

Zinc dyshomeostasis in rats with aldosteronism. Response to spironolactone

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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* 293: H2361–H2366, 2007. First published July 6, 2007; doi:10.1152/ajpheart.00200.2007.—Zinc is a structural constituent of many proteins, including Cu/Zn superoxide dismutase (SOD), an endogenous antioxidant enzyme. Hypozincemia has been found in patients hospitalized with congestive heart failure, where neurohormonal activation, including the renin-angiotensin-aldosterone system (RAAS), is expected and oxidative stress is present. This study was undertaken to elucidate potential pathophysiological mechanisms involved in Zn dyshomeostasis in aldosteronism. In rats receiving aldosterone/salt treatment (ALDOST) alone for 1 and 4 wk or in combination with spironolactone (Spiro), an ALDO receptor antagonist, we monitored 24-h urinary and fecal Zn excretion and tissue Zn levels in heart, liver, and skeletal muscle, together with tissue metallothionein (MT)-I, a Zn²⁺-binding protein, and Cu/Zn-SOD activities in plasma and tissues. When compared with unoperated, untreated, age-/sex-matched controls, urinary and, in particular, fecal Zn losses were markedly increased ($P < 0.05$) at days 7 and 28 of ALDOST, leading to hypozincemia and a fall ($P < 0.05$) in plasma Cu/Zn-SOD activity. Microscopic scars and perivascular fibrosis of intramural coronary arteries first appeared in the right and left ventricles at week 4 of ALDOST and were accompanied by increased ($P < 0.05$) tissue Zn, MT-I, and Cu/Zn-SOD activity, which were not found in uninjured liver or skeletal muscle. Spiro cotreatment prevented cardiac injury and Zn redistribution to the heart. Thus increased urinary and fecal Zn losses, together with their preferential translocation to sites of cardiac injury, where MT-I overexpression and increased Cu/Zn-SOD activity appeared, contribute to Zn dyshomeostasis in rats with aldosteronism, which were each prevented by Spiro. These findings may shed light on Zn dyshomeostasis found in patients with decompensated heart failure.

aldosterone; oxidative stress; antioxidant defenses; excretory zinc losses; hypozincemia

ENDOGENOUS ANTIOXIDANT DEFENSES are integral to combating oxidative stress. These defenses include intra- and extracellular Cu/Zn-SOD, the activities of which are dependent on the bioavailability of these essential trace minerals. Zinc, the sixth most abundant cation in the body, is an essential micronutrient predominantly derived from dietary sources; it is stored largely in liver and skeletal muscle (24). Reduced antioxidant defenses can occur when dietary Zn intake is inadequate, excretory Zn losses are excessive relative to intake, Cu/Zn-SOD is utilized in the dismutation of superoxide without adequate replenishment, or a combination of these circumstances (49). In response to dietary Zn deficiency, plasma Zn levels and tissue Zn stores both decline and are associated with reduced tissue

metallothionein (MT), a cysteine-rich Zn²⁺-binding protein involved in Zn²⁺ homeostasis and free radical scavenging, and are also associated with reduced serum Cu/Zn-SOD activity. This contrasts with Zn insufficiency seen with states of stress associated with renin-angiotensin-aldosterone system (RAAS) activation, such as extremes of heat or coronary artery bypass surgery (5, 48), or in response to tissue injury involving brain, kidney, lung, or heart (7, 11, 34, 43, 45, 46, 55, 56), where circulating Zn levels decline while Zn is redistributed to injured tissues based, in part, on upregulated MT-I expression and where tissue Cu/Zn-SOD expression and activity are increased (17, 28). Albeit of uncertain origin(s), hypozincemia has been reported in patients hospitalized because of their symptoms and signs of congestive heart failure (CHF) and where RAAS activation is expected (4, 10, 19, 44, 52).

Little is known about urinary and fecal Zn excretion, Zn distribution within tissues relative to MT-I expression, and the activity of Cu/Zn-SOD in blood and solid tissues in rats with aldosteronism. Our working hypothesis suggests that, like the origins of the hypocalcemia and hypomagnesemia found in aldosteronism (13, 25), urinary and fecal Zn wasting accompanies aldosterone/salt treatment leading to hypozincemia. However, contrary to dietary Zn deficiency, aldosteronism is associated with increased MT-I, a heterogenous tissue redistribution of Zn, and increased Cu/Zn-SOD activity at involved sites. To investigate potential mechanisms of Zn dyshomeostasis, uninephrectomized rats were administered a regimen of aldosterone/salt (1% NaCl) in drinking water (ALDOST) alone for 1 and 4 wk, or together with spironolactone (Spiro) cotreatment, an aldosterone receptor antagonist. At the end of each treatment period, we monitored urinary Zn losses; plasma zinc; tissue Zn; Cu/Zn-SOD activity; and MT-I in heart, liver, and skeletal muscle, together with morphological evidence of injury. These experimental groups were compared with age-/sex-matched, untreated control rats.

METHODS

Animal Model

Male, 8–12-wk-old Sprague-Dawley rats (Harlan, IN) were used in this study approved by the Institutional Animal Care and Use Committee. As reported elsewhere (2, 21, 42), ALDOST consists of uninephrectomized rats receiving ALDO (0.75 $\mu\text{g}/\text{h}$) by implanted minipump (Alzet, Cupertino, CA) together with 1% NaCl/0.4% KCl in drinking water (ALDOST), and standard laboratory chow (Harlan Teklad 22/5 Rodent Diet, Harlan Teklad, Madison, WI) containing 78.84 mg of Zn/kg chow. Uninephrectomy alone, or a 1% NaCl diet alone, does not lead to the pathophysiological responses associated

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with ALDOST (reviewed in Ref. 53). A separate group of rats received ALDOST plus Spiro (A + Spiro) ($150 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ in divided doses by twice-daily gavage). Animals were anesthetized and euthanized, and the blood, heart, liver, and rectus femoris muscle were harvested. Each experimental group consisted of six rats. Unoperated, untreated, age-/sex-matched rats served as controls.

Urinary and Fecal Zinc Excretion

As reported previously, metabolic studies were used to monitor urinary and fecal excretion of Ca^{2+} and Mg^{2+} in rats receiving ALDOST (13). The same protocol was used for the collection of minerally uncontaminated 24-h specimens to monitor Zn losses during A + Spiro and in controls. Urinary and fecal Zn concentrations were determined using an atomic absorption spectrophotometer, with excretion rates expressed as micrograms per 24 h.

Thymus gland atrophy with apoptosis is a feature of Zn insufficiency. To elucidate thymus involution in this study, the gland was removed, weighed, and then frozen in isopentane with dry ice for detection of apoptosis at weeks 1 and 4 of ALDOST. Apoptosis was detected by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assay using an Apop Tag Fluorescein kit (Intergen, Norcross, GA). Evidence of thymus gland atrophy indicative of Zn insufficiency was seen at weeks 1 and 4 ALDOST, whereas organ weight (0.35 ± 0.02 and 0.23 ± 0.01 gm, respectively) was significantly less ($P < 0.05$) than that found in untreated, age-/sex-matched controls (0.59 ± 0.01 and 0.33 ± 0.01 gm, respectively). With the use of the TUNEL assay, spontaneous thymocyte apoptosis was evident throughout the thymus gland of 9- and 12-wk-old controls to account for the expected regression in organ weight. The extent of these apoptotic cells in both cortex and medulla of the thymus was accentuated in rats receiving 1 and 4 wk ALDOST to account for their markedly accelerated regression in thymus weight. These findings suggest that our model of ALDOST is in keeping with Zn insufficiency and differs from dietary Zn deficiency, where Zn levels are reduced in plasma and tissues and tissue MT expression is suppressed (16, 36).

Plasma and Tissue Zinc Concentrations

Microanalysis of Zn in plasma and tissue was performed using atomic absorption spectroscopy, as previously reported (6, 8), and expressed as $\mu\text{g}/\text{dl}$ and ng/mg fat-free dry tissue (FFDT), respectively.

Total, Cu/Zn- and Mn-SOD Activities

Heart, skeletal muscle, and liver tissue samples were homogenized in 10 volumes of ice-cold 20 mM HEPES buffer (pH 7.2) containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose and were sonicated. After centrifugation at 1,500 g for 5 min, the supernatant was kept frozen at -80°C for later batch analysis of total, Cu/Zn- and Mn-SOD activities. Plasma extracellular SOD activity was measured directly, following dilution with 20 mM HEPES buffer (pH 7.2) containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose. Plasma extracellular SOD and tissue total SOD activities were measured utilizing a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine (Cayman Chemical Superoxide Dismutase Assay, Ann Arbor, MI). One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Tissue Mn-SOD activity was measured following the addition of 3 mM KCN to the assay mixture to inhibit Cu/Zn-SOD. Tissue Cu/Zn-SOD activities were, therefore, calculated as the difference between total and Mn-SOD activities.

Tissue MT-I

MT-I protein levels in the myocardium were measured by Western blot analysis. Briefly, left ventricles were dissected free and homogenized in lysis buffer and then separated by 12% SDS-PAGE. After

electrophoresis, samples were transferred to polyvinylidene fluoride membranes and incubated with antibody against MT-I. Blots were subsequently incubated with peroxidase-conjugated secondary antibody. After being washed, the blots were developed with the enhanced chemiluminescence method. The amount of protein detected by each antibody was measured by a computer image analysis system.

Cardiac, Liver, and Skeletal Muscle Pathology

The presence of injury was examined in heart, liver, and skeletal muscle based on the presence of fibrosis, which was assessed by collagen-specific picrosirius red staining in $6 \mu\text{m}$ cardiac sections and observed by light microscopy, as previously reported (42).

Statistical Analysis

Data were analyzed using analysis of variance. Significant differences between individual means were determined using the Bonferroni multiple comparisons test. Significance was assigned to $P < 0.05$, and values presented are expressed as means \pm SE.

RESULTS

Urinary and Fecal Zinc Excretion

Twenty-four hours urinary Zn excretion for controls, ALDOST, and A + Spiro on days 7 and 28 is presented in Fig. 1, top. In controls, urinary excretion was $\sim 4 \mu\text{g}/24 \text{ h}$ and was increased fivefold ($P < 0.05$) to $\sim 20 \mu\text{g}/24 \text{ h}$ at both weeks 1 and 4 of ALDOST. This early and persistent loss of urinary Zn was prevented ($P < 0.05$) by Spiro cotreatment ($\sim 5 \mu\text{g}/24 \text{ h}$ at both time points).

Fecal Zn excretion in controls was $743 \pm 30 \mu\text{g}/24 \text{ h}$ and rose ($P < 0.05$) to $5,650 \pm 856 \mu\text{g}/24 \text{ h}$ at week 1 and $5,994 \pm 1,268 \mu\text{g}/24 \text{ h}$ at week 4 ALDOST (see Fig. 1, bottom). In

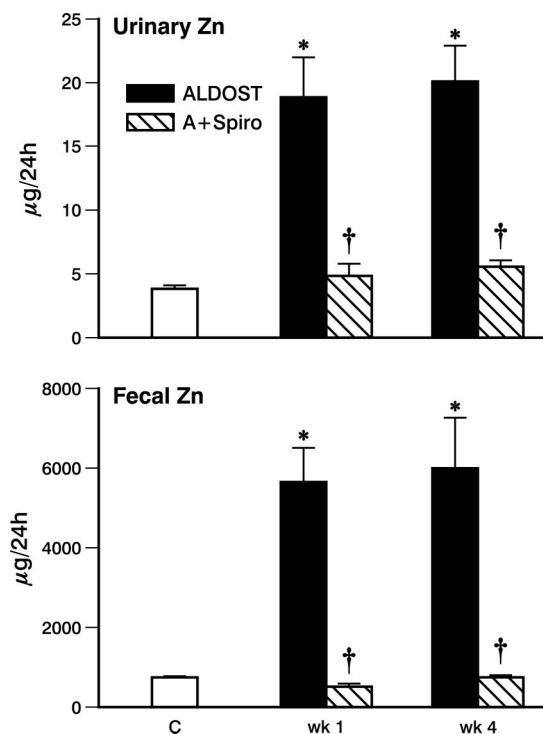


Fig. 1. Twenty-four hour urinary (top) and fecal (bottom) Zn excretion for controls (C) and at the end of weeks 1 and 4 of aldosterone/salt treatment (ALDOST), and aldosterone/salt treatment plus spironolactone (A + Spiro). * $P < 0.05$ for ALDOST vs. C; † $P < 0.05$ A + Spiro vs. ALDOST.

response to Spiro cotreatment, fecal Zn excretion was attenuated ($P < 0.05$) at *weeks 1* and *4* ALDOST (513 ± 77 and $747 \pm 52 \mu\text{g}/24 \text{ h}$, respectively).

Plasma Zinc Concentration and Cu/Zn-SOD Activity

Plasma Zn in controls was $81 \pm 3 \mu\text{g}/\text{dl}$, with all samples falling within the expected normal range of $75\text{--}140 \mu\text{g}/\text{dl}$ (49). It was reduced ($P < 0.05$) at *weeks 1* and *4* ALDOST by $\sim 50\%$ (see Fig. 2, *top*), in keeping with increased urinary and fecal Zn losses and a negative daily Zn balance. Cotreatment with Spiro attenuated ($P < 0.05$) this decline in plasma Zn at both time points, which was consistent with its reducing urinary and fecal Zn losses. A fall ($P < 0.05$) in plasma Cu/Zn-SOD activity seen at *weeks 1* and *4* ALDOST was also prevented ($P < 0.05$) by Spiro cotreatment (see Fig. 2, *bottom*).

Tissue Zinc

Heart. The Zn concentration in hearts of controls was $79 \pm 5 \text{ ng}/\text{mg}$ FFDT and remained unchanged at *week 1* ALDOST (see Fig. 3). At *week 4*, however, cardiac Zn rose ($P < 0.05$) to $93 \pm 3 \text{ ng}/\text{mg}$ FFDT. Cardiac Zn was no different from controls at *weeks 1* and *4* ALDOST in response to Spiro cotreatment (see Fig. 3).

Liver and skeletal muscle zinc. Tissue Zn levels in liver and skeletal muscle in controls were 111 ± 2 and $37 \pm 3 \text{ ng}/\text{mg}$ FFDT, respectively. These levels did not change significantly at *week 4* ALDOST (112 ± 5 and $48 \pm 7 \text{ ng}/\text{mg}$ FFDT, respectively) or in response to cotreatment with Spiro (95 ± 2 and $47 \pm 2 \text{ ng}/\text{mg}$ FFDT, respectively).

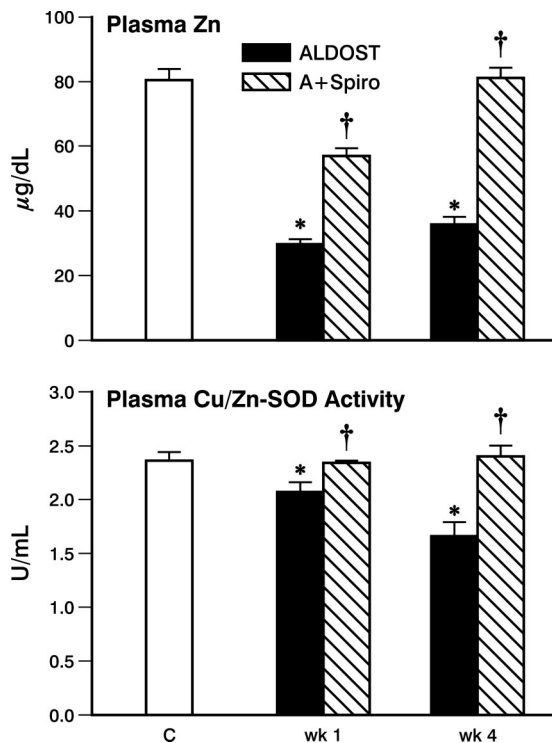


Fig. 2. Plasma Zn (*top*) levels fell ($*P < 0.05$) during *weeks 1* and *4* of ALDOST compared with C. This was attenuated ($\dagger P < 0.05$) with A + Spiro cotreatment. Plasma Cu/Zn-SOD activity (*bottom*) was reduced ($P < 0.05$ vs. C) during *weeks 1* and *4* ALDOST, which was prevented in A + Spiro at each time point.

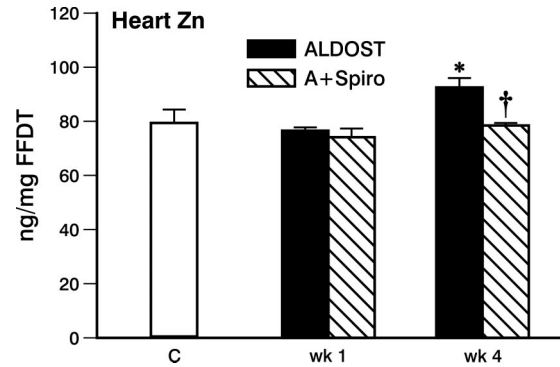


Fig. 3. Zn levels in the heart from C were no different at *week 1* ALDOST, but were increased ($*P < 0.05$ vs. C) at *week 4*. A + Spiro cotreatment prevented this response ($\dagger P < 0.05$ vs. ALDOST). FFDT, fat-free dry tissue.

Tissue MT-I

Heart. MT-I protein expression by Western blot analysis in control hearts was 75 ± 4 optical density (OD) (see Fig. 4, *top*) and was significantly ($P < 0.05$) increased at *week 1* (110 ± 9 OD) and *week 4* ALDOST (123 ± 9 OD), respectively. Spiro cotreatment prevented the increased expression of this Zn-binding protein at *weeks 1* and *4* ALDOST to suggest an ALDO-mediated response.

Liver and skeletal muscle. Tissue MT-I values for liver and skeletal muscle were 145 ± 10 OD and 180 ± 19 OD, respectively. These values were essentially unchanged during ALDOST (159 ± 16 and 192 ± 17 OD, respectively) or with Spiro cotreatment (152 ± 12 and 183 ± 18 OD, respectively).

Tissue Cu/Zn-SOD Activities

Heart. In hearts harvested from controls, Cu/Zn-SOD activity was $0.56 \pm 0.10 \text{ U}/\text{mg}$ protein (see Fig. 5), which represented more than 85% of total SOD activity found in heart tissue. This level remained unchanged at *week 1* ALDOST but increased ($P < 0.05$) to $0.93 \pm 0.12 \text{ U}/\text{mg}$ protein at *week 4*. In response to Spiro cotreatment, the rise in cardiac Cu/Zn-

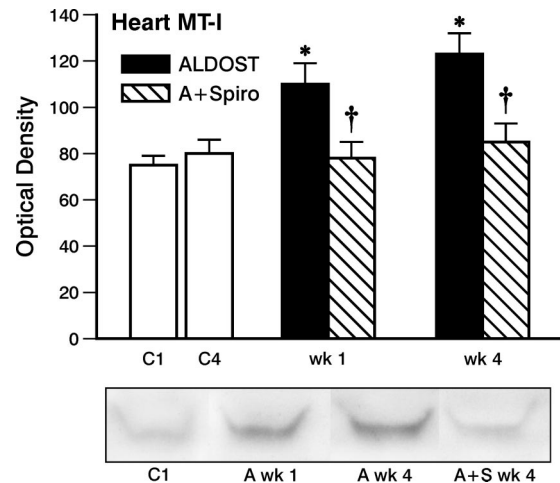


Fig. 4. *Top*: cardiac metallothionein (MT-I) in 9- and 12-wk-old controls (C1 and C4, respectively) was increased ($*P < 0.05$ vs. C1 and C4) ($\dagger P < 0.05$ vs. ALDOST) at *weeks 1* and *4* of ALDOST, which was not seen with A + Spiro cotreatment. A representative Western blot for MT-I is shown for C, 1 and 4 wks ALDOST, and 4 wks A + Spiro.

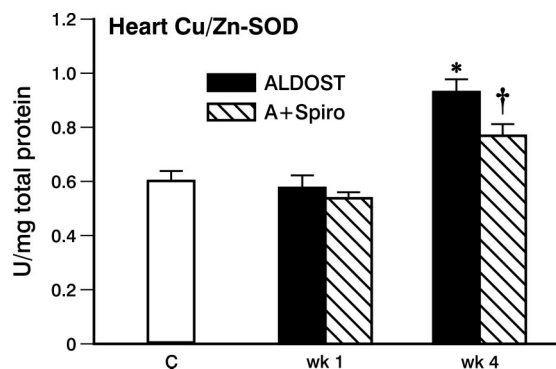


Fig. 5. Cu/Zn-SOD activity in the heart was first increased at *week 4* ALDOST (* $P < 0.05$ vs. C) and was attenuated by A + Spiro cotreatment († $P < 0.05$ vs. ALDOST).

SOD activity seen at *week 4* ALDOST was attenuated to 0.77 ± 0.04 U/mg protein ($P < 0.05$). Mn-SOD activity represented a smaller fraction of total SOD activity in control rats (0.08 ± 0.04 U/mg protein) and was unchanged from controls at *weeks 1* and *4* ALDOST (0.07 ± 0.02 and 0.07 ± 0.02 U/mg protein, respectively) alone or with Spiro cotreatment (0.09 ± 0.02 and 0.07 ± 0.02 U/mg protein, respectively).

Liver and skeletal muscle. Cu/Zn-SOD activities in liver and skeletal muscle were 1.27 ± 0.20 and 0.72 ± 0.16 U/mg protein, respectively, representing the predominant source of total SOD activity in these tissues (1.34 ± 0.20 and 0.79 ± 0.20 U/mg protein, respectively), and was unchanged during ALDOST at *weeks 1* and *4* alone or with Spiro cotreatment. The relatively small fraction of total SOD activity attributed to Mn-SOD activity in liver and skeletal muscle and was unchanged from controls (0.06 ± 0.02 and 0.07 ± 0.03 U/mg protein, respectively) during ALDOST or with Spiro cotreatment.

Cardiac Pathology

Evidence of a perivascular fibrosis involving intramural coronary arteries of the right and left ventricles was first seen at *week 4* of ALDOST. In addition to this reactive fibrosis, microscopic scars, representing a replacement fibrosis for necrotic cardiomyocytes, were also seen at this time point. Cotreatment with Spiro prevented the appearance of both the reactive and replacement fibrosis.

DISCUSSION

Our study led to several major findings. First, chronic aldosteronism is accompanied by increased 24-h urinary and fecal Zn^{2+} losses that appear early (*week 1*) and are persistent (*week 4*), contributing to the appearance of hypozincemia and reduction in plasma Cu/Zn-SOD activity, an established biomarker of Zn status (32, 33). These augmented losses of Zn are prevented by Spiro cotreatment. Mechanisms responsible for increased urinary and fecal losses of Zn in aldosteronism are not clear. An acidification of urine and feces by excessive renal production of ammonia, related to the regulation of aldosterone of a Na^+/H^+ exchanger at these sites, accounts for the characteristic metabolic alkalosis seen in aldosteronism (14, 20, 54). Acidification also contributes to Ca^{2+} losses in aldosteronism (13, 26).

Second, the reduction in plasma Zn seen with ALDOST is also related to a redistribution of Zn^{2+} from plasma to tissues.

The extent of this Zn^{2+} redistribution is heterogeneous among tissues and appears to be related to the differential expression of MT-I, a physiological Zn^{2+} binder and donor, at the site of tissue injury. Herein, we found an upregulation of MT-I expression in the heart, an early detoxifying protein involved in endogenous defenses, evoked by peptide and steroid hormones, including aldosterone, to protect cells against oxidative stress, which was prevented by Spiro cotreatment (49). The appearance of cardiac injury at *week 4* ALDOST, but not in uninjured liver and skeletal muscle, may represent yet another stimulus to MT-I overexpression and consequent Zn^{2+} redistribution in a tissue-specific manner, together with oxidative stress-induced MT-disulfide bond formation that favors the release of Zn at these sites (18, 30, 47). This redistribution of Zn^{2+} to the injured heart during aldosteronism is different from dietary Zn^{2+} deficiency, where Zn levels are reduced in plasma and tissues, and tissue MT expression is suppressed (16, 36). Spiro cotreatment prevented cardiac injury, oxidative stress, and thereby Zn^{2+} redistribution and MT-I expression during ALDOST. Leopold et al. (27) have recently reported that aldosterone-associated vasculopathy, characterized by increased oxidative stress and decreased bioavailability of nitrous oxide, may involve a deficiency of glucose-6-phosphate dehydrogenase and thereby impaired antioxidant defense mechanisms within the endothelium.

Reduced serum Zn levels appear during periods of stress (5, 48) in patients hospitalized with decompensated heart failure (6) and in those with hyperparathyroidism (29). Increased circulating parathyroid hormone (PTH) is accompanied by Zn^{2+} redistribution to tissues (12). Secondary hyperparathyroidism (SHPT) is a covariant of aldosteronism due to augmented urinary and fecal losses of Ca^{2+} and Mg^{2+} with subsequent reduction in plasma-ionized Ca^{2+} and Mg^{2+} concentrations, each providing potent stimuli to the elaboration of PTH by the parathyroid glands (6, 13, 23, 25, 37, 38, 50). Spiro prevents these heightened losses of Ca^{2+} and Mg^{2+} and thereby prevents SHPT (6, 13, 22, 38). In response to elevations in circulating PTH, there is a Ca^{2+} overloading of diverse tissues, including heart, skeletal muscle, platelets, and peripheral blood mononuclear cells that can be prevented by parathyroidectomy or Ca^{2+} channel blocker (1, 50). The excessive intracellular Ca^{2+} accumulation is accompanied by an induction of oxidative stress preventable by each of these interventions (1, 50). Enhanced Ca^{2+} entry can lead to a simultaneous rise in intracellular Zn^{2+} since one of the potential mechanisms of Zn entry involves voltage-sensitive Ca^{2+} channels (40). A 30–40% increase in rat cardiac tissue Ca^{2+} occurs at *week 4* of ALDOST (13, 50). This approximates the rise in cardiac tissue Zn we found in the present study and that was reported in hamsters with hypertrophic cardiomyopathy and hereditary muscular dystrophy, a disorder marked by intracellular Ca^{2+} overloading (15). Zinc also enters cells through Zn^{2+} -sensitive membrane transporters (40).

Our third major finding relates to a key antioxidant enzyme, Cu/Zn-SOD activity, involved in superoxide detoxification following cell injury that was increased in the injured heart at *week 4* ALDOST, in keeping with increased cardiac Zn concentration and MT-I overexpression. Increased cardiac MT-I expression is seen in mice in response to streptozocin-induced diabetic cardiomyopathy and in rats in response to cadmium or isoproterenol-induced cardiac injury (9, 41). In the silencing of

MT expression with a small interfering mRNA, this cardioprotective response is selectively abolished (51). Our findings, however, suggest oxidative stress overwhelmed endogenous antioxidant defenses and could not prevent cardiac injury. We have previously shown a Zn ionophore, pyrrolidine dithiocarbamate, to be cardioprotective during ALDOST (42). Unlike the heart, there was no microscopic evidence of fibrosis (or injury) in liver or skeletal muscle that replaced necrotic cells during ALDOST. We therefore speculate that the absence of increased Zn and MT-I overexpression in these tissues is in keeping with the absence of injury at these sites.

We are aware of certain limitations of our present study. In the setting of constant Zn losses, such as occurs via feces and urine in rats receiving ALDOST or via breast milk in lactating mothers, the need to replenish Zn must include increased dietary Zn intake. In our study, however, rats remained on standard chow with its fixed Zn content. In the absence of increased dietary sources, some other homeostatic mechanisms must be invoked to compensate for Zn losses. Such mechanisms include increased Zn absorption and reduced fecal and urinary excretion. However, persistent Zn losses accompany chronic ALDOST. Therefore, an alternative mechanism is needed to sustain Zn homeostasis during these losses to draw on endogenous pools of Zn, such as those found in bone and skin. Thirty percent of total body Zn is associated with bone, and bone resorption provides a source of circulating Zn (31). We have not monitored bone Zn content in the present study; however, our previous studies identified a marked reduction in bone mineral density, including Ca^{2+} and Mg^{2+} content, in rats with SHPT that accompanies ALDOST. In lactating mothers, maternal bone resorption and reduction in bone Zn have been seen (31). More specific studies using ^{65}Zn would address its preferential distribution in various tissues, including the source of increased tissue Zn seen at 4 wks ALDOST. Future studies are needed to address this. The distribution of Zn^{2+} , including cytosolic-free or biologically active Zn^{2+} in cardiomyocytes, expression and activity of Zn^{2+} importers and exporters, and a metal-responsive transcription factor in regulating intracellular Zn^{2+} kinetics must also be addressed. The utility of a Zn-supplemented diet to preserve Zn^{2+} balance would also be needed to explore these issues more thoroughly. Supplemental dietary Zn induces expression of MT-I and protects against daunorubicin-induced cardiac and liver toxicity in rats, and adriamycin-related cardiac injury in mice (3, 39). The role of a Ca^{2+} channel blocker in preventing intracellular Zn^{2+} loading and that of PTH, a calcitropic hormone, in promoting intracellular Zn^{2+} translocation should also provide mechanistic insights. Future studies are needed to address these issues.

In conclusion, the hypozincemia that accompanies aldosteronism is related to enhanced urinary and fecal losses of Zn and its redistribution from the circulation to injured cardiac tissue. Furthermore, aldosteronism is associated with the appearance of oxidative stress, MT-I overexpression, and increased Cu/Zn-SOD activity in the injured heart, which regulate Zn^{2+} distribution and antioxidant defenses. The Zn^{2+} dyshomeostasis seen in aldosteronism is not unlike the stress response that appears following injury to brain or lung and may, therefore, explain the reduction in serum Zn found in patients with decompensated heart failure (6) and contribute to the hitherto unappreciated efficacy of Spiro treatment in these patients (35).

Given that antioxidant defenses are dependent on this diet-derived vital trace mineral, its pathophysiological role in the genesis and progression of heart failure has been brought into question, including the potential role of dietary Zn^{2+} supplements in its overall management.

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