Association of Circulating Renin and Aldosterone With Osteocalcin and Bone Mineral Density in African Ancestry Families

Allison L. Kuipers, Candace M. Kammerer, J. Howard Pratt, Clareann H. Bunker, Victor W. Wheeler, Alan L. Patrick, Joseph M. Zmuda

Abstract—Hypertension is associated with accelerated bone loss, and the renin–angiotensin–aldosterone system is a key regulator of blood pressure. Although components of this system are expressed in human bone cells, studies in humans are sparse. Thus, we studied the association of circulating renin and aldosterone with osteocalcin and bone mineral density. We recruited 373 African ancestry family members without regard to health status from 6 probands (mean family size: 62 and relative pairs: 1687). Participants underwent a clinical examination, dual-energy x-ray absorptiometry, and quantitative computed tomographic scans. Renin activity, aldosterone concentration, and osteocalcin were measured in fasting blood samples. Aldosterone/renin ratio was calculated as aldosterone concentration/renin activity. All models were analyzed using pedigree-based variance components methods. Full models included adjustment for age, sex, body composition, comorbidities, lifestyle factors, blood pressure, and antihypertensive medication. Higher renin activity was significantly associated with lower total osteocalcin and with higher trabecular bone mineral density (both P<0.01). There were also significant genetic correlations between renin activity and whole-body bone mineral density. There were no associations with aldosterone concentration in any model and results for aldosterone/renin ratio were similar to those for renin activity. This is the first study to report a significant association between renin activity and a marker of bone turnover and bone mineral density in generally healthy individuals. Also, there is evidence for significant genetic pleiotropy and, thus, there may be a shared biological mechanism underlying both the renin–angiotensin–aldosterone system and bone metabolism that is independent of hypertension. (Hypertension. 2016;67:00-00. DOI: 10.1161/HYPERTENSIONAHA.115.06837.) • Online Data Supplement

Key Words: Aldosterone • Bone Density • Hypertension • Osteocalcin • Renin

The renin–angiotensin–aldosterone system (RAAS) regulates blood pressure and fluid balance. The RAAS is a major contributor to essential hypertension, and inhibitors of this system are some of the most common treatments for hypertension. Hypertension, or high blood pressure, has been associated with osteoporosis and low bone mass in some studies. Components of the RAAS are expressed in human bone cells and can activate a local RAAS response that leads to increased bone turnover and decreased bone density. Indeed, there is also evidence for shared genetic determinants of the RAAS and bone mineral density (BMD).

Research on the RAAS and bone metabolism in humans has focused on patients with primary aldosteronism (PA) or on the effect of RAAS inhibitor therapies on bone. The majority of studies on the effect of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers on bone, including those in humans and animal models, suggest that pharmacological inhibition of the RAAS pathway can lead to decreased fracture risk and increased bone mass. However, some studies in humans have found the opposite association. There have only been 2 studies, each with <50 participants, which examined the association of circulating RAAS measures with BMD in humans. Both studies found that renin activity was directly correlated with BMD, although it is unclear if the results are not be generalizable to healthy cohorts because they were conducted in highly selected samples of hemodialysis patients or premenopausal women. Therefore, in this study, we aimed to expand on these previous studies by using a larger family study of generally healthy men and women to further assess the association of circulating RAAS measures, including plasma renin activity (PRA), plasma aldosterone concentration (PAC), and the aldosterone/
The Modification of Diet in Renal Disease Study formula was used. Assay variation was 4.8% for total osteocalcin. Serum creatinine, carboxylated and uncarboxylated osteocalcin equivalently. Intra-

The study was approved by the Tobago Division of Health and Boards. All procedures followed the principles of the Declaration of Helsinki, and were in accordance of institutional guidelines.

Study Sample
Participants for this analysis were from the Tobago Family Health Study. Briefly, 8 individual probands were identified and recruited for the study without regard to their medical history on the Caribbean island of Tobago. Each proband was eligible if they had a spouse willing to participate and had at least 6 living offspring or siblings aged ≥18 years and who were residing in Tobago. All first-, second-, and third-degree relatives of the probands and their spouses were invited to participate. Participants had extensive clinical examinations, medical history interviews, and BMD assessment. This analysis includes data on 373 individuals recruited from 6 large families (mean family size=62; relative pairs=1687) who have complete data on osteocalcin, BMD, and RAAS measures, which include PRA, PAC, and ARR. Written informed consent was obtained from each participant. The study was approved by the Tobago Division of Health and Social Services and the University of Pittsburgh Institutional Review Boards. All procedures followed the principles of the Declaration of Helsinki and were in accordance of institutional guidelines.

Blood Sample Assays
Plasma and serum samples were collected on all individuals in the morning after 8+ hours of fasting and then frozen at −80°C at the time of collection until analysis. The PRA (ng/mL per hour) was measured using a Clinical Assays GammaCoat radioimmunoassay kit (Baxter Healthcare, Cambridge, MA). The PAC (ng/dL) was measured by radioimmunoassay with antisera from Diagnostic Products Corp (Los Angeles, CA). The ARR (ng/dL per mg/L per hour) was calculated as PAC/PRA.

Serum concentrations of total osteocalcin were measured, in duplicate, in previously unlabeled specimens by radioimmunoassay, as previously described elsewhere. Briefly, osteocalcin is measured in serum using a radioimmunoassay using an antibody made against osteocalcin purified from human bone. This antibody recognizes both carboxylated and uncarboxylated osteocalcin equivalently. Intra-assay variation was 4.8% for total osteocalcin. Serum creatinine was quantitatively determined by the VITROS CREA Slide method. The Modification of Diet in Renal Disease Study formula was used to estimate glomerular filtration rate (GFR) as: (ml/min per 1.73 m²) = 175 × (serum creatinine [mg/dL] − 0.193 × [age years] − 0.098 if female) ÷ (1.212, because they are all of African ancestry).

BMD Measures
Integral areal BMD at the proximal femur were measured by dual-energy x-ray absorptiometry using a Hologic QDR-4500W densitometer (Hologic Inc, Bedford, MA). The short-term in vivo precision of the dual-energy x-ray absorptiometry measurements for 12 subjects were all ≤1.16%. Trabecular and cortical volumetric BMD at the left tibia was measured by peripheral quantitative computed tomography using an XCT-2000 scanner (Stratec Medizintechnik, Pforzheim, Germany). A single axial slice of 2.5-mm thickness with a voxel size of 0.5 mm and a speed of 20 mm/s was taken at 33% (cortical) and 4% (trabecular) of the tibia length sites. Image processing was performed using the Stratec software package (version 5.5E). The short-term in vivo precision of the peripheral quantitative computed tomography measurements for 15 subjects ranged from 0.65% (cortical BMD) to 2.1% (trabecular BMD).

Covariates
Demographic, lifestyle and medical history variables were collected by trained clinic staff through administration of a questionnaire and interview. Blood pressure was measured while seated 3x throughout the visit. The average of the second and third readings for systolic and diastolic blood pressures is used in this analysis. Thirty-six participants were on antihypertensive medication including 7 on angiotensin-converting enzyme inhibitors, 6 on thiazide diuretics, 5 on methyldopa, 2 on calcium channel blockers, 1 on a β-blocker, 4 on combination therapy, and 11 reported being on antihypertensive medication but did not bring it to the clinic visit. Diabetes mellitus was defined as a fasting glucose level ≥126 mg/dL or current use of diabetes mellitus medication. Smoking status was classified as either current or not (yes/no). Participants reporting ever smoking <100 cigarettes in their lifetime were considered nonsmokers. Alcohol consumption was assessed by questionnaire and was defined as having ≥3 drink per week (yes/no) as there was a low prevalence of substantial alcohol intake. Physical activity was dichotomized active or not active after asking participants if they had walked for exercise at all in the past week.

Statistical Analysis
Trait distributions were assessed and, if necessary, transformed by natural logarithms to reduce non-normality. Outliers, defined as ±4 SD from the mean, were removed for each trait to reduce undue influence, and no more than 2 observations were removed from any trait. Means with SDs and medians with interquartile range were calculated for normal and non-normal traits, respectively. All analyses of mean differences between men and women were tested using the variance components framework implemented in the Sequential Oligogenic Linkage Analysis Routines program that accounts for complex familial relationships. Sequential Oligogenic Linkage Analysis Routines not only controls for this correlated structure but also estimates the proportion of the trait variation that is attributable to genetic, covariate, and error effects.

To determine the association of RAAS measures with osteocalcin and BMD, we calculated the percent difference in osteocalcin or BMD per 1 SD increased RAAS measure (calculated as RAAS measure [β]-specificified mean osteocalcin or BMD <100%). These analyses were first done without covariates (unadjusted model). Next, we incorporated major determinants of bone (base model), including age, sex, height, and body weight. Finally, to account for all potential confounding of these relationships, we added additional parameters (multivariable model) for menopausal status, systolic blood pressure, diastolic blood pressure, hypertensive medication, estimated GFR, diabetes mellitus, current smoking, drinking, walking, and calcium supplementation.

To assess the potential shared genetic covariance between RAAS and bone measures, we estimated the residual heritability (H²) and genetic correlations (ρ G) in Sequential Oligogenic Linkage Analysis Routines. All heritability and genetic correlations were estimated while simultaneously incorporating all multivariable covariates used in the full, multivariable model. We first estimated the residual heritability (that is, the genetic heritability estimated after removing known covariate effects) and next determined the extent of genetic correlation between the variance components of PRA and ARR and osteocalcin and BMD measures.

The statistical significance of ρ G ≠ 0 was tested by a likelihood ratio test of models in which the parameter was or was not constrained.

Results
Study Characteristics
The mean age across family members was 42 years with a range of 18 to 86 years; 62% were females (Table 1). On the basis of mean BMI, participants were overweight and women were more overweight than men (P<0.0001). Women also had a higher prevalence of antihypertensive medication use compared with men (12.6% versus 4.9%; P=0.01), although men had higher systolic blood pressure (P=0.01). Compared with men, women had lower kidney function as assessed by estimated GFR (P=0.04) and a higher prevalence of type II
diabetes mellitus \( (P=0.03) \). However, men were more likely to have poorer lifestyle habits including current smoking, drinking \( \geq 3 \) alcoholic beverages a week and use of calcium supplementation. Women had greater PAC \( (P=0.01) \) than men but similar PRA and ARR (Table 2). Although osteocalcin was similar between men and women, women had lower whole-body, total hip, and trabecular BMD than men \( (P<0.0001 \) for all).

**Association of RAAS Measures With Osteocalcin and BMD**

Higher PRA was associated with lower total osteocalcin in unadjusted, minimally adjusted, and fully adjusted models (Table 3). Specifically, in fully adjusted multivariable models, a 1-SD increase in PRA was associated with \( \approx 10\% \) lower osteocalcin \( (P<0.0001; \) Table S1 in the online-only Data Supplement). PRA was not associated with whole-body, total hip, or cortical BMD in any model \( (P>0.05 \) for all). However, PRA was associated with trabecular BMD in all models. We found that a 1 SD greater PRA was associated with 1.6\% greater trabecular BMD in fully adjusted models \( (P=0.01) \). However, PRA was not associated with trabecular BMD in all models. We found that a 1 SD greater PRA was associated with a 1.6\% greater trabecular BMD in fully adjusted models \( (P=0.01) \). Similarly, a 1 SD greater ARR was associated with 1.7\% lower trabecular BMD \( (P=0.01) \) but was not associated with other BMD measures (Table S1). There was also no evidence of a statistically significant interaction effect of PRA or ARR on BMD or osteocalcin by hypertension status \( (P>0.1; \) data not show). There was no significant association of PAC concentrations with osteocalcin or BMD (Table S1).

**Genetic Correlations of RAAS Measures With Osteocalcin and BMD**

PRA, ARR, osteocalcin, and BMD measures were each significantly heritable (Tables 4; Table S2). PRA had a significant, negative genetic correlation with whole-body BMD \( (\rho_G=-0.40; \) \( P=0.02) \), whereas ARR had significant, positive genetic correlation with total osteocalcin \( (\rho_G=0.32; \) \( P=0.02; \) Table S2). Because there were no significant phenotypic associations of PAC with osteocalcin or BMD, we did not perform further genetic correlation analyses for these measures.

**Discussion**

We found that higher PRA or lower ARR, but not PAC, was associated with higher trabecular BMD and lower osteocalcin in African ancestry families. In addition to these phenotypic associations, this is the first study to report significant genetic pleiotropy between RAAS measures and indices of bone health. We assessed and controlled for potential confounding factors in our analyses and all results were independent of hypertension and its treatment, demographic characteristics, comorbidities, and lifestyle factors. Taken together, these findings suggest that RAAS measures and bone metabolism may be physiologically linked independent of blood pressure and hypertension.

Two existing studies\(^{19,20}\) reported a direct correlation between PRA and BMD, which is consistent with our findings. These findings in humans are also consistent with observations in a mouse model.\(^1\) Ours is the first study to examine the possible relationship between RAAS measures and cortical and trabecular bone in humans. We found that the association of PRA with BMD seems to be specific for trabecular bone, which has also been suggested by mouse models of osteoporosis.\(^{27,28}\) We saw similar results, though in the expected opposite direction (as PRA increases, ARR decreases), for ARR. This suggests that both renin activity and the relative levels of renin to aldosterone may be important for skeletal health, though aldosterone alone does not seem to be a significant factor.

To further evaluate the relationship of RAAS measures with bone, we assessed whether there was an association...
with osteocalcin, a major protein component of bone matrix that is released into the circulation during bone turnover.29 Thus, higher serum osteocalcin is associated with more bone turnover and, generally, lower BMD.29 Although there are no previous reports of a link between RAAS measures and osteocalcin in humans, glucocorticoid activation of the RAAS in trabecular bone increases circulating osteocalcin and osteoporosis risk in rabbits.28 Our results show that PRA and ARR are much more strongly associated with circulating osteocalcin levels than BMD and in a direction consistent with the BMD findings. Therefore, we hypothesize that renin may have a primary effect on osteocalcin, which in turn leads to secondary effects on BMD. Additional studies will be needed to test this hypothesis more directly.

Individuals with PA, a major source of secondary hypertension, have lower BMD than controls.7–9 Although we had no individuals with PA in our study, we found no significant association between normal PAC and measures of bone metabolism. However, we did find significant associations of the ARR, which is a marker of PA, with osteocalcin and BMD measures. Greater ARR was associated with lower BMD, consistent with studies in patients with PA.7–9 We also found that greater ARR was significantly associated with greater osteocalcin (P<0.0001), and there was significant genetic pleiotropy underlying these measures. Our findings raise the possibility that BMD may only be affected at clinically high levels of aldosterone or when RAAS pathway homeostasis is disrupted.

The potential mechanisms underlying these associations are not clear, but it seems that at least part of the association is because of shared genetic determinants. The significant genetic correlation between PRA and ARR with osteocalcin and BMD suggests that some of the genetic variation that affects RAAS measures also influences bone metabolism. These findings replicate results from a broader study of genetic correlation throughout the human genome, which found evidence of shared genetic determinants between RAAS measures and BMD.5 However, there is also evidence that there is a direct interaction of these circulating factors in the skeletal environment. In model organisms, local activation of the RAAS on bone cells activates angiotensin II receptors expressed in osteoblasts.1 This activation leads to increased osteoclastogenesis and bone turnover, and, thus, to lower bone mass.1,27,28 This mechanistic work in animals is further strengthened by studies that show inhibition of the RAAS pathway using angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers leads to decreased fracture risk and increased bone mass in humans.10–13,16–18 The findings of this article are consistent with these previous conclusions, and extend them in a larger study of healthy adults who were recruited without regard to hypertension or skeletal health status.

### Table 2. Means and SDs of RAAS, Bone Mineral Density, and Osteocalcin Measures in African Ancestry Families

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall (n=373)</th>
<th>Men (n=143)</th>
<th>Women (n=230)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma renin activity, ng/mL per h*</td>
<td>3.0 (1.0–6.2)</td>
<td>2.9 (0.9–5.6)</td>
<td>3.1 (1.1–6.5)</td>
<td>0.721</td>
</tr>
<tr>
<td>Plasma aldosterone concentration, ng/dL*</td>
<td>9.0 (5.6–13.4)</td>
<td>8.5 (5.4–11.7)</td>
<td>9.5 (5.7–15.2)</td>
<td>0.014</td>
</tr>
<tr>
<td>Aldosterone/renin ratio, ng/dL per ng/mL per h*</td>
<td>3.1 (1.3–8.3)</td>
<td>3.2 (1.3–8.1)</td>
<td>3.0 (1.3–8.5)</td>
<td>0.613</td>
</tr>
<tr>
<td>Total osteocalcin, μg/L</td>
<td>4.50±2.81</td>
<td>4.59±2.85</td>
<td>4.45±2.79</td>
<td>0.396</td>
</tr>
<tr>
<td>Whole body BMD, g/cm²</td>
<td>1.19±0.13</td>
<td>1.26±0.11</td>
<td>1.15±0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total hip BMD, g/cm²</td>
<td>1.11±0.17</td>
<td>1.19±0.14</td>
<td>1.06±0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cortical BMD, g/cm²</td>
<td>1182±30</td>
<td>1181±25</td>
<td>1184±22</td>
<td>0.418</td>
</tr>
<tr>
<td>Trabecular BMD, g/cm²</td>
<td>251±36</td>
<td>264±35</td>
<td>243±34</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMD indicates bone mineral density; and RAAS, renin–angiotensin–aldosterone system.

*Renin and aldosterone are displayed as median (interquartile range) because of non-normality.

### Table 3. Association of PRA With Serum Osteocalcin and BMD in African Ancestry Families

<table>
<thead>
<tr>
<th>Bone Measure</th>
<th>Model</th>
<th>Difference (%) in Bone Measure per 1 SD PRA*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total osteocalcin</td>
<td>Unadjusted</td>
<td>−6.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Base†</td>
<td>−6.90</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Multivariable‡</td>
<td>−8.26</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Whole-body BMD</td>
<td>Unadjusted</td>
<td>−0.15</td>
<td>0.803</td>
</tr>
<tr>
<td></td>
<td>Base†</td>
<td>0.25</td>
<td>0.617</td>
</tr>
<tr>
<td></td>
<td>Multivariable‡</td>
<td>0.51</td>
<td>0.301</td>
</tr>
<tr>
<td>Total hip BMD</td>
<td>Unadjusted</td>
<td>0.59</td>
<td>0.462</td>
</tr>
<tr>
<td></td>
<td>Base†</td>
<td>1.19</td>
<td>0.067</td>
</tr>
<tr>
<td></td>
<td>Multivariable‡</td>
<td>1.19</td>
<td>0.070</td>
</tr>
<tr>
<td>Cortical BMD</td>
<td>Unadjusted</td>
<td>−0.11</td>
<td>0.445</td>
</tr>
<tr>
<td></td>
<td>Base†</td>
<td>−0.17</td>
<td>0.195</td>
</tr>
<tr>
<td></td>
<td>Multivariable‡</td>
<td>−0.14</td>
<td>0.244</td>
</tr>
<tr>
<td>Trabecular BMD</td>
<td>Unadjusted</td>
<td>1.78</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>Base†</td>
<td>1.94</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Multivariable‡</td>
<td>1.61</td>
<td>0.012</td>
</tr>
</tbody>
</table>

BMD indicates bone mineral density; and PRA, plasma renin activity.

*Values shown are the percent difference in bone measure per 1 SD (4.5 ng/mL per h) greater PRA.

†Base model includes adjustment for age, sex, height, and weight.

‡Multivariable model includes adjustment for age, sex, height, weight, menopausal status, systolic blood pressure, diastolic blood pressure, hypertensive medication, estimated glomerular filtration rate, diabetes mellitus, current smoking, drinking, walking, and calcium supplementation.
Although we had the ability to assess many potential confounders including lifestyle habits, comorbidities and hypertension, there remain several potential study limitations. First, we did not have data on dietary intake of vitamin K, which may be an important confounder as it is a cofactor required for carboxylation of osteocalcin.\(^\text{10}\) However, as our study uses total osteocalcin level, not only carboxylated osteocalcin, we do not think vitamin K would have a major effect on our findings. Our study was relatively small and cross-sectional by design, which limits determination of temporality or causality. In addition, this study was conducted in African ancestry families, which limits generalizability to other racial/ethnic or geographic groups. Our study also lacks measurement of the full array of RAAS pathway components including angiotensin-converting enzyme, angiotensin, or angiotensinogen and, therefore, there may be additional factors of the RAAS pathway that may be missed by these analyses. However, this study is the first to test for an association between any RAAS measures and osteocalcin, and it is larger than the 2 previous studies of the association of circulating RAAS with BMD in humans.\(^\text{11,12}\) In addition, it is the first study conducted in a sample recruited without regard to health status and may, therefore, be more generalizable to a broader population of healthy adults.

### Perspectives

This is currently the largest study of the association between serum biomarkers of the RAAS pathway and BMD. It is also the first study to extend the analyses to bone turnover markers, be skeletal compartment specific, and to investigate the potential for shared genetic determinants underlying these relationships. In a sample of African ancestry families, we have identified significant associations between higher PRA (or lower ARR) and higher trabecular BMD and lower osteocalcin, independent of hypertension. We have also presented the first evidence that shared genetic determinants may underlie these associations. Therefore, variation in the RAAS pathway because of pharmacological or physiological means may have previously unrecognized effects on bone metabolism that need to be identified. It is also possible that existing therapeutics that alter the RAAS pathway could be useful to maintain or improve skeletal health. Future studies will be needed to replicate these results in additional populations and to evaluate the mechanisms linking RAAS and bone metabolism, including identifying potential shared genetic pathways.

### Acknowledgments

We thank Caren Gundberg, PhD, at Yale School of Medicine for her assistance in measuring and interpreting osteocalcin levels.

### Sources of Funding

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### Disclosures

None.

### References


Novelty and Significance

What Is New?
- This is the largest study to test for an association of circulating renin–angiotensin–aldosterone system measures with measures of bone mineral density.
- It is also the first in African ancestry individuals, the first to also include information on bone turnover markers, and the first to test for shared genetic determinants underlying these traits.

What Is Relevant?
- The association of plasma renin activity and aldosterone/renin ratio, and bone mineral density seems to be restricted to trabecular bone.
- They are also strongly associated with osteocalcin, a marker of bone turnover. Also, these associations show evidence of having some shared genetic determination (pleiotropy).

Summary
We found that circulating renin–angiotensin–aldosterone system measures were associated with greater bone mineral density and lower bone turnover independent of the confounding effect of hyper- tension. In addition, there is evidence that there are shared genetic pathways underlying these associations. Finally, we found no association of serum aldosterone concentration with bone in African ancestry individuals.
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ASSOCIATION OF CIRCULATING RENIN AND ALDOSTERONE WITH OSTEOCALCIN AND BONE MINERAL DENSITY IN AFRICAN ANCESTRY FAMILIES

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Table S1. Multivariable* Association of Plasma Aldosterone Concentration and Aldosterone to Renin Ratio with Osteocalcin and Bone Mineral Density in African Ancestry Families

<table>
<thead>
<tr>
<th>Bone Measure</th>
<th>Difference (%) in bone measure per 1 SD PAC †</th>
<th>P-value</th>
<th>Difference (%) in bone measure per 1 SD ARR ‡</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total OC (ug/l)</td>
<td>0.23</td>
<td>0.881</td>
<td>8.69</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Whole Body BMD (g/cm²)</td>
<td>-0.62</td>
<td>0.185</td>
<td>-0.80</td>
<td>0.121</td>
</tr>
<tr>
<td>Total Hip BMD (g/cm²)</td>
<td>-0.22</td>
<td>0.730</td>
<td>-1.26</td>
<td>0.072</td>
</tr>
<tr>
<td>Cortical BMD (g/cm³)</td>
<td>0.02</td>
<td>0.896</td>
<td>0.07</td>
<td>0.577</td>
</tr>
<tr>
<td>Trabecular BMD (g/cm³)</td>
<td>0.07</td>
<td>0.907</td>
<td>-1.69</td>
<td>0.014</td>
</tr>
</tbody>
</table>

*Multivariable model includes adjustment for age, sex, height, weight, menopausal status, systolic blood pressure, diastolic blood pressure, hypertensive medication, eGFR, diabetes, current smoking, drinking, walking and calcium supplementation.

†Values shown are the percent difference in bone measure per 1 SD (8.1 ng/dL) greater plasma aldosterone concentration.

‡Values shown are the percent difference in bone measure per 1 SD (17.9 ng/dL per ng/mL/h) greater aldosterone to renin ratio.
Table S2. Multivariable* Adjusted Genetic Correlations of Aldosterone to Renin Ratio with Osteocalcin and Bone Mineral Density

<table>
<thead>
<tr>
<th>Bone Measure</th>
<th>Heritability (H²ᵣ ± SE)</th>
<th>Aldosterone to Renin Ratio (H²ᵣ ± SE = 0.705 ± 0.10)</th>
<th>ρ_G</th>
<th>ρ_G P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total OC (ug/l)</td>
<td>0.727 ± 0.11</td>
<td>0.323</td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>Whole Body BMD (g/cm²)</td>
<td>0.623 ± 0.12</td>
<td>0.175</td>
<td>0.288</td>
<td></td>
</tr>
<tr>
<td>Total Hip BMD (g/cm²)</td>
<td>0.696 ± 0.10</td>
<td>-0.052</td>
<td>0.717</td>
<td></td>
</tr>
<tr>
<td>Cortical BMD (g/cm³)</td>
<td>0.444 ± 0.12</td>
<td>0.199</td>
<td>0.243</td>
<td></td>
</tr>
<tr>
<td>Trabecular BMD (g/cm³)</td>
<td>0.622 ± 0.11</td>
<td>0.118</td>
<td>0.455</td>
<td></td>
</tr>
</tbody>
</table>

*Multivariable models include adjustment for age, sex, height, weight, menopausal status, systolic blood pressure, diastolic blood pressure, hypertensive medication, eGFR, diabetes, current smoking, drinking, walking and calcium supplementation.

H²ᵣ: residual genetic heritability estimate after adjustment; ρ_G estimated genetic bivariate correlation.