Loss of bone minerals and strength in rats with aldosteronism

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Chhokar, Vikram S., Yao Sun, Syamal K. Bhattacharya, Robert A. Ahokas, Linda K. Myers, Zhiqiang Xing, Richard A. Smith, Ivan C. Gerling, and Karl T. Weber. Loss of bone minerals and strength in rats with aldosteronism. Am J Physiol Heart Circ Physiol 287: H2023–H2026, 2004; doi:10.1152/ajpheart.00477.2004.—Congestive heart failure (CHF) is a clinical syndrome with origins rooted in a salt-avid state largely mediated by effector hormones of the circulating renin-angiotensin-aldosterone system. Other participating neurohormones include catecholamines, endothelin-1, and arginine vasopressin. CHF is accompanied by a systemic illness of uncertain causality. Features include the appearance of oxidative/nitrosative stress and a wasting of tissues including bone. Herein we hypothesized that inappropriate (relative to dietary Na+) elevations in plasma aldosterone (Aldo) contribute to an altered redox state, augmented excretion of divalent cations, and in turn, a loss of bone minerals and strength. In uninephrectomized rats that received chronic Aldo and 1% NaCl treatment for 4–6 wk, we monitored plasma α1-antiproteinase activity, which is an inverse correlate of oxidative/nitrosative stress; plasma concentrations of ionized Mg2+ and Ca2++; urinary Mg2+ and Ca2+ excretion; and bone mineral composition and strength to flexure stress. Compared with controls, we found reductions in plasma α1-antiproteinase activity and ionized Mg2+ and Ca2+ together with persistently elevated urinary Mg2+ and Ca2+ excretion, a progressive loss of bone mineral density and content with reduced Mg2+ and Ca2+ concentrations, and a reduction in cortical bone strength. Thus the hypermagnesuria and hypercalcemia that accompany chronic Aldo-1% NaCl treatment contribute to the systemic appearance of oxidative/nitrosative stress and a wasting of bone minerals and strength.

aldosterone; congestive heart failure; peripheral bone mononuclear cells; antiproteinase; parathyroid hormone

CONGESTIVE HEART FAILURE (CHF), a clinical syndrome with characteristic signs and symptoms, arises from circulating renin-angiotensin-aldosterone system activation (38) and is accompanied by a systemic illness that includes oxidative/nitrosative stress in the heart, systemic organs, circulating immune cells, and blood (10, 20, 34, 37) and a catabolic state with wasting of lean tissue, fat, and bone (2, 3, 22, 32). Aldosteronism in rats, which is defined as inappropriate (relative to dietary Na+) and chronic elevations in plasma aldosterone (Aldo) comparable to those seen in CHF, ultimately leads to yet another component of this illness, namely, bone wasting.

MATERIALS AND METHODS

Male 8-wk-old Sprague-Dawley rats (Harlan, IN) were used in this study, which was approved by the institution’s Animal Care and Use Committee. Unoperated and untreated age- and gender-matched rats served as controls. Uninephrectomized rats received Aldo (0.75 μg/h) via implanted minipump (Alzet; Cupertino, CA) together with 1% NaCl and 0.4% KCl in drinking water (Aldost) and standard laboratory diet (Harlan Tekland 2215 Rodent Diet). Plasma α1-antiproteinase (α1-AP) was assessed using the α1AP-410 assay system (Oxis Research; Portland, OR; Ref. 16). Concentrations of plasma ionized Mg2+ and Ca2+ were determined via the direct ion-selective electrode technique using a Nova 8 Analyzer (Nova Biomedical; Waltham, MA).

For the study of urinary Mg2+ and Ca2+ excretion, each animal was placed in a minerally decontaminated and distilled-deionized water-rinsed metabolic cage and allowed to continue to drink water that contained 1% NaCl. Food was withheld during the 24-h period of urine collection. Urine was frozen for later determination of Mg2+ and Ca2+ quantities. After each use, the cages were manually cleaned with deionized water, and all nonmetallic parts were washed with diluted (~3 N) hydrochloric acid and rinsed again with distilled-deionized water. Urinary Mg2+ and Ca2+ concentrations were determined as reported previously (7) using an atomic absorption spectrophotometer. By multiplying the respective concentrations (in μg/ml) by the 24-h urine volumes (in ml/24 h), we calculated the urinary excretion rates (expressed in μg/24 h).

Bone mineral density (BMD) and bone mineral content (BMC) values were determined for excised, manually cleaned tibias and femurs via peripheral dual-energy X-ray absorptiometry using GE Lunar PIXImus2 (GE Healthcare; Fairfield, CT) as previously validated for rat leg bones (25). Quality control and calibration were carried out within 24 h before each scanning period. Total tibia concentrations of Mg2+ and Ca2+ were determined using the atomic absorption spectrophotometer (Ref. 1; expressed in μg/mg of fat-free dry bone).

An Instron Universal Test System (Instron; Canton, MA) was used for mechanical testing (12). Flexure stress of the femurs was determined by breaking the bones with three-point bending. The femurs broke in the middiaphyseal region, and data were recorded as flexure stress in megapascals.

Values are presented as means ± SE. Data were analyzed with ANOVA. Significant differences between individual means were
determined using the post hoc Bonferroni multiple-comparisons test. Significance was assigned to $P < 0.05$.

RESULTS AND DISCUSSION

This study led to several major findings. The first is the presence of an altered redox state during Aldost. The $\alpha_1$-AP, which is a major inhibitor of serine proteinases, is inactivated by peroxynitrite and hypochlorous acid. In control rats, plasma $\alpha_1$-AP activity was 39.8 ± 2.1 μmol/min; it was significantly reduced (24.3 ± 1.2 μmol/min) at 4 wk Aldost. This decrease in plasma $\alpha_1$-AP activity is in keeping with the presence of reactive oxygen and nitrogen species at a systemic level (40). Although $\alpha_1$-AP is frequently used as a biomarker to assess the presence of such species, we would caution that other factor(s) (e.g., a deficiency in $\alpha_1$-AP production and secretion as seen in hepatic disease or damage) could, albeit unlikely, account for some of the decreased plasma activity we found with Aldost. The cellular origin of this altered redox state is not well understood and likely involves multiple tissues such as heart and skeletal muscle, which have been reported as sites of oxidative/nitrosative stress in CHF (10, 20, 34, 37). We have understood and likely involves multiple tissues such as heart and skeletal muscle, which have been reported as sites of oxidative/nitrosative stress in both PBMCs and in the monocyte-phagocyte system based on the appearance of oxidative/nitrosative stress in CHF (10, 20, 34, 37). We have

The pathogenic basis for the induction of oxidative/nitrosative stress in PBMCs during Aldost has been linked to the reduced cytosolic free Mg$^{2+}$ concentration ([Mg$^{2+}$]) together with intracellular Ca$^{2+}$ loading (1, 16) of these cells. In human lymphocytes, a Na$^+$-dependent reduction in [Mg$^{2+}$] appears in response to incubation with physiological concentrations of Aldo (13). This response is abrogated by an Aldo receptor antagonist, a transcription inhibitor, as well as by a protein-synthesis inhibitor; a Na$^+$/[Mg$^{2+}$] exchanger was implicated as the responsible mechanism (13). Lymphocyte [Mg$^{2+}$], is reduced in patients with primary aldosteronism (13). Findings in our study raise the prospect that additional factors could regulate intracellular concentrations of these PBMC cations (see below) and will need to be considered in future studies.

In control rats, the plasma ionized Mg$^{2+}$ was 0.35 ± 0.02 mmol/l. At 4 wk of Aldost, the ionized Mg$^{2+}$ was significantly reduced to 0.19 ± 0.02 mmol/l ($P < 0.05$). Likewise, the plasma ionized Ca$^{2+}$ at 4 wk of Aldost (0.70 ± 0.02 mmol/l) was less than the level found in unoperated controls (0.96 ± 0.03 mmol/l; $P < 0.05$). These reductions in plasma ionized Mg$^{2+}$ and Ca$^{2+}$ accompanied the marked increase in 24-h urinary Mg$^{2+}$ and Ca$^{2+}$ excretion levels during Aldost (see Table 1). The observed hypermagnesuria and hypercalciumia was persistent over 4–6 wk of Aldost and represent a second major finding of this study. Short-term studies in rats, dogs, and humans using such mineralocorticoid hormones as either deoxycorticosterone or fludrocortisone together with dietary Na$^+$ loading have each demonstrated the dietary Na$^+$-dependent increase in urinary Ca$^{2+}$ excretion that occurs within the terminal segment of the nephron (8, 15, 24, 35, 42). Similar findings have been noted in humans with primary aldosteronism (27). Others (6, 21, 29) have shown that such a regimen in rats and patients with primary aldosteronism increases the urinary excretion of Mg$^{2+}$, which normalizes after surgical removal of the aldosterone-producing adenoma.

Table 1. Daily urine volume and urinary Mg$^{2+}$ and Ca$^{2+}$ excretion in response to Aldost

<table>
<thead>
<tr>
<th>Urine Volume, ml/24 h</th>
<th>Urinary Excretion Rate, μg/24 h</th>
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<tbody>
<tr>
<td>Control</td>
<td>Aldost</td>
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<tr>
<td>Control</td>
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Values are means ± SE; $n = 5$ rats. Aldost, treatment with aldosterone; NaCl, and KCl. *$P < 0.05$, all Aldost values statistically different from controls.

Table 2. Tibial and femoral BMD and BMC in response to Aldost

<table>
<thead>
<tr>
<th>BMD, g/cm$^2$</th>
<th>BMC, g/cm$^2$</th>
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<tbody>
<tr>
<td>Tibial</td>
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<td>Tibial</td>
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Values are means ± SE; $n = 5$ rats. BMD, bone mineral density; BMC, bone mineral content. *$P < 0.05$, all Aldost values significantly different from controls.

Table 3. Tibial total Mg$^{2+}$ and Ca$^{2+}$ concentrations in response to Aldost

<table>
<thead>
<tr>
<th>Total Mg$^{2+}$, μg/mg FFDB</th>
<th>Total Ca$^{2+}$, μg/mg FFDB</th>
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<tbody>
<tr>
<td>Control</td>
<td>Aldost</td>
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Values are means ± SE; control, $n = 6$; Aldost, $n = 5$ rats. FFDB, fat-free dry bone. *$P < 0.05$, all Aldost values statistically different from controls.

Fig. 1. Three-point bending data from isolated rat femurs. Rats received aldosterone (0.75 μg/h) together with 1% NaCl and 0.4% KCl in drinking water (Aldost). Data are means ± SD expressed in megapascals (MPa); $n = 5$ rats. *$P < 0.05$, statistically different from control.
removal of diseased adrenal tissue. Although the stimulus for the hypermagnesuria and hypercalciuria is uncertain, it is not related to elevations in arterial pressure (24, 35) or the mineralocorticoid hormone per se in the absence of dietary Na+ (35); it appears despite enhanced secretion of parathyroid hormone (PTH; Ref. 6). In rats and humans, marked dietary salt loading alone (e.g., 8% NaCl) is accompanied by hypercalciuria (11, 17, 23, 31, 33). In either setting, an expansion of the extravascular space is considered to be the likely mechanism leading to increased urinary excretion of these divalent cations (24, 35), which may be facilitated by nitric oxide-mediated increments in medullary blood flow (18, 44).

A third and hitherto unreported finding is the adverse effect of Aldost on bone. Coincident with marked urinary loss of Mg2+ and Ca2+ are rapid and profound reductions in BMD and BMC of both the tibia and femur (see Table 2). This loss of bone minerals was sustained over 4–6 wk of Aldost. We found comparable reductions in tibial total Mg2+ and Ca2+ concentrations (see Table 3). At week 6 of Aldost, this amounted to nearly a 50% reduction in the concentration of these divalent cations. Similar reductions in bone Mg2+ concentration together with systemic evidence of oxidative stress have been observed with dietary Mg2+ deficiency (30, 39). In conjunction with reduced BMD and BMC, there was a decrease in bone strength during Aldost compared with controls (Fig. 1). Cortical bone strength, as measured by three-point bending of the middiaphyseal region of the femur, was significantly reduced at weeks 5 and 6 of Aldost. In this study, whole femoral BMD decreased by 22% and BMC by 32% at 6 wk of Aldost compared with controls. The BMD and BMC levels for tibial bones decreased even more dramatically (53% and 71%, respectively). The femur contains more cortical bone, which may account for its losing less BMD and BMC. The decrease in BMD and BMC for the tibias also indicated a decrease in the overall size of the bones. The three-point bending measurements at 6 wk of Aldost showed a 30% decrease in femoral cortical bone strength (middiaphyseal region) compared with control rats. This region is almost entirely cortical bone and is an area of slow turnover compared to trabecular bone. The 30% decrease in cortical bone strength shown here is dramatic. The compressive strength of trabecular bone is roughly proportional to the square of its apparent density (9, 19). If bone density decreases by a factor of 2, the compressive strength decreases by a factor of 4. It is difficult to know fracture threshold from a rat model; however, the decreases in BMD and bone strength shown in this study are remarkable.

The urinary loss of Mg2+ and Ca2+ that accompanied Aldost will have profound systemic effects that were not addressed in the present study. It can be assumed that the loss of these divergent cations and reductions in their plasma ionized concentrations would induce hormonal responses that attempt to maintain plasma Mg2+ and Ca2+ homeostasis. These responses would include enhanced PTH secretion and formation of biologically active vitamin D metabolites. As noted above, we found reduced concentrations of serum ionized Mg2+ and Ca2+ at 4 wk of Aldost; this would serve to upregulate PTH secretion. Others have shown that plasma ionized Ca2+ and total plasma Ca2+ concentrations are reduced in rats receiving deoxycorticosterone and salt treatment (5, 41, 42), serum PTH and urinary excretion of cAMP are increased, which is a marker of parathyroid activity, and the presence of urinary hydroxyproline, which is a marker of bone resorption (4, 5). Endocrine properties of circulating PTH may be involved in the loss of bone minerals (5). PTH regulates renal production of 1,25(OH)2D3, which would serve to enhance gastrointestinal absorption of Ca2+. Increased gastrointestinal Ca2+ absorption, mediated by 1,25(OH)2D3, accompanies the hypercalciuria induced by deoxycorticosterone and salt treatment of several weeks duration (42). These early compensatory responses, however, may not be adequate to preserve plasma ionized Ca2+ concentrations during prolonged Aldost, as we noted herein, and this would represent a continuous stimulus to increased PTH secretion. As such, hyperparathyroidism has been observed in association with primary aldosteronism (14). Improvements in serum ionized Ca2+ and circulating PTH levels occur after adrenal surgery in primary aldosteronism (28, 29). Additional studies are needed to systematically address these important issues including the role of these hormones in regulating PBMC Mg2+ and Ca2+.

The ramifications of this study to the secondary aldosteronism found in human CHF could explain, at least in part, the appearance of a systemic illness with altered redox state and tissue wasting, including bone. Our findings also raise the possibility that older patients who have CHF with aldosteronism may be at increased risk for bone fracture. The large and rapid decrease in BMD and bone strength makes investigation of Aldo and sodium in humans essential. Even short-term hyperaldosteronism in humans may have significant effects on osteoporosis and risk of fracture later in life. Also, the effects of drugs such as bisphosphonates, which are used to treat various bone metabolism disorders by reducing Ca2+ lost by bone, should be investigated for their effects in patients with CHF. Bone loss together with elevated PTH levels are often found in normocalcemic patients with advanced CHF who await cardiac transplantation (3, 22, 32). Increased circulating levels of PTH and PTH-related protein have also been observed in patients with treated heart failure of less-advanced severity (26, 43). The pathogenesis of this secondary hyperparathyroidism, albeit uncertain and likely multifactorial, has included considerations of dietary Ca2+ deficiency, prerenal azotemia, hypovitaminosis D, reduced cardiac output, and the use of furosemide, which is a potent loop diuretic that promotes the excretion of divalent cations (32). On the basis of the findings of our study, we suggest that the consequences of Aldo (together with dietary NaCl) on urinary Mg2+ and Ca2+ excretion could also be contributory.

GRANTS
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