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ASSOCIATION OF CIRCULATING RENIN AND ALDOSTERONE WITH OSTEOCALCIN AND BONE MINERAL DENSITY IN AFRICAN ANCESTRY FAMILIES

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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; relative pairs: 1687). Participants underwent a clinical exam, dual energy x-ray absorptiometry, and quantitative computed tomography scans. Renin activity, aldosterone concentration, and osteocalcin were measured in fasting blood samples. Aldosterone to 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, co-morbidities, 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 to 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 biologic mechanism underlying both the renin-angiotensin-aldosterone system and bone metabolism that is independent of hypertension.

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CONFLICTS OF INTEREST AND FINANACIAL DISCLOSURES

None

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Keywords

renin; aldosterone; osteocalcin; bone mineral density; African ancestry

INTRODUCTION

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[1–4]. Components of the RAAS are expressed in human bone cells[5] and can activate a local RAAS response that leads to increased bone turnover and decreased bone density[1]. Indeed, there is also evidence for shared genetic determinants of the RAAS and bone mineral density (BMD)[6].

Research on the RAAS and bone metabolism in humans has focused on patients with primary aldosteronism (PA)[7–9] or on the effect of RAAS inhibitor therapies on bone[10–15]. The majority of studies on the effect of angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARB) on bone, including those in humans[10–13] and animal models[16–18], suggest that pharmacologic 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[14, 15]. There have only been two studies, each with less than 50 participants, which examined the association of circulating RAAS measures with BMD in humans[19, 20]. 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[20] or premenopausal women[19]. Therefore, in the current study, we aimed to expand on these previous studies by using a larger family study of generally healthy men and women in order to further assess the association of circulating RAAS measures, including plasma renin activity, plasma aldosterone concentration and the aldosterone to renin ratio, with indices of bone turnover and BMD. The family study design also allowed us to estimate heritability of these traits and to determine if there may be shared genetic determinants underlying these traits.

METHODS

Study Sample

Participants for this analysis were from the Tobago Family Health Study[21]. Briefly, 8 individual probands were identified and recruited for the study without regard to their medical history on the Caribbean island of Tobago[21]. Each proband was eligible if they had a spouse willing to participate and had at least six living offspring and/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 exams, medical history interviews and bone mineral density assessment. The current analysis includes data on 373 individuals recruited from 6 large families (mean family size = 62; relative pairs: 1687) who have complete data on osteocalcin (OC), BMD, and RAAS

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measures, which include plasma renin activity (PRA), plasma aldosterone concentration (PAC) and aldosterone to renin ratio (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 time of collection until analysis. The PRA (ng/mL/h) was measured using a Clinical Assays GammaCoat radioimmunoassay kit (Baxter Healthcare, Cambridge, MA). The PAC (ng/dL) was measured by radioimmunoassay with antiserum from Diagnostic Products Corp. (Los Angeles, CA). The ARR (ng/dL per ng/mL/h) was calculated as PAC/PRA.

Serum concentrations of total OC were measured, in duplicate, in previously unthawed specimens by radioimmunoassay, as previously described elsewhere[22]. Briefly, OC is measured in serum using a radioimmunoassay utilizing an antibody made against OC purified from human bone. This antibody recognizes both carboxylated and uncarboxylated OC equivalently. Intra-assay variation was 4.8% for total OC. 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 as: GFR [mL · min^{-1} · (1.73 m^2) $^{-1}$] = 175 × (serum creatinine [mg/dL] $^{-1.154}$× age [years] $^{-0.203}$[× 0.742, if female] [× 1.212, because they are all of African ancestry][23].

Bone Mineral Density Measures

Integral areal BMD at the proximal femur were measured by dual energy X-ray absorptiometry (DXA) using a Hologic QDR-4500W densitometer (Hologic Inc., Bedford, MA). The short-term *in vivo* precision of the DXA measurements for 12 subjects were all ≤1.16%. Trabecular and cortical volumetric BMD at the left tibia was measured by peripheral quantitative computed tomography (pQCT) 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 pQCT 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 three times throughout the visit. The average of the 2nd and 3rd readings for systolic and diastolic blood pressures are used in this analysis. Thirty-six participants were on antihypertensive medication including 7 on ACE inhibitors, 6 on thiazide diuretics, 5 on methyldopa, 2 on calcium channel blockers, 1 on a beta-blocker, 4 on combination therapy, and 11 reported being on antihypertensive medication but did not bring it to the clinic visit.

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Diabetes was defined as a fasting glucose level ≥126 mg/dl or current use of diabetes medication[24]. Smoking status was classified as either current or not (yes/no). Participants reporting ever smoking <100 cigarettes in their lifetime were considered non-smokers. Alcohol consumption was assessed by questionnaire and was defined as having ≥3 drink per week (yes/no) as there was a very 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 logrithms 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 standard deviations 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 (SOLAR) program[25] that accounts for complex familial relationships. SOLAR 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 OC and BMD, we calculated the percent difference in OC or BMD per 1 SD increased RAAS measure [(calculated as RAAS measure beta coefficient/mean OC 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, in order 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, eGFR, diabetes, current smoking, drinking, walking and calcium supplementation.

To assess the potential shared genetic covariance between RAAS and bone measures, we estimated the residual heritability (h^2_r) and genetic correlations (ρ_G) in SOLAR[25]. 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 OC and BMD measures[25, 26]. The statistical significance of $\rho_G \neq 0$ 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 18–86 years; 62% were females (Table 1). Based on mean BMI, participants were overweight and women were more

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overweight than men (P<0.0001). Women also had a higher prevalence of antihypertensive medication use compared to men (12.6% vs. 4.9%; p=0.01), although men had higher systolic blood pressure (p=0.01). Compared to men, women had lower kidney function as assessed by eGFR (p=0.04) and a higher prevalence of type II diabetes (p=0.03). However, men were more likely to have poorer lifestyle habits including current smoking, drinking ≥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 OC 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 Bone Mineral Density

Higher PRA was associated with lower total OC 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 8% lower total OC (p<0.0001). Additionally, 1SD greater ARR was associated with ~10% higher total OC (p<0.0001; Table S1). PRA was not associated with whole body, total hip or cortical BMD in any model (Table 3; 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). 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 OC by hypertension status (all interaction P>0.1; data not show). There was no significant association of PAC concentrations with OC or BMD (Table S1).

Genetic Correlations of RAAS Measures with Osteocalcin and BMD

PRA, ARR, OC and BMD measures were each significantly heritable (Tables 4 and S2). PRA had a significant, negative genetic correlation with whole body BMD (ρ_G=−0.40; p=0.02), while ARR had significant, positive genetic correlation with total OC (ρ_G=0.32; p=0.02; Table S2). Since there were no significant phenotypic associations of PAC with OC 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 OC 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, co-morbidities 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

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RAAS measures and cortical and trabecular bone in humans. We found that the association of PRA with BMD appears 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 appear to be a significant factor.

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

Individuals with primary aldosteronism (PA), a major source of secondary hypertension, have lower BMD than controls[7–9]. While 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 OC and BMD measures. Greater ARR was associated with lower BMD, consistent with studies in PA patients[7–9]. We also found that greater ARR was significantly associated with greater OC ($p<0.0001$), 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 and/or when RAAS pathway homeostasis is disrupted.

The potential mechanisms underlying these associations are not clear, but it appears that at least part of the association is due to shared genetic determinants. The significant genetic correlation between PRA and ARR with OC and BMD suggests that some of the genetic variation that impacts RAAS measures, also influences bone metabolism. These findings replicates results from a broader study of genetic correlation throughout the human genome, which found evidence of shared genetic determinants between RAAS measures and BMD[6]. 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 2 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 ACE inhibitors or angiotensin II receptor blockers leads to decreased fracture risk and increased bone mass in humans[10–13, 16–18]. The findings of the current paper 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.

While we had the ability to assess many potential confounders including lifestyle habits, co-morbidities and hypertension, there remain several potential study limitations. First, we did

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not have data on dietary intake of vitamin K, which may be an important confounder as it is a co-factor required for carboxylation of OC[30]. However, since our study uses total OC level, not only carboxylated OC, we do not believe 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. Additionally, 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 ACE, 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 OC and it is larger than the two previous studies of the association of circulating RAAS and BMD in humans[14, 15]. 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 OC, independent of hypertension. We have also presented the first evidence that shared genetic determinants may underlie these associations. Therefore, variation in the RAAS pathway due to pharmacological and/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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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NOVELTY AND SIGNIFICANCE

1. What is New? This is the largest study to test for an association of circulating RAAS 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.
2. What is Relevant? The association of plasma renin activity and aldosterone to 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 (pleioptropy).
3. Summary: We found that circulating RAAS measures were associated with greater bone mineral density and lower bone turnover independent of the confounding effect of hypertension. Additionally, there is evidence that there are shared genetic pathways underlying these associations. Lastly, we found no association of serum aldosterone concentration with bone in African ancestry individuals.

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Table 1

Characteristics of the African Ancestry Families

Characteristic	Overall (N=373)	Men (N=143)	Women (N=230)	P-value
Age (years)	42.3±16.8	41.6±17.2	42.7±16.6	0.714
Female (%)	61.7	N/A	N/A	N/A
Post-Menopausal (%)	19.4	N/A	31.7	N/A
Height (cm)	170.6±8.6	177.4±7.2	166.4±6.5	<0.001
Weight (kg)	82.2±18.9	84.0±17.4	81.1±19.5	0.122
BMI (kg/m^2)	28.3±6.4	26.6±5.0	29.3±6.9	<0.001
Systolic Blood Pressure (mmHg)	121.5±24.0	126.1±20.3	118.6±25.6	0.012
Diastolic Blood Pressure (mmHg)	75.2±13.0	76.7±12.5	74.3±13.2	0.157
Antihypertensive Medication (%)	9.7	4.9	12.6	0.010
eGFR (ml/min/1.73 m^2)	104.3±27.0	107.1±23.1	102.5±29.0	0.043
Diabetes (%)	15.1	10.6	18.0	0.034
Current Smoking (%)	4.6	11.9	0.0	<0.001
≥3 Alcoholic drinks/week (%)	11.3	26.6	1.8	<0.001
Walk for exercise in past week (%)	69.9	70.6	69.4	0.806
Taking Calcium Supplement (%)	19.6	9.1	26.1	<0.001

Values are presented as mean±SD or %, as appropriate.

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Table 2

Means and standard deviations of RAAS, Bone Mineral Density and Osteocalcin Measures in African Ancestry Families

Variable	Overall (N=373)	Men (N=143)	Women (N=230)	P-value
Plasma Renin Activity (ng/mL/h) *	3.0 (1.0–6.2)	2.9 (0.9–5.6)	3.1 (1.1–6.5)	0.721
Plasma Aldosterone Concentration (ng/dL) *	9.0 (5.6–13.4)	8.5 (5.4–11.7)	9.5 (5.7–15.2)	0.014
Aldosterone to Renin Ratio (ng/dL per ng/mL/h) *	3.1 (1.3–8.3)	3.2 (1.3–8.1)	3.0 (1.3–8.5)	0.613
Total OC (ug/l)	4.50±2.81	4.59±2.85	4.45±2.79	0.396
Whole Body BMD (g/cm^2)	1.19±0.13	1.26±0.11	1.15±0.12	<0.001
Total Hip BMD (g/cm^2)	1.11±0.17	1.19±0.14	1.06±0.17	<0.001
Cortical BMD (g/cm^3)	1182±30	1181±25	1184±22	0.418
Trabecular BMD (g/cm^3)	251±36	264±35	243±34	<0.001

*Renin and aldosterone are displayed as median(interquartile range) due to non-normality

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Table 3

Association of Plasma Renin Activity with Serum Osteocalcin and Bone Mineral Density in African Ancestry Families

Bone Measure	Model	Difference (%) in bone measure per 1 SD PRA*	P-value
Total Osteocalcin (ug/l)	**Unadjusted**	**−6.27**	**<0.0001**
	Base†	**−6.90**	**<0.0001**
	Multivariable‡	**−8.26**	**<0.0001**
Whole Body BMD (g/cm^2)	Unadjusted	−0.15	0.803
	Base†	0.25	0.617
	Multivariable‡	0.51	0.301
Total Hip BMD (g/cm^2)	Unadjusted	0.59	0.462
	Base†	1.19	0.067
	Multivariable‡	1.19	0.070
Cortical BMD (g/cm^3)	Unadjusted	−0.11	0.445
	Base†	−0.17	0.195
	Multivariable‡	−0.14	0.244
Trabecular BMD (g/cm^3)	**Unadjusted**	**1.78**	**0.020**
	Base†	**1.94**	**0.003**
	Multivariable‡	**1.61**	**0.012**

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

†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, eGFR, diabetes, current smoking, drinking, walking and calcium supplementation.

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Table 4

Multivariable* Adjusted Genetic Correlations of Plasma Renin Activity with Osteocalcin and Bone Mineral Density

Bone Measure	Heritability	Plasma Renin Activity (H^2_r ± SE = 0.515 ± 0.10)	
	(H^2_r ± SE)	ρ_G	ρ_G P-value
Total OC (ug/l)	0.727 ± 0.11	−0.230	0.148
Whole Body BMD (g/cm^2)	0.623 ± 0.12	**−0.394**	**0.023**
Total Hip BMD (g/cm^2)	0.696 ± 0.10	−0.051	0.755
Cortical BMD (g/cm^3)	0.444 ± 0.12	−0.266	0.148
Trabecular BMD (g/cm^3)	0.622 ± 0.11	−0.266	0.139

*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.

H^2_r: residual genetic heritability estimate after adjustment; ρ_G estimated genetic bivariate correlation.