshold (38) and as depicted in Figure 2. Together with membrane depolarization and ATP depletion, there ensues a nonphysiologic cyclophilin D-based opening of mitochondrial inner membrane permeability transition pore (mPTP) and osmotic swelling with structural and functional degeneration. This pathophysiologic scenario triggers the pronecrotic phenotype with cell death. It represents a *mitochondriocentric signal-transducer-effector pathway* to nonischemic cardiomyocyte necrosis operative during either acute hyperadrenergic or chronic stressor states (see Figure 3) (39).

Necrosis is followed by the spillage of cell contents, including the leakage of troponins, which appear in the circulation as a biomarker confirmatory of necrosis. Proteins released by necrotic cardiomyocytes serve as “danger signals” activating the immune system (33).

A series of site-directed, sequential pharmacologic interventions targeted along cellular-subcellular cascades were carried out to block downstream events leading to PTH-mediated intracellular Ca^{2+} overloading, oxidative stress and mPTP opening to salvage cardiomyocytes (reviewed in 40).

Zn²⁺ DYSHOMEOSTASIS IN STRESSOR STATES

Zinc, the body's sixth most abundant cation, is an essential micronutrient predominantly derived from dietary sources; it is present in most tissues, but stored at greater proportion in liver and skeletal muscle. Reduced antioxidant defenses can occur when dietary Zn intake is inadequate, excretory Zn^{2+} losses are excessive relative to intake, Cu/Zn-SOD is utilized in the dismutation of superoxide without adequate replenishment, or a combination of these scenarios (41).

Acute Stressor States

The importance of Zn^{2+} and Zn^{2+} deficiency was recognized by Prasad 50 years ago (42). In response to *dietary Zn²⁺ deficiency* plasma Zn^{2+} levels and tissue Zn^{2+} stores both decline and are associated with reduced tissue MT1, a cysteine-rich Zn^{2+} -binding protein involved

in Zn^{2+} homeostasis and free radical scavenging, together with reduced serum Cu/Zn-SOD activity. This contrasts to Zn^{2+} insufficiency seen with stressor states (43, 44). Rapid reductions in circulating Zn^{2+} occur in response to activation of the hypothalamic-pituitary-adrenal axis which accompanies *acute stressor states*, such as bodily injury, hyper- or hypothermia, acute myocardial infarction, or coronary artery bypass surgery (45–47). Hypozincemia can be reproduced by adrenocorticotropin hormone (ACTH) infusion. The degree of hypozincemia correlates with the severity of injury. Zn^{2+} is translocated to sites of injury and to liver for storage, facilitated by upregulated expression of MT1 (6, 21). In response to tissue injury involving heart, kidney or lung, where circulating Zn^{2+} levels decline while Zn^{2+} is redistributed to injured tissues based, in part, on MT1 upregulation, and where tissue Cu/Zn-SOD expression and activity are increased (48–51). At the site of injury, Zn^{2+} participates in wound healing, including: initiation of gene transcription and protein synthesis; immune cell replication and functions; and activation of metalloproteinases (6, 52). The hypokalemia and ionized hypocalcemia and hypomagnesemia seen on admission with acute hyperadrenergic states is based on catecholamine-mediated translocation of these cations to skeletal muscle and adipose tissue, respectively (reviewed in 53).

Chronic Stressor State

CHF is a *chronic stressor state*. The underlying pathophysiologic basis for hypozincemia in CHF is multifactorial. Most importantly is renin-dependent (or secondary) aldosteronism that accompanies RAAS activation, in which a marked increment in fecal and urinary Zn^{2+} excretion occurs (see Figure 1) and can be blocked by an aldosterone receptor antagonist (6). There is also marked excretory losses of Ca^{2+} and Mg^{2+} with ultimate appearance of ionized hypocalcemia and hypomagnesemia leading to SHPT (see Figure 1) (23, 54). Hyperparathyroidism itself raises urinary Zn^{2+} excretion (55, 56).

Other factors contributing to hypozincemia in CHF include: *i*) urinary Zn^{2+} losses associated with ACE inhibitor treatment (7); *ii*) reduced dietary Zn^{2+} intake and/or impaired small intestinal Zn^{2+} absorption (8); and *iii*) Zn^{2+} translocation for storage within the liver, muscle and bone with subsequent release for purposes of tissue repair, e.g., during recurrent episodes of myocyte necrosis (21, 52). Resultant Zn^{2+} deficiency contributes to the systemic illness of CHF by causing: *i*) prooxidant phenotype with oxidative stress-induced damage to cardiomyocytes, tissue DNA, proteins and lipids (57); and *ii*) an immunostimulatory state with activated immune cells elaborating proinflammatory cytokines.

Zn^{2+} TRAFFICKING IN CARDIOMYOCYTES

Nano level quantitation and intracellular Zn^{2+} trafficking studies have made possible the advent of fluorescent proteins and sensor fluorescence energy transfer spectroscopy to monitor cytosolic $[\text{Zn}^{2+}]_i$ and nuclear $[\text{Zn}^{2+}]_i$, together with total tissue Zn content by atomic absorption spectroscopy (54, 58, 59). Using a fluorescent dye, FluoZin-3, LeWinter et al. (60) identified Zn^{2+} entry via sarcolemmal Ca^{2+} channels to further implicate $[\text{Zn}^{2+}]_i$ as an endogenous competitive inhibitor of Ca^{2+} entry. Both extra- and intracellular cardiomyocyte Zn^{2+} are inhibitory of ATP-induced Ca^{2+} entry, including that which accompanies β -adrenergic receptor stimulation by catecholamines (61, 62).

Pathophysiologic responses in $[Zn^{2+}]_i$, as antioxidant, and $[Ca^{2+}]_i$, as prooxidant, are depicted in Figure 4.

More than 99% of total cardiomyocyte $[Zn]$ is inactive and bound to MT1; upon its release from MT1 $[Zn^{2+}]_i$ rises (60). Nitric oxide (NO) mobilizes cardiomyocytes Zn^{2+} from MT1-thiol clusters raising $[Zn^{2+}]_i$ as an antioxidant in the attenuation of cardiac mitochondrial oxidative damage following reperfusion (14). The multifaceted antioxidant profile of nebivolol, a β_1 blocker, relates to its dual action as agonist of β_3 adrenergic receptors, which, in turn, activates eNOS to enhance NO generation and raise $[Zn^{2+}]_i$, via its mobilization from the inactive MT-bound store (63).

Total Zn content of normal human heart is reported to be 20–30 mg/g wet tissue (64). This approximates our findings in normal rat and hamster myocardial tissue and reported as 82 ± 3 and 79 ± 5 ng/mg fat-free dried tissue, respectively (9, 59). The overall Zn content of cardiomyocytes is predominantly regulated by membrane-bound Zn^{2+} transporters: *i*) ZnT-based pumps which modulate Zn^{2+} efflux; and *ii*) Zip transporters that regulate Zn^{2+} influx (reviewed in 65). A lesser amount ($\approx 30\%$) enters via L-type Ca^{2+} channels (9). Cytosolic $[Zn^{2+}]_i$ is further regulated by its sensor, MTF1. The rise in $[Zn^{2+}]_i$ seen with aldosteronism is facilitated by the upregulation of membranous Zn^{2+} importers and exporters (9). Because of this tight regulation of $[Zn^{2+}]_i$, Zn^{2+} cytotoxicity is relatively uncommon.

Intrinsically Coupled Zn^{2+} and Ca^{2+} Dyshomeostasis

The dyshomeostasis of extra- and intracellular Zn^{2+} and Ca^{2+} that accompanies aldosteronism contributes to a deleterious but reversible dysequilibrium between them. Intrinsically coupled dyshomeostasis of intracellular Zn^{2+} and Ca^{2+} occurs spontaneously to regulate the integrated redox state of cardiomyocytes and mitochondria. These cations were monitored in their cytosolic free state using relevant dyes and fluorescence spectroscopy with ALDOST alone or in combination with either spironolactone or amlodipine cotreatment. As shown in Figure 5, aldosteronism was accompanied by increased total Zn and Ca in myocardial tissue (top panel), cardiomyocytes (middle panel), and in subsarcolemmal mitochondria (lower panel). Increased $[Ca^{2+}]_i$ and $[Zn^{2+}]_i$, together with elevated subsarcolemmal mitochondrial $[Ca^{2+}]_m$ and $[Zn^{2+}]_m$, could be prevented by spironolactone, acting to prevent SHPT, and significantly attenuated by amlodipine (9). These salutary iterations in divalent cation composition corroborated well with reduced biomarkers of oxidative stress in mitochondria and were coincident with increased activities of Cu/Zn-SOD and GPX (9, 39, 54). Adaptive modulation in $[Zn^{2+}]_i$ was accompanied by the contemporaneous upregulation of MT1, both a Zn^{2+} importer (Zip1) and exporter (ZnT-1), and MTF1.

The temporal responses in coupled Ca^{2+} and Zn^{2+} dyshomeostasis, reflecting the tenuous prooxidant:antioxidant equilibrium, indicate elevations in $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ are coupled with $[Zn^{2+}]_i$ and $[Zn^{2+}]_m$ and accompanied by increased mitochondrial H_2O_2 production, cardiomyocyte xanthine oxidase activity, and cardiac and mitochondrial 8-isoprostane levels (9). This prooxidant state is initially counterbalanced by increased activity of antioxidant proteins, enzymes, and the nonenzymatic antioxidants, or cumulative antioxidant capacity, as well as by the augmented antioxidant defenses. However, these defenses ultimately prove

inadequate in combating the persistent intracellular Ca^{2+} overloading and the consequential marked rise in cardiac tissue 8-isoprostane (39).

Uncoupling Zn^{2+} and Ca^{2+} Dyshomeostasis Confers Cardioprotection

Whether intrinsically coupled Ca^{2+} and Zn^{2+} dyshomeostasis and prooxidant:antioxidant dysequilibrium (9) seen with aldosteronism could be uncoupled in favor of antioxidants was examined using cotreatment with a ZnSO_4 supplement, pyrrolidine dithiocarbamate (PDTTC), a Zn^{2+} ionophore (see Figure 4), or ZnSO_4 in combination with a Ca^{2+} channel blocker, amlodipine (10). Cotreatment with ZnSO_4 alone, PDTTC alone, or ZnSO_4 plus amlodipine augmented the rise in cardiomyocyte $[\text{Zn}^{2+}]_i$ beyond that seen with aldosteronism, while attenuating the rise in $[\text{Ca}^{2+}]_i$ which together served to reduce biomarkers of oxidative stress in cardiomyocytes and their mitochondria that proved to be cardioprotective (10, 30, 54). ZnSO_4 supplement alone, however, does not reverse hypocalcemia and hypomagnesemia (54). Hence there is a need for a polycation supplement to correct the deficits of Zn^{2+} , Ca^{2+} and Mg^{2+} that arise because of their excessive excretory losses.

The extent to which the coupled dyshomeostasis between Zn^{2+} and Ca^{2+} of aldosteronism could be uncoupled by ZnSO_4 supplement or Zn^{2+} ionophore was far exceeded by nebivolol cotreatment (63, 66). The reciprocal regulation between $[\text{Zn}^{2+}]_i$ and $[\text{Ca}^{2+}]_i$ seen in ALDOST alone, ALDOST plus ZnSO_4 , and ALDOST plus nebivolol cotreatment is depicted in Figure 6. The increment in $[\text{Zn}^{2+}]_i$ seen with nebivolol related to its binding to β_3 receptors with activation of eNOS and NO generation that releases intracellular Zn^{2+} bound to MT1. This marked rise in $[\text{Zn}^{2+}]_i$ seen with nebivolol was associated with a nearly complete abrogation to the rise in $[\text{Ca}^{2+}]_i$ and accompanied by marked reduction in H_2O_2 production by mitochondria and abrogation in biomarkers of lipid peroxidation by these organelles and in cardiac tissue (63). The degree of cardioprotection seen with nebivolol was not found with another β_1 receptor blocker, atenolol, which is devoid of β_3 receptor binding (63, 67).

Zinc-Finger Transcription Factors

In addition to NO-based release of metallothionein-bound cytosolic Zn^{2+} , it will also induce the release of Zn^{2+} residing within cell nuclei (13). This would suggest zinc-finger proteins are NO targets and that NO modulates zinc-finger transcription factors and thereby gene regulation (13).

Zinc-finger proteins recognize DNA sequences. They may therefore serve in drug discovery and therapeutics through their DNA binding properties to zinc-finger transcription factors (68). Engineered zinc-finger proteins could be used to regress disease-related genes in heart failure (69).

Clinical Correlates

SHPT has now been recognized as an independent predictor of decompensated heart failure and the need for hospitalization (70, 71). Moreover, PTH is emerging as an independent risk

factor for mortality and cardiovascular events in community-dwelling individuals (72–74). The prognostic significance of PTH is independent of glomerular filtration rate (75, 76).

Hypozincemia has been widely reported in patients with CHF irrespective of whether they had an ischemic or idiopathic cardiomyopathy, or had been in sinus rhythm or atrial fibrillation (2–5). In patients who had gastric bypass surgery for the treatment of morbid obesity, chronic deficiencies of Zn^{2+} and Se^{2+} are common, together with the appearance of a dilated cardiomyopathy with reduced cations and activity of glutathione peroxidase (GPX) in the myopathic heart (77). Six months' treatment with supplemental Zn^{2+} and Se^{2+} , together with standard of care, resulted in normalized tissue cations and GPX activity and improved ventricular function. Supplemental Zn^{2+} reduces oxidative stress and chronic inflammation in chronic diseases (42). It prevents myocardial injury that accompanies: a hyperadrenergic state (78, 79); streptozocin-induced diabetes (80); and ischemia/reperfusion (15, 81). Likewise, supplemental Se^{2+} reverses a dilated cardiomyopathy known to be associated with dietary Se^{2+} deficiency (82). The minimal daily requirement for Zn^{2+} is 8–10 mg. As a supplement for acute or chronic Zn^{2+} deficiency, 50–100 mg/day ZnSO_4 is suggested (83).

SUMMARY AND CONCLUSIONS

The neurohormonal activation that accompanies CHF includes chronic aldosteronism with secondary hyperparathyroidism leading to intrinsically coupled Zn^{2+} and Ca^{2+} dyshomeostasis. This includes their excessive excretory losses that account for hypozincemia and ionized hypocalcemia. Zn^{2+} losses are also potentiated by ACE inhibitor treatment. Coupled with reduced dietary Zn^{2+} intake, a Zn^{2+} deficiency arises to compromise antioxidant defenses and predispose to the mitochondriocentric signal-transducer-effector pathway with PTH-mediated Ca^{2+} overloading and oxidative stress-induced cardiomyocyte necrosis.

Cardiomyocyte cytosolic free $[\text{Zn}^{2+}]_i$ has multiple biologic functions, none of which are more evident than its enhancement of antioxidant capacity thereby to confer cardioprotection in preventing nonischemic necrotic cell death and consequent microscopic scarring of myocardium. These antioxidant properties include: endogenous inhibitor to entry of prooxidant Ca^{2+} ; catalytic element to Cu/Zn-SOD activity; and $[\text{Zn}^{2+}]_i$ -based activation of its sensor, MTF1, which regulates antioxidant defense genes, including MT1.

In the failing heart, compromised antioxidant reserves can be reversed by exogenous Zn^{2+} , such as with a nutraceutical like ZnSO_4 , and from endogenous inactive cytosolic Zn^{2+} pool, using nebivolol, a β_3 receptor agonist, wherein inactive cytosolic Zn^{2+} bound to MT1 is released by nitric oxide. Thus, the spectrum of $[\text{Zn}^{2+}]_i$ as multifaceted antioxidant can be preferentially exploited promoting cardioprotection and counteracting the prooxidant heart failure phenotype.

Acknowledgments

This work was supported, in part, by NIH grants R01HL073043, R01HL090867 and R01HL096813 (KTW). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

We wish to express our gratitude to Richard A. Parkinson, MEd, for editorial assistance and scientific illustrations.

References

1. King, JC.; Cousins, RJ. Zinc. In: Shils, ME.; Shike, M.; Ross, AC.; Caballero, B.; Cousins, RJ., editors. *Modern Nutrition in Health and Disease*. 10. Philadelphia: Lippincott, Williams & Wilkins; 2006. p. 271-285.
2. Ghaemian A, Salehifar E, Jalalian R, Ghasemi F, Azizi S, Masoumi S, Shiraj H, Mohammadpour RA, Bagheri GA. Zinc and copper levels in severe heart failure and the effects of atrial fibrillation on the zinc and copper status. *Biol Trace Elem Res*. 2011; 143:1239–1246. [PubMed: 21258970]
3. Salehifar E, Shokrzadeh M, Ghaemian A, Aliakbari S, Saeedi Saravi SS. The study of Cu and Zn serum levels in idiopathic dilated cardiomyopathy (IDCMP) patients and its comparison with healthy volunteers. *Biol Trace Elem Res*. 2008; 125:97–108. [PubMed: 18716717]
4. Topuzoglu G, Erbay AR, Karul AB, Yensel N. Concentrations of copper, zinc, and magnesium in sera from patients with idiopathic dilated cardiomyopathy. *Biol Trace Elem Res*. 2003; 95:11–17. [PubMed: 14555795]
5. Arroyo M, LaGuardia SP, Bhattacharya SK, Nelson MD, Johnson PL, Carbone LD, Newman KP, Weber KT. Micronutrients in African-Americans with decompensated and compensated heart failure. *Transl Res*. 2006; 148:301–308. [PubMed: 17162251]
6. Thomas M, Vidal A, Bhattacharya SK, Ahokas RA, Sun Y, Gerling IC, Weber KT. Zinc dyshomeostasis in rats with aldosteronism. Response to spironolactone. *Am J Physiol Heart Circ Physiol*. 2007; 293:H2361–H2366. [PubMed: 17616752]
7. Golik A, Modai D, Averbukh Z, Sheffy M, Shamis A, Cohen N, Shaked U, Dolev E. Zinc metabolism in patients treated with captopril versus enalapril. *Metabolism*. 1990; 39:665–667. [PubMed: 2195291]
8. Hughes CM, Woodside JV, McGartland C, Roberts MJ, Nicholls DP, McKeown PP. Nutritional intake and oxidative stress in chronic heart failure. *Nutr Metab Cardiovasc Dis*. 2012; 22:376–382. [PubMed: 21186107]
9. Kamalov G, Deshmukh PA, Baburyan NY, Gandhi MS, Johnson PL, Ahokas RA, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Coupled calcium and zinc dyshomeostasis and oxidative stress in cardiac myocytes and mitochondria of rats with chronic aldosteronism. *J Cardiovasc Pharmacol*. 2009; 53:414–423. [PubMed: 19333130]
10. Kamalov G, Ahokas RA, Zhao W, Zhao T, Shahbaz AU, Johnson PL, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Uncoupling the coupled calcium and zinc dyshomeostasis in cardiac myocytes and mitochondria seen in aldosteronism. *J Cardiovasc Pharmacol*. 2010; 55:248–254. [PubMed: 20051880]
11. Maret W. The function of zinc metallothionein: a link between cellular zinc and redox state. *J Nutr*. 2000; 130:1455S–1458S. [PubMed: 10801959]
12. Oteiza PI. Zinc and the modulation of redox homeostasis. *Free Radic Biol Med*. 2012; 53:1748–1759. [PubMed: 22960578]
13. Kröncke KD, Carlberg C. Inactivation of zinc finger transcription factors provides a mechanism for a gene regulatory role of nitric oxide. *FASEB J*. 2000; 14:166–173. [PubMed: 10627291]
14. Jang Y, Wang H, Xi J, Mueller RA, Norfleet EA, Xu Z. NO mobilizes intracellular Zn²⁺ via cGMP/PKG signaling pathway and prevents mitochondrial oxidant damage in cardiomyocytes. *Cardiovasc Res*. 2007; 75:426–433. [PubMed: 17570352]
15. Karagulova G, Yue Y, Moreyra A, Boutjdir M, Korichneva I. Protective role of intracellular zinc in myocardial ischemia/reperfusion is associated with preservation of protein kinase C isoforms. *J Pharmacol Exp Ther*. 2007; 321:517–525. [PubMed: 17322024]
16. Wang J, Song Y, Elsherif L, Song Z, Zhou G, Prabhu SD, Saari JT, Cai L. Cardiac metallothionein induction plays the major role in the prevention of diabetic cardiomyopathy by zinc supplementation. *Circulation*. 2006; 113:544–554. [PubMed: 16432057]
17. Radovanovic S, Savic-Radojevic A, Pljesa-Ercegovac M, Djukic T, Suvakov S, Krotin M, Simic DV, Matic M, Radojicic Z, Pekmezovic T, Simic T. Markers of oxidative damage and antioxidant

enzyme activities as predictors of morbidity and mortality in patients with chronic heart failure. *J Card Fail.* 2012; 18:493–501. [PubMed: 22633308]

18. Shane E, Mancini D, Aaronson K, Silverberg SJ, Seibel MJ, Adesso V, McMahon DJ. Bone mass, vitamin D deficiency, and hyperparathyroidism in congestive heart failure. *Am J Med.* 1997; 103:197–207. [PubMed: 9316552]
19. Alsafwah S, LaGuardia SP, Nelson MD, Battin DL, Newman KP, Carbone LD, Weber KT. Hypovitaminosis D in African Americans residing in Memphis, Tennessee with and without heart failure. *Am J Med Sci.* 2008; 335:292–297. [PubMed: 18414068]
20. LaGuardia SP, Dockery BK, Bhattacharya SK, Nelson MD, Carbone LD, Weber KT. Secondary hyperparathyroidism and hypovitaminosis D in African-Americans with decompensated heart failure. *Am J Med Sci.* 2006; 332:112–118. [PubMed: 16969139]
21. Selektor Y, Parker RB, Sun Y, Zhao W, Bhattacharya SK, Weber KT. Tissue ⁶⁵zinc translocation in a rat model of chronic aldosteronism. *J Cardiovasc Pharmacol.* 2008; 51:359–364. [PubMed: 18427278]
22. Massry SG, Smogorzewski M. Mechanisms through which parathyroid hormone mediates its deleterious effects on organ function in uremia. *Semin Nephrol.* 1994; 14:219–231. [PubMed: 8036356]
23. Chhokar VS, Sun Y, Bhattacharya SK, Ahokas RA, Myers LK, Xing Z, Smith RA, Gerling IC, Weber KT. Hyperparathyroidism and the calcium paradox of aldosteronism. *Circulation.* 2005; 111:871–878. [PubMed: 15710759]
24. Fujita T, Palmieri GM. Calcium paradox disease: calcium deficiency prompting secondary hyperparathyroidism and cellular calcium overload. *J Bone Miner Metab.* 2000; 18:109–125. [PubMed: 10783844]
25. Boluyt MO, Bing OH, Lakatta EG. The ageing spontaneously hypertensive rat as a model of the transition from stable compensated hypertrophy to heart failure. *Eur Heart J.* 1995; 16 (Suppl N): 19–30. [PubMed: 8682057]
26. Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing OHL. Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat. *Circulation.* 1995; 91:161–170. [PubMed: 7805198]
27. Kuster GM, Kotlyar E, Rude MK, Siwik DA, Liao R, Colucci WS, Sam F. Mineralocorticoid receptor inhibition ameliorates the transition to myocardial failure and decreases oxidative stress and inflammation in mice with chronic pressure overload. *Circulation.* 2005; 111:420–427. [PubMed: 15687129]
28. Shapiro BP, Owan TE, Mohammed S, Kruger M, Linke WA, Burnett JC Jr, Redfield MM. Mineralocorticoid signaling in transition to heart failure with normal ejection fraction. *Hypertension.* 2008; 51:289–295. [PubMed: 18086943]
29. Mohammed SF, Ohtani T, Korinek J, Lam CS, Larsen K, Simari RD, Valencik ML, Burnett JC Jr, Redfield MM. Mineralocorticoid accelerates transition to heart failure with preserved ejection fraction via “nongenomic effects”. *Circulation.* 2010; 122:370–378. [PubMed: 20625113]
30. Sun Y, Zhang J, Lu L, Chen SS, Quinn MT, Weber KT. Aldosterone-induced inflammation in the rat heart. Role of oxidative stress. *Am J Pathol.* 2002; 161:1773–1781. [PubMed: 12414524]
31. Kane GC, Karon BL, Mahoney DW, Redfield MM, Roger VL, Burnett JC Jr, Jacobsen SJ, Rodeheffer RJ. Progression of left ventricular diastolic dysfunction and risk of heart failure. *JAMA.* 2011; 306:856–863. [PubMed: 21862747]
32. Dunlay SM, Roger VL, Weston SA, Jiang R, Redfield MM. Longitudinal changes in ejection fraction in heart failure patients with preserved and reduced ejection fraction. *Circ Heart Fail.* 2012; 5:720–726. [PubMed: 22936826]
33. Matzinger P. The danger model: a renewed sense of self. *Science.* 2002; 296:301–305. [PubMed: 11951032]
34. Ulasova E, Gladden JD, Chen Y, Zheng J, Pat B, Bradley W, Powell P, Zmijewski JW, Zelickson BR, Ballinger SW, Darley-USmar V, Dell'Italia LJ. Loss of interstitial collagen causes structural and functional alterations of cardiomyocyte subsarcolemmal mitochondria in acute volume overload. *J Mol Cell Cardiol.* 2011; 50:147–156. [PubMed: 21059354]

35. Sam F, Kerstetter DL, Pimental DR, Mulukutla S, Tabae A, Bristow MR, Colucci WS, Sawyer DB. Increased reactive oxygen species production and functional alterations in antioxidant enzymes in human failing myocardium. *J Card Fail.* 2005; 11:473–480. [PubMed: 16105639]
36. Borch E, Bargelli V, Stillitano F, Giordano C, Sebastiani M, Nassi PA, d'Amati G, Cerbai E, Nediani C. Enhanced ROS production by NADPH oxidase is correlated to changes in antioxidant enzyme activity in human heart failure. *Biochim Biophys Acta.* 2010; 1802:331–338. [PubMed: 19892017]
37. Sawyer DB, Colucci WS. Mitochondrial oxidative stress in heart failure: “oxygen wastage” revisited. *Circ Res.* 2000; 86:119–120. [PubMed: 10666404]
38. Abramov AY, Duchon MR. Measurements of threshold of mitochondrial permeability transition pore opening in intact and permeabilized cells by flash photolysis of caged calcium. *Methods Mol Biol.* 2011; 793:299–309. [PubMed: 21913108]
39. Kamalov G, Ahokas RA, Zhao W, Johnson PL, Shahbaz AU, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Temporal responses to intrinsically coupled calcium and zinc dyshomeostasis in cardiac myocytes and mitochondria during aldosteronism. *Am J Physiol Heart Circ Physiol.* 2010; 298:H385–H394. [PubMed: 19915175]
40. Rutledge MR, Farah V, Adeboye AA, Seawell MR, Bhattacharya SK, Weber KT. Parathyroid hormone, a crucial mediator of pathologic cardiac remodeling in aldosteronism. *Cardiovasc Drugs Ther.* 2013; 27:161–170. [PubMed: 22373564]
41. Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. *Physiol Rev.* 1993; 73:79–118. [PubMed: 8419966]
42. Prasad AS. Impact of the discovery of human zinc deficiency on health. *J Am Coll Nutr.* 2009; 28:257–265. [PubMed: 20150599]
43. Lowry SF, Goodgame JT Jr, Smith JC, Maher MM, Makuch RW, Henkin RI, Brennan MF. Abnormalities of zinc and copper during total parenteral nutrition. *Ann Surg.* 1979; 189:120–128. [PubMed: 103506]
44. Lowry SF, Smith JC Jr, Brennan MF. Zinc and copper replacement during total parenteral nutrition. *Am J Clin Nutr.* 1981; 34:1853–1860. [PubMed: 6792897]
45. Ohno H, Yahata T, Yamashita K, Kuroshima A. Effect of acute cold exposure on ACTH and zinc concentrations in human plasma. *Jpn J Physiol.* 1987; 37:749–755. [PubMed: 2448512]
46. Uhari M, Pakarinen A, Hietala J, Nurmi T, Kouvalainen K. Serum iron, copper, zinc, ferritin, and ceruloplasmin after intense heat exposure. *Eur J Appl Physiol Occup Physiol.* 1983; 51:331–335. [PubMed: 6685031]
47. Antila H, Salo M, Nanto V, Irjala K, Brenner R, Vapaavuori M. Serum iron, zinc, copper, selenium, and bromide concentrations after coronary bypass operation. *J Parenter Enteral Nutr.* 1990; 14:85–89.
48. Suzuki H, Matsumori A, Matoba Y, Kyu BS, Tanaka A, Fujita J, Sasayama S. Enhanced expression of superoxide dismutase messenger RNA in viral myocarditis. An SH-dependent reduction of its expression and myocardial injury. *J Clin Invest.* 1993; 91:2727–2733. [PubMed: 8390488]
49. Yin X, Wu H, Chen Y, Kang YJ. Induction of antioxidants by adriamycin in mouse heart. *Biochem Pharmacol.* 1998; 56:87–93. [PubMed: 9698092]
50. Takahashi T, Itano Y, Noji S, Matsumoto K, Taga N, Mizukawa S, Toda N, Matsumi M, Morita K, Hirakawa M. Induction of renal metallothionein in rats with ischemic renal failure. *Res Commun Mol Pathol Pharmacol.* 2001; 110:147–160. [PubMed: 12760485]
51. Wesselkamper SC, McDowell SA, Medvedovic M, Dalton TP, Deshmukh HS, Sartor MA, Case LM, Henning LN, Borchers MT, Tomlinson CR, Prows DR, Leikauf GD. The role of metallothionein in the pathogenesis of acute lung injury. *Am J Respir Cell Mol Biol.* 2006; 34:73–82. [PubMed: 16166738]
52. Shahbaz AU, Zhao T, Zhao W, Johnson PL, Ahokas RA, Bhattacharya SK, Sun Y, Gerling IC, Weber KT. Calcium and zinc dyshomeostasis during isoproterenol-induced acute stressor state. *Am J Physiol Heart Circ Physiol.* 2011; 300:H636–H644. [PubMed: 21076021]

53. Borkowski BJ, Cheema Y, Shahbaz AU, Bhattacharya SK, Weber KT. Cation dyshomeostasis and cardiomyocyte necrosis. The Fleckenstein hypothesis revisited. *Eur Heart J*. 2011; 32:1846–1853. [PubMed: 21398641]
54. Gandhi MS, Deshmukh PA, Kamalov G, Zhao T, Zhao W, Whaley JT, Tichy JR, Bhattacharya SK, Ahokas RA, Sun Y, Gerling IC, Weber KT. Causes and consequences of zinc dyshomeostasis in rats with chronic aldosteronism. *J Cardiovasc Pharmacol*. 2008; 52:245–252. [PubMed: 18806605]
55. Malette LE, Henkin RI. Altered copper and zinc metabolism in primary hyperparathyroidism. *Am J Med Sci*. 1976; 272:167–174. [PubMed: 1008079]
56. Chen SM, Kuo CD, Liao JF, Ho LT. Effects of parathyroid hormone on plasma zinc concentration in rat with chronic renal failure. *Chin J Physiol*. 2005; 48:121–128. [PubMed: 16304838]
57. Eide DJ. The oxidative stress of zinc deficiency. *Metallomics*. 2011; 3:1124–1129. [PubMed: 21789324]
58. Miranda JG, Weaver AL, Qin Y, Park JG, Stoddard CI, Lin MZ, Palmer AE. New alternately colored FRET sensors for simultaneous monitoring of Zn^{2+} in multiple cellular locations. *PLoS One*. 2012; 7:e49371. [PubMed: 23173058]
59. Crawford AJ, Bhattacharya SK. Excessive intracellular zinc accumulation in cardiac and skeletal muscles of dystrophic hamsters. *Exp Neurol*. 1987; 95:265–276. [PubMed: 3803514]
60. Palmer BM, Vogt S, Chen Z, Lachapelle RR, LeWinter MM. Intracellular distributions of essential elements in cardiomyocytes. *J Struct Biol*. 2006; 155:12–21. [PubMed: 16621603]
61. Alvarez-Collazo J, Díaz-García CM, López-Medina AI, Vassort G, Alvarez JL. Zinc modulation of basal and β -adrenergically stimulated L-type Ca^{2+} current in rat ventricular cardiomyocytes: consequences in cardiac diseases. *Pflugers Arch*. 2012; 464:459–470. [PubMed: 23007464]
62. Musat S, Wang X, Dhalla NS. Modification of the ATP-Induced increase in $[Ca^{2+}]_i$ by copper and zinc in rat cardiomyocytes. *J Cardiovasc Pharmacol Ther*. 1998; 3:291–298. [PubMed: 10684511]
63. Khan MU, Zhao W, Zhao T, Al Darazi F, Ahokas RA, Sun Y, Bhattacharya SK, Gerling IC, Weber KT. Nebivolol: a multifaceted antioxidant and cardioprotectant in hypertensive heart disease. *J Cardiovasc Pharmacol*. 2013; 62:445–451. [PubMed: 23921314]
64. Henkin, RI.; Apgar, J.; Cole, JF.; Coleman, JE.; Cottrell, CH.; Fleischer, M.; Goyer, RA.; Greifer, B.; Knezek, BD.; Mushak, P.; Piscator, M.; Stillings, BR.; Taylor, JK.; Wolfe, DA. Zinc in humans. In: Henkin, RI., editor. *Zinc*. Baltimore: University Park Press; 1979. p. 123–172.
65. Cousins RJ, Blanchard RK, Moore JB, Cui L, Green CL, Liuzzi JP, Cao J, Bobo JA. Regulation of zinc metabolism and genomic outcomes. *J Nutr*. 2003; 133 (5 Suppl 1):1521S–1526S. [PubMed: 12730457]
66. Cheema Y, Sherrod JN, Zhao W, Zhao T, Ahokas RA, Sun Y, Gerling IC, Bhattacharya SK, Weber KT. Mitochondriocentric pathway to cardiomyocyte necrosis in aldosteronism: cardioprotective responses to carvedilol and nebivolol. *J Cardiovasc Pharmacol*. 2011; 58:80–86. [PubMed: 21558884]
67. Kramer JH, Mak IT, Freedman AM, Weglicki WB. Propranolol reduces anoxia/reoxygenation-mediated injury of adult myocytes through an anti-radical mechanism. *J Mol Cell Cardiol*. 1991; 23:1231–1244. [PubMed: 1687065]
68. Jamieson AC, Miller JC, Pabo CO. Drug discovery with engineered zinc-finger proteins. *Nat Rev Drug Discov*. 2003; 2:361–368. [PubMed: 12750739]
69. Zhang HS, Liu D, Huang Y, Schmidt S, Hickey R, Guschin D, Su H, Jovin IS, Kunis M, Hinkley S, Liang Y, Hinh L, Spratt SK, Case CC, Rebar EJ, Ehrlich BE, Gregory PD, Giordano FJ. A designed zinc-finger transcriptional repressor of phospholamban improves function of the failing heart. *Mol Ther*. 2012; 20:1508–1515. [PubMed: 22828502]
70. Sugimoto T, Tanigawa T, Onishi K, Fujimoto N, Matsuda A, Nakamori S, Matsuoka K, Nakamura T, Koji T, Ito M. Serum intact parathyroid hormone levels predict hospitalisation for heart failure. *Heart*. 2009; 95:395–398. [PubMed: 19001003]
71. Hagström E, Ingelsson E, Sundström J, Hellman P, Larsson TE, Berglund L, Melhus H, Held C, Michaëlsson K, Lind L, Ärnlov J. Plasma parathyroid hormone and risk of congestive heart failure in the community. *Eur J Heart Fail*. 2010; 12:1186–1192. [PubMed: 20797986]

72. Pilz S, Tomaschitz A, Drechsler C, Ritz E, Boehm BO, Grammer TB, März W. Parathyroid hormone level is associated with mortality and cardiovascular events in patients undergoing coronary angiography. *Eur Heart J*. 2010; 31:1591–1598. [PubMed: 20439261]
73. Hagström E, Hellman P, Larsson TE, Ingelsson E, Berglund L, Sundström J, Melhus H, Held C, Lind L, Michaëlsson K, Arnlöv J. Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. *Circulation*. 2009; 119:2765–2771. [PubMed: 19451355]
74. Kestenbaum B, Katz R, de Boer I, Hoofnagle A, Sarnak MJ, Shlipak MG, Jenny NS, Siscovick DS. Vitamin D, parathyroid hormone, and cardiovascular events among older adults. *J Am Coll Cardiol*. 2011; 58:1433–1441. [PubMed: 21939825]
75. Björkman M, Sorva A, Tilvis R. Parathyroid hormone as a mortality predictor in frail aged inpatients. *Gerontology*. 2009; 55:601–606. [PubMed: 19752529]
76. Schierbeck LL, Jensen TS, Bang U, Jensen G, Køber L, Jensen JE. Parathyroid hormone and vitamin D--markers for cardiovascular and all cause mortality in heart failure. *Eur J Heart Fail*. 2011; 13:626–632. [PubMed: 21415099]
77. Frustaci A, Sabbioni E, Fortaner S, Farina M, del Torchio R, Tafani M, Morgante E, Ciriolo MR, Russo MA, Chimenti C. Selenium- and zinc-deficient cardiomyopathy in human intestinal malabsorption: preliminary results of selenium/zinc infusion. *Eur J Heart Fail*. 2012; 14:202–210. [PubMed: 22186680]
78. Chvapil M, Owen JA. Effect of zinc on acute and chronic isoproterenol induced heart injury. *J Mol Cell Cardiol*. 1977; 9:151–159. [PubMed: 839549]
79. Singal PK, Dhillon KS, Beamish RE, Dhalla NS. Protective effect of zinc against catecholamine-induced myocardial changes electrocardiographic and ultrastructural studies. *Lab Invest*. 1981; 44:426–433. [PubMed: 7230729]
80. Li B, Tan Y, Sun W, Fu Y, Miao L, Cai L. The role of zinc in the prevention of diabetic cardiomyopathy and nephropathy. *Toxicol Mech Methods*. 2013; 23:27–33. [PubMed: 23039870]
81. Chanoit G, Lee S, Xi J, Zhu M, McIntosh RA, Mueller RA, Norfleet EA, Xu Z. Exogenous zinc protects cardiac cells from reperfusion injury by targeting mitochondrial permeability transition pore through inactivation of glycogen synthase kinase-3 β . *Am J Physiol Heart Circ Physiol*. 2008; 295:H1227–H1233. [PubMed: 18660440]
82. Reeves WC, Marcuard SP, Willis SE, Movahed A. Reversible cardiomyopathy due to selenium deficiency. *JPEN J Parenter Enteral Nutr*. 1989; 13:663–665. [PubMed: 2614867]
83. Saper RB, Rash R. Zinc: an essential micronutrient. *Am Fam Physician*. 2009; 79:768–772. [PubMed: 20141096]

Figure 1.

Acute and chronic stressor states with excess catecholamines and aldosteronism, respectively, lead to hypozincemia, ionized hypocalcemia and hypomagnesemia with secondary hyperparathyroidism (SHPT), that provoke bone resorption and enhanced absorption of these cations from gut and kidney. Paradoxically, elevations in plasma parathyroid hormone (PTH) raise intracellular Ca^{2+} to induce oxidative stress and promote cardiomyocyte necrosis. See text. Adapted from Kamalov G, et al. *J Cardiovasc Pharmacol.* 2010;56:320–328.

Figure 2.

The putative association between mitochondrial (mt) total [Ca²⁺]_m and their production of H₂O₂ during preclinical and pathologic stages of aldosteronism/salt treatment (ALDOST) when cardiomyocytes cross from prosurvival to pronecrotic phenotype. Necrosis occurs when a threshold in [Ca²⁺]_m has been surpassed. Actual data points are shown as closed circles.

Figure 3.

A mitochondriocentric signal-transducer-effector pathway to nonischemic cardiomyocyte necrosis. Aldosterone/salt treatment (ALDOST) and associated elevations in plasma PTH promote cytosolic free $[Ca^{2+}]_i$ and mitochondrial $[Ca^{2+}]_m$ overloading and ensuing oxidative stress. Together with lost membrane potential and ATP synthesis, a pathologic opening of the subsarcolemmal (SSM) mitochondrial permeability transition pore (mPTP) follows leading to degeneration of these organelles and cardiomyocyte necrosis with leakage of troponins. Adapted from Rutledge MR, et al. *Cardiovasc Drugs Ther.* 2013;27:161–170.

Figure 4.

An evolving schema of the pathophysiologic paradigm contributing to the dyshomeostasis of cardiomyocyte cytosolic free $[Ca^{2+}]_i$ and subsarcolemmal mitochondria $[Ca^{2+}]_m$ as prooxidant, together with $[Zn^{2+}]_i$ and $[Zn^{2+}]_m$ as antioxidant, during chronic aldosteronism with SHPT. Elevations in plasma PTH cause intracellular Ca^{2+} overloading via L-type Ca^{2+} channels (LTCC), and the induction of mitochondrial reactive oxygen species (mtROS) generation contributing to mitochondrial permeability transition pore (mPTP) opening and ensuing cardiomyocyte necrosis with scarring. Antioxidant defenses are activated to counteract the prooxidant state. These include: ROS-mediated or nebivolol β_3 adrenergic receptors (β_3R) binding-induced generation of nitric oxide (NO) from endothelial NOS (eNOS) producing cyclic guanosine monophosphate (cGMP) via guanylyl cyclase (GC) which inhibits LTCC Ca^{2+} entry. NO also promotes release of inactive cytosolic Zn^{2+} bound to metallothionein (Zn/MT) raising both $[Zn^{2+}]_i$ and $[Zn^{2+}]_m$. The rise in $[Zn^{2+}]_i$ activates its sensor, metal-responsive transcription factor (MTF)-1 which, in turn, upregulates transcription of antioxidant defense genes. Furthermore, Zn^{2+} (Zip) transporters, induced by

ROS, contribute to increased Zn^{2+} entry and consequent rise in $[\text{Zn}^{2+}]_i$ and $[\text{Zn}^{2+}]_m$. Zn^{2+} supplement or Zn^{2+} ionophore will raise $[\text{Zn}^{2+}]_i$, which regulates Cu/Zn-superoxide dismutase (SOD) activity. Adapted from Cheema Y, et al. *J Cardiovasc Pharmacol*. 2011;58:80–86.

Figure 5.

The responses to intrinsically coupled Zn^{2+} and Ca^{2+} dyshomeostasis that occur in chronic stressor state invoked by 4 wks ALDOST raise myocardial total Ca^{2+} and Zn^{2+} (top panel), cytosolic free cardiomyocyte $[\text{Ca}^{2+}]_i$ and $[\text{Zn}^{2+}]_i$ (middle panel), as well as mitochondrial total $[\text{Ca}^{2+}]_m$ and $[\text{Zn}^{2+}]_m$ (lower panel). * $p < 0.05$ vs. control. Adapted from Kamalov G, et al. *J Cardiovasc Pharmacol.* 2009;53:414–423.

Figure 6.

An integrated reciprocal regulation between cytosolic free $[Zn^{2+}]_i$ and $[Ca^{2+}]_i$ is revealed by their percent increase in cardiomyocytes at: 4 wks ALDOST; ALDOST+ $ZnSO_4$ supplement (10); and ALDOST+nebivolol (Neb) (63). Reprinted from Khan MU, et al. *J Cardiovasc Pharmacol.* 2013;62:445–451.