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MYOCARDIAL REMODELING IN LOW-RENIN HYPERTENSION. MOLECULAR PATHWAYS TO CELLULAR INJURY IN RELATIVE ALDOSTERONISM

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Abstract

The pathologic hypertrophy of hypertensive heart disease is related to the quality not quantity of myocardium; the presence of fibrosis is inevitably linked to structural and functional insufficiencies with increased cardiovascular risk. Inappropriate (relative to dietary Na⁺) elevations in plasma aldosterone, or relative aldosteronism, are accompanied by suppressed plasma renin activity, elevation in arterial pressure, and dyshomeostasis of divalent cations. The accompanying hypocalcemia, hypomagnesemia, and hypozincemia of aldosteronism contribute to the appearance of secondary hyperparathyroidism. Parathyroid hormone-mediated intracellular Ca²⁺ overloading of cardiac myocytes and mitochondria leads to the induction of oxidative stress and molecular pathways associated with cardiomyocyte necrosis and scarring of myocardium, while the dyshomeostasis of Zn²⁺ compromises antioxidant defenses. This dyshomeostasis of Ca²⁺ and Zn²⁺ is intrinsically coupled as pro- and antioxidant, respectively, raising the prospect for therapeutic strategies designed to mitigate intracellular Ca²⁺ overloading while enhancing Zn²⁺-mediated antioxidant defenses, thus preventing adverse myocardial remodeling with fibrosis, associated diastolic dysfunction, and cardiac arrhythmias.

Keywords

hypocalcemia; hypomagnesemia; hypozincemia; parathyroid hormone; oxidative stress; aldosteronism

Introduction

Concentric left ventricular hypertrophy (LVH) accompanies either intermittent or sustained elevations in arterial pressure [1,2]. Despite comparable enlargement in LV mass seen with

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isometric exercise training or hypertensive heart disease (HHD), the latter is frequently associated with abnormalities in LV diastolic function [3–5]. Moreover, the LVH that accompanies HHD is a common risk factor for other adverse cardiovascular events, including ventricular arrhythmias [2,6,7]. This would suggest that it is not the quantity of myocardium, but rather the pathologic quality of its structure that proves detrimental to its function.

Morphologic studies of the myocardium in HHD, obtained postmortem or by endomyocardial biopsy, reveal an abnormal accumulation of fibrillar collagen [5,8–10]. Two distinct morphologic patterns of fibrosis have emerged. A *reparative fibrosis*, or microscopic scarring, which is a footprint of prior cardiomyocyte necrosis. The programmed death of these cells is not accompanied by either inflammatory cell or fibroblast infiltration, and therefore apoptosis does not beget a fibrous tissue response. A *reactive perivascular fibrosis* unequivocally follows the vasculopathy of the intramural coronary circulation from which fibrillar collagen extends to initiate *interstitial fibrosis*. The extent to which the myocardium is remodeled by fibrous tissue infiltration correlates well with the degree of LVH and abnormalities of myocardial stiffness [4,5,10].

Amongst hypertensive African-Americans (AA), LVH is an important prognostic risk factor for ventricular arrhythmias independent of coronary artery disease [11,12]. AA patients most commonly have low-renin hypertension, where plasma aldosterone (ALDO) levels are not fully suppressed by dietary Na⁺ loading. The clinical presentation of this relative aldosteronism includes symptoms and signs of expanded intra- and extravascular volumes due to reduced urinary Na⁺ excretion. Referred to as “wet” hypertension it is managed by a diuretic with proven efficacy in correcting edema and controlling blood pressure [13,14]. Herein we review studies that address pathways leading to cardiomyocyte necrosis with scarring and relevant cardioprotective strategies which could prevent adverse myocardial remodeling in low-renin hypertension associated with elevated or inappropriate levels of plasma ALDO, which hereafter we refer to simply as aldosteronism.

Low-Renin Hypertension in Humans

Brunner et al. [13] categorized patients having essential hypertension into 3 groups based on plasma renin activity relative to urinary Na⁺ excretion. Those with low renin activity, in whom aldosterone excretion failed to be suppressed during dietary Na⁺ loading, were considered to have low-renin hypertension with a functional derangement in ALDO secretion [15,16]. These patients have a tendency to Na⁺ retention and often present with salt-induced hypertension.

In studies reported by Resnick and coworkers in the 1980s, the metabolic-hormonal profile found in patients with low-renin hypertension was elucidated (see Table 1). The profile had the following features: (i) reduced plasma renin activity coupled with inappropriate elevations in plasma ALDO, which failed to be adequately suppressed by salt loading; (ii) ionized hypocalcemia accompanied by elevations in serum parathyroid hormone (PTH); and (iii) increased intracellular Ca²⁺ (e.g., platelets) [17]. These investigators proposed an association between Ca²⁺ and Na⁺ metabolism that contributes to the pathophysiology of salt-induced hypertension and the blood pressure lowering effects of: a) oral Ca²⁺ supplementation, which corrects the ionized hypocalcemia, and b) Ca²⁺ channel blockade, which retards PTH-mediated intracellular Ca²⁺ overloading. Secondary hyperparathyroidism (SHPT) is a well-recognized clinical feature of aldosteronism. Rossi et al. demonstrated that elevations in serum PTH found in patients with primary aldosteronism could be reduced by either spironolactone treatment or by adrenal surgery [18]. The increased melanin content of skin in AA serves as a natural sunscreen and consequently results in the prevalence of hypovitaminosis D, often of marked severity, that compromises Ca²⁺ homeostasis predisposing these patients to hypocalcemia and subsequent SHPT [19–21]. Hypomagnesemia is also a recognized feature of aldosteronism,

which too is corrected by spironolactone or adrenal surgery [22,23]. In addition to the ionized hypocalcemia and hypomagnesemia that accompany increased urinary and fecal losses of these divalent cations, studies by others in subsequent years would identify a concomitant dyshomeostasis of zinc with hypozincemia [24,25].

Therefore, the metabolic-hormonal profile shown in Table 1 for low-renin hypertension with aldosteronism depicts a dyshomeostasis of multiple nutrients that include Ca^{2+} , Mg^{2+} , Zn^{2+} , and vitamin D. Other factors that may play critical roles in the genesis of compromised Ca^{2+} stores and appearance of SHPT in AA include: a) reduced dietary Ca^{2+} intake because of lactose intolerance and an active avoidance of dairy products rich in Ca^{2+} ; and b) a preference for a high Na^+ diet that enhances urinary Ca^{2+} excretion. A high salt diet normally suppresses plasma renin activity in healthy subjects, but fails to do so in patients with low-renin hypertension; it is accompanied by increased excretory losses of Ca^{2+} with the resultant calciuria predisposing to ionized hypocalcemia and, in turn, SHPT with a resorption of bone invoked to restore extracellular Ca^{2+} homeostasis. Over time, osteopenia and osteoporosis accompany the calciuria of long-term dietary Na^+ excess predisposing to atraumatic bone fractures [26]. Hence, the link between Na^+ -induced alterations in Ca^{2+} metabolism, where increased urinary Na^+ promotes augmented urinary Ca^{2+} excretion is again noted and is also the case with salt-induced hypertension and low-renin hypertension.

The Calcium Paradoxes of Low-Renin Human Hypertension

In 1964, Fleckenstein and coworkers [27] advanced their hypothesis that excessive intracellular Ca^{2+} accumulation is cytotoxic to cardiomyocytes. Referred to as intracellular Ca^{2+} overloading, they proposed this sequelae would accompany the acute administration of a catecholamine or with chronic elevations of a calcitropic hormone, such as vitamin D. They reported their findings in 1967 elaborating on the cardioprotective properties of a Ca^{2+} channel blocker, which attenuated Ca^{2+} entry in response to a hormonal challenge [27].

Fujita & Palmieri [28] reported that elevations in plasma PTH, elicited in response to falling extracellular Ca^{2+} , where SHPT is invoked to promote bone mineral resorption to compensate for hypocalcemia, would lead to excessive intracellular Ca^{2+} accumulation. This intracellular Ca^{2+} overloading contemporaneous to falling extracellular Ca^{2+} was coined by them as a *Ca^{2+} paradox* and proposed to be an underlying pathophysiologic mechanism involved in various diseases, including hypertension. Years earlier, Massry and coworkers [29] had identified the role of PTH-mediated intracellular Ca^{2+} overloading that accompanied the SHPT associated with chronic renal failure, which also involved cardiomyocytes and vascular smooth muscle cells. Increased Ca^{2+} entry is PTH-receptor and G-protein-mediated and is associated with activation of L-type Ca^{2+} channels, as well as mobilization of Ca^{2+} from sarcoplasmic reticulum and reduced Ca^{2+} efflux [30]. Ca^{2+} channel blockade prevents PTH-mediated intracellular Ca^{2+} overloading and subsequent increase in contractility of cardiomyocytes [31,32]. Noncontractile cells also become Ca^{2+} overloaded during SHPT. These included lymphocytes and monocytes (or peripheral blood mononuclear cells, PBMC), which contribute to an immunostimulatory state and the proinflammatory vascular phenotype of the heart, kidneys, and mesentery [29,33–36].

McCarron et al. [37] raised the prospect of yet another *Ca^{2+} paradox*. In essential hypertension, the presence of intracellular Ca^{2+} overloading of vascular smooth cells and the accompanying elevation in arterial pressure could each be remedied by a dietary Ca^{2+} supplement. In correcting the hypocalcemia, the Ca^{2+} supplementation abrogates SHPT and the ensuing altered vasomotor reactivity of systemic arterioles.

Regression of Left Ventricular Hypertrophy

In patients with HHD the reduction in left ventricular mass can be achieved with antihypertensive agents that include Ca^{2+} channel blockers, ACE inhibitors, and angiotensin (AT_1) receptor antagonists, and to a lesser extent by beta blockers and diuretics [38]. The regression of LVH is associated with reduced cardiovascular risk for cardiovascular-related death, heart failure, atrial fibrillation, myocardial infarction, as well as stroke, and is independent of blood pressure reduction and treatment modality [39,40]. African-Americans with low-renin hypertension and LVH, however, may not respond favorably to AT_1 receptor blockers compared to Caucasians [41].

An Animal Model of Low-Renin Hypertension

Eight-week-old male Sprague-Dawley rats are uninephrectomized, followed by the implantation of an osmotic minipump containing ALDO. The infusion of ALDO ($0.75 \mu\text{g/h}$) is combined with 1% NaCl in drinking water which is further fortified with 0.4% KCl to prevent hypokalemia. We refer to this model as aldosterone/salt treatment (ALDO/ST). Over the course of several weeks of ALDO/ST, arterial pressure rises gradually and is accompanied by LVH [42]. Pathophysiologic responses that contribute to the rise in blood pressure are multifactorial and beyond the scope of this brief review. At wk 1 of ALDO/ST, animals are healthy and the myocardium appears normal by light microscopy. At wk 2 and beyond, this *preclinical stage* gives way to anorexia and a failure to gain weight, compared to untreated age-/sex-matched controls, and later the appearance of cardiac pathology emerges by wk 4. This *pathologic stage* features the appearance of microscopic scarring scattered throughout both the right and left atria and ventricles [43]. A perivascular/interstitial fibrosis involving the coronary, renal and mesenteric circulations also appears at wk 4. This topic has been extensively reviewed elsewhere [44].

A series of studies have addressed the relevance of hypertension vis-à-vis aldosteronism in contributing to cardiac fibrosis. As reviewed extensively [45], studies which have concluded hemodynamic factors are not involved include: a) the presence of fibrosis in nonpressure-overloaded right atria and ventricle; b) the absence of fibrosis with the LV pressure overload associated with infrarenal aortic banding; c) the prevention of fibrosis with either a small (nondepressor) or large (depressor) dose of spironolactone, which respectively fails to or does prevent hypertension; and d) intracerebroventricular infusion of a mineralocorticoid receptor antagonist, which prevents hypertension, but not fibrosis [46]. Furthermore, Garnier, et al. [47] have reported that a cardiac-specific upregulated expression of aldosterone synthase with increased tissue levels of ALDO is not accompanied by cardiac fibrosis. Thus, the evidence gathered to date indicates the adverse remodeling of myocardium during ALDO/ST is: a) independent of hypertension; and b) not related to ALDO per se, but some circulating factor that accompanies aldosteronism (vide infra).

Pathways Leading to Cardiomyocyte Necrosis in Aldosteronism

Several molecular pathways and biochemical processes may account for cardiomyocyte necrosis and subsequent reparative fibrosis, or scarring, found at 4 wks ALDO/ST.

Oxidative stress

Extensive evidence of oxidative stress in the myocardium during chronic mineralocorticoidism has been reported by several laboratories [33–35,48,49]. This includes: a) the presence of 3-nitrotyrosine, a byproduct of the reaction involving superoxide and nitric oxide; b) an activation of the $\text{gp91}^{\text{phox}}$ subunit of NADPH oxidase found in inflammatory cells invading the injured myocardium and which contributes to superoxide generation; c) upregulated redox-sensitive

nuclear transcription factor (NF)- κ B and a proinflammatory gene cascade it regulates and which includes intercellular adhesion molecule (ICAM)-1, monocyte chemoattractant protein (MCP)-1, and tumor necrosis factor (TNF)- α ; and d) increased tissue levels of 8-isoprostane and malondialdehyde, biomarkers of lipid peroxidation. There is also considerable evidence of oxidative stress in blood and urine in keeping with the systemic nature of an altered redox state during ALDO/ST. But how does this occur?

Intracellular Ca^{2+} overloading

Our hypothesis would draw upon Fleckenstein's original concept that intracellular Ca^{2+} overloading is an integral and adverse pathophysiologic feature of various stress states [27], such as catecholamine excess which has since been validated for ischemia/reperfusion injury [50]. Accordingly, we monitored intracellular Ca^{2+} levels in the heart and peripheral blood mononuclear cells (PBMC) in rats receiving 1 and 4 wks ALDO/ST. We found increased Ca^{2+} levels in the myocardium and PBMC at these time points accompanied by biomarker-mediated evidence of oxidative stress, such as increased levels of malondialdehyde and 8-isoprostane in the heart and increased H_2O_2 production by PBMC [49,51–53]. Nevertheless, the underlying pathogenetic mechanisms responsible for intracellular Ca^{2+} overloading during ALDO/ST deserve to be further investigated.

Calcium and magnesium dyshomeostasis

Elevations in dietary Na^+ , albeit modest but inappropriate in this model, are accompanied by increased tubular Na^+ and, in turn, urinary concentrations of Ca^{2+} and Mg^{2+} . ALDO promotes distal tubular epithelial cell channel reabsorption of Na^+ without influencing Ca^{2+} and Mg^{2+} , which then accounts for the marked excretory urinary losses of Ca^{2+} and Mg^{2+} [51]. A similar scenario unfolds in the Na^+ channels of the colon's epithelial cells that represents another site of high density ALDO receptor binding. It is therefore not surprising that fecal excretion of Ca^{2+} and Mg^{2+} have been found to be many fold greater than their urinary losses [51].

Secondary hyperparathyroidism (SHPT)

Our metabolic studies in rats receiving ALDO/ST identified the marked increase in both the urinary and fecal excretion of Ca^{2+} and Mg^{2+} , which led to plasma ionized hypocalcemia and hypomagnesemia and, in turn, to increased plasma PTH levels [51]. SHPT was manifested by a marked and progressive resorption of bone and reduction in bone mineral density and bone strength [54]. We therefore hypothesized that the intracellular Ca^{2+} overloading and induction of oxidative stress that accompanies ALDO/ST is unequivocally PTH-mediated (see Figure 1), and represents a classic scenario embodying the Ca^{2+} paradox of SHPT reported by Fujita & Palmieri [28]. PTH-mediated intracellular Ca^{2+} overloading is paradoxically coupled to an induction of oxidative stress in diverse tissues that includes cardiomyocytes and their mitochondria. At these sites, especially in cardiac mitochondria, reactive oxygen and nitrogen species overwhelm antioxidant defenses. In mitochondria, Ca^{2+} overloading and oxidative stress lead to an opening of the mitochondrial permeability transition pore together with the structural and functional degeneration of these organelles that triggers the final common pathway to cardiomyocyte necrosis [55].

A series of experiments had to be conducted in rats receiving ALDO/ST to validate this hypothesis and later to prevent this pathologic sequelae of events. Effective interventions included: a) cotreatment with spironolactone (Spiro), an ALDO receptor antagonist, which attenuated the enhanced urinary and fecal losses of these cations to prevent hypocalcemia and hypomagnesemia, and thereby abrogated SHPT [51]; b) cotreatment with a Ca^{2+} and Mg^{2+} -supplemented diet, together with vitamin D, to prevent SHPT, which is analogous to the paradox described by McCarron [56], given the degree of intracellular Ca^{2+} overloading [57]; c) parathyroidectomy, performed prior to starting ALDO/ST [58]; d) cotreatment with

cinacalcet, a calcimimetic that resets the Ca^{2+} -sensing receptor of the parathyroid glands to prevent SHPT despite marked hypocalcemia [59]; e) cotreatment with amlodipine (Amlod), a Ca^{2+} channel blocker, which prevents intracellular Ca^{2+} overloading [52]; and finally f) cotreatment with N-acetylcysteine, an antioxidant [35].

Thus, the multitude of evidence gathered to date congruently supports that PTH-mediated intracellular Ca^{2+} overloading is the mechanism involved in the induction of oxidative stress during aldosteronism, where reactive oxygen and nitrogen species overwhelm cellular antioxidant defenses. However, it raises the question whether this scenario is based solely on the excessive generation of prooxidants, or whether the endogenous antioxidant defenses that combat reactive oxygen and nitrogen species has been compromised and overwhelmed by the overproduction of prooxidants under the pathogenetic stimuli propagated by PTH-mediated intracellular Ca^{2+} overloading.

Zinc dyshomeostasis

Commensurate with the colonic and renal losses of Ca^{2+} and Mg^{2+} seen with aldosteronism, the chronic excess of this mineralocorticoid is accompanied by increased excretory Zn^{2+} losses, the appearance of hypozincemia, and a fall in plasma Cu/Zn-superoxide dismutase (SOD) activity [60]. Like Ca^{2+} and Mg^{2+} losses, the hyperzincuria seen with ALDO/ST is due to urinary acidification that contributes to a consequent metabolic alkalosis [49]. Also contributory to hypozincemia is a coordinated selective translocation of Zn^{2+} to the sites of tissue injury facilitated by upregulated expression of metallothionein (MT)-1, a Zn^{2+} -binding protein [49,60].

To monitor Zn^{2+} kinetics more systematically in this model, we used ^{65}Zn as a radioactive tracer. We found a simultaneous fall in plasma ^{65}Zn and a selective accumulation of ^{65}Zn at sites of injury, which included its translocations to the damaged skin, acutely incised to implant the minipump at wk 1, as well as the injured heart and kidneys at wk 4. Intriguingly, this pathophysiology-driven intracellular Zn^{2+} trafficking to injured tissues was accompanied by the upregulation of MT-1 [61]. However, at wk 4 there was a decline in ^{65}Zn in healed skin and bone, which served as reservoirs, in an attempt to restore the fall in extracellular Zn^{2+} . Thus, the rapid translocation of circulating Zn^{2+} to injured tissues contributes to hypozincemia found with ALDO/ST, where increased tissue Zn^{2+} is involved in wound healing at these sites [62]. Since a synchronized dyshomeostasis of Zn^{2+} is recognized as another integral feature of aldosteronism-mediated myocardial remodeling, it is crucial to investigate whether the rise in cardiac tissue Zn^{2+} involves its cardiac myocytes and mitochondria.

Zinc and antioxidant defenses

In cardiomyocytes and mitochondria harvested from the heart at wk 4 of ALDO/ST, the pathologic stage when necrotic cell death and scarring occurs, we found increased cytosolic free $[\text{Zn}^{2+}]_i$ in cardiac myocytes and total Zn^{2+} concentrations in mitochondria [49]. The rise in cardiomyocyte Zn^{2+} was facilitated by the increased expression of membranous Zn^{2+} transporters. Increased $[\text{Zn}^{2+}]_i$ serves to augment the antioxidant defenses of cardiomyocytes, including their upregulation of MT-1 and activation of metal-responsive transcription factor (MTF)-1, which encodes genes related to various antioxidant defenses, such as Cu/Zn-SOD, MT-1, and glutathione synthase. So too were biomarkers of oxidative stress, such as 8-isoprostane and malondialdehyde [63].

The efficacy of a ZnSO_4 supplementation in attenuating these adverse responses, while enhancing antioxidant defenses during ALDO/ST, was then addressed. We found ZnSO_4 cotreatment could prevent hypozincemia, but not ionized hypocalcemia. It also attenuated oxidative stress and microscopic scarring without preventing the vasculitis and perivascular

fibrosis attendant with PBMC activation [49]. Thus, increased tissue Zn^{2+} in the heart serves as an antioxidant while intracellular Ca^{2+} overloading as prooxidant, whereas cardiomyocyte necrosis highlights the intrinsic codependency between these two biologically crucial divalent cations. A Zn^{2+} supplement has proven cardioprotective in mice with streptozocin-induced diabetic cardiomyopathy, in a model of ischemia/reperfusion in rat hearts, and following isoproterenol administration in rats [49,64–66].

Coupled Ca^{2+} and Zn^{2+} dyshomeostasis

The dyshomeostasis of extra- and intracellular Ca^{2+} and Zn^{2+} which accompany ALDO/ST contributes to a dysequilibrium between pro- and antioxidants. We hypothesized that an intrinsically coupled dyshomeostasis of intracellular Ca^{2+} and Zn^{2+} in aldosteronism alters the redox state of cardiac myocytes and mitochondria. Toward this end, we harvested hearts from rats receiving 4 wks ALDO/ST alone or cotreated with Spiro or Amlod. Compared to untreated, age-/sex-matched controls, we found (see Figure 2) increased cardiomyocyte cytosolic free $[\text{Ca}^{2+}]_i$ and $[\text{Zn}^{2+}]_i$, together with increased mitochondrial $[\text{Ca}^{2+}]_m$ and $[\text{Zn}^{2+}]_m$ in rats with ALDO/ST, each of which could be prevented by Spiro and attenuated by Amlod cotreatment [63].

These iterations in divalent cation composition were accompanied by increased levels of 3-nitrotyrosine and 4-hydroxy-2-nonenal in cardiomyocytes, together with increased H_2O_2 production, malondialdehyde and oxidized GSSG in mitochondria that were coincident with increased activities of Cu/Zn-SOD and glutathione peroxidase (GSH-Px) [49,55,63]. These changes in intracellular Zn^{2+} were accompanied by the increased expression of MT-1, Zn^{2+} transporters (Zip1 and ZnT-1) and MTF-1. Thus, in cardiac myocytes and mitochondria from rats with ALDO/ST, an intrinsically coupled dyshomeostasis of intracellular Ca^{2+} and Zn^{2+} serves to alter the redox state via induction of oxidative stress and generation of antioxidant defenses, respectively. These findings underscore the importance of therapeutic strategies that can uncouple these crucial cations and modulate them in favor of sustained antioxidant defenses. The coupled Ca^{2+} and Zn^{2+} dyshomeostasis seen in aldosteronism is reminiscent of the Ca^{2+} overloading and oxidative stress that exists in the cardiac and skeletal muscles of hamsters with hereditary muscular dystrophy and hypertrophic cardiomyopathy which is also accompanied by increased tissue Zn^{2+} [67–69]. This divalent cation dyshomeostasis seen in muscular dystrophy can be prevented by parathyroidectomy [67], a Ca^{2+} channel blocker [68], or by a nitric oxide synthase antagonist. Furthermore, our findings in rats with ALDO/ST resemble the protective role of increased $[\text{Zn}^{2+}]_i$ induced by a Zn^{2+} supplement or Zn^{2+} ionophore when intracellular $[\text{Ca}^{2+}]_i$ overloading of the heart is present [64,65].

Future studies will address the relative merits of ZnSO_4 cotreatment by further raising intracellular Zn^{2+} in favor of antioxidant defenses, as well as the synergistic efficacy of ZnSO_4 combined with Amlod to simultaneously reduce Ca^{2+} entry.

Summary and Conclusions

In both man and rats, inappropriate elevations in plasma ALDO (relative to dietary Na^+), which cannot be suppressed by dietary Na^+ loading, are accompanied by reduced plasma renin activity, elevated arterial pressure, and consequent increased LV mass, together with the heightened excretory losses of Ca^{2+} , Mg^{2+} , and Zn^{2+} . The resultant hypocalcemia, hypomagnesemia, and hypozincemia that accompany low-renin hypertension individually and collectively contribute to the appearance of SHPT, where elevations in plasma PTH seek to restore extracellular homeostasis of these divalent cations through their resorption from bone. At the same time, however, PTH-mediated intracellular Ca^{2+} overloading occurs in diverse cell types, including cardiac myocytes. This Ca^{2+} paradox, which includes mitochondria, accounts for the induction of oxidative stress and resultant cardiomyocyte necrosis. The

subsequent reparative fibrosis, or scarring, contributes to the adverse structural remodeling of the hypertrophied LV and nonhypertrophied RV by fibrous tissue, with its attending pathologic influences on myocardial stiffness and substrate for re-entrant arrhythmias.

Several pathways are involved in the necrosis of cardiomyocytes that accompanies aldosteronism. Most common and clinically relevant amongst them are PTH-mediated intracellular Ca^{2+} overloading and induction of oxidative stress, which can be abrogated by a Ca^{2+} supplement to prevent hypocalcemia—a Ca^{2+} paradox. Another pathway relates to impaired antioxidant defenses that accompany Zn^{2+} dyshomeostasis with hypozincemia. Therefore, it is intriguing to speculate that the dyshomeostasis of intracellular Ca^{2+} and Zn^{2+} in cardiac myocytes and mitochondria are intrinsically coupled where Ca^{2+} serves as prooxidant and Zn^{2+} as antioxidant. This raises the prospect for pharmacologic uncoupling of Ca^{2+} and Zn^{2+} in favor of antioxidant defenses through Zn^{2+} supplementation alone, or in combination with a Ca^{2+} supplement or Ca^{2+} channel blocker. In addressing the importance of a dyshomeostasis of macro- and micronutrients in low-renin hypertension with aldosteronism, such as seen in African-Americans, it may be possible to prevent the adverse structural remodeling of myocardium which contributes to increased cardiovascular risk.

As Díez has recently suggested [70], a new pathophysiologic paradigm of hypertensive heart disease is emerging; it is not simply an adaptation to the hemodynamic burden of arterial hypertension. Understanding the molecular pathways leading to tissue injury will enable us to develop novel strategies that combine current pharmacologic interventions with nutraceutical adjuvants to achieve the greatest therapeutic potential in combating adverse myocardial remodeling in hypertensive heart disease.

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Figure 1.

The appearance of secondary hyperparathyroidism (SHPT) during aldosterone/salt treatment is associated with increased excretory losses of Ca^{2+} and Mg^{2+} and the consequent appearance of plasma ionized hypocalcemia and hypomagnesemia. Elevations in parathyroid hormone (PTH) seek to restore extracellular homeostasis of these divalent cations through their resorption from bone and their absorption from gut and reabsorption by kidney promoted by the steroid hormone $1,25(\text{OH})_2\text{D}_3$. Paradoxically, PTH elaboration is responsible for intracellular Ca^{2+} overloading and the induction of oxi-/nitrosative stress, which leads to a proinflammatory vascular phenotype, elevation in blood pressure (BP), and subsequent left ventricular hypertrophy (LVH).

Figure 2.

Our current understanding of the pathways involving intrinsically coupled dyshomeostasis of Ca^{2+} and Zn^{2+} found with aldosterone/salt treatment. Increased excretory losses of these divalent cations lead to hypocalcemia and hypozincemia. Consequent secondary hyperparathyroidism with persistent elevations in circulating parathyroid hormone (PTH) are accompanied by uncontrolled Ca^{2+} entry via L-type Ca^{2+} channels (LTCC) to saturate intracellular binding and storage sites, and ultimately to intracellular $[\text{Ca}^{2+}]_i$ overloading and excessive Ca^{2+} sequestration within mitochondria. An induction of oxidative stress and generation of reactive oxygen species (ROS) ensues involving mitochondria. The rise in $[\text{Zn}^{2+}]_i$ and $[\text{Zn}^{2+}]_m$ involves increased Zn^{2+} entry via LTCC to a minor extent, while the

majority of $[Zn^{2+}]_i$ is regulated by membrane-bound Zn transporters, including Zip1 and ZnT-1, and its binding to metallothionein (MT) to minimize cytotoxicity.

We would speculate ROS serve as signals that initiate an activation of molecular transducers:

a) stress-response kinases, MAPKs, and their activation of redox-sensitive nuclear transcription factor (NF)- κ B and a proinflammatory gene cascade it induces including adhesion molecules (ICAM-1), chemokines (MCP-1) and cytokines (TNF- α and TGF- β); and b)

activation of inducible or endothelial nitric oxide synthases (iNOS and eNOS), where nitric oxide (NO) generation releases Zn^{2+} from the Zn/MT complex. Increased $[Zn^{2+}]_i$ serves to activate its sensor, metal-response element transcription factor (MTF)-1, and the antioxidant genes it induces, including MT-1, Cu/Zn-SOD, and glutathione (GSH) synthase. Increased $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ are prooxidants, which in toxic amounts threaten the fate of cardiomyocytes, while increments in $[Zn^{2+}]_i$ and $[Zn^{2+}]_m$ inhibit apoptosis and necrosis. TGF-

β , transforming growth factor β ; TNF- α , tumor necrosis factor α . Reprinted, with permission, from Kamalov G, Deshmukh PA, Baburyan NY, *et al.*: **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.

Table 1

Metabolic-hormonal profile in low-renin hypertension with relative aldosteronism.

<ul style="list-style-type: none">• increased plasma aldosterone ($\uparrow$ urinary and fecal Ca^{2+} and Mg^{2+} excretion)• plasma ionized hypocalcemia and hypomagnesemia• increased plasma parathyroid hormone (PTH)• PTH-mediated intracellular $[\text{Ca}^{2+}]_i$ overloading• hypozincemia ($\uparrow$ urinary and fecal Zn^{2+} excretion)• $\downarrow$ BP by dietary Ca^{2+} supplement• $\downarrow$ BP by Ca^{2+} channel blocker

Table 2

Factors contributing to Ca^{2+} and Zn^{2+} dyshomeostasis in low-renin hypertension with relative aldosteronism.

<ul style="list-style-type: none">• hypovitaminosis D• reduced dietary Ca^{2+} (lactose intolerance)• increased dietary Na^+ (calciuria)• loop diuretics (urinary Ca^{2+} losses)• aldosteronism (excretory Ca^{2+} and Zn^{2+} losses)