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THE *FOK1* VITAMIN D RECEPTOR GENE POLYMORPHISM IS ASSOCIATED WITH PLASMA RENIN ACTIVITY IN CAUCASIANS

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Abstract

Objectives—25-hydroxyvitamin D (25[OH]D) deficiency and excess activity of the renin-angiotensin system (RAS) are both associated with cardiovascular disease. Vitamin D interacts with the vitamin D receptor (VDR) to negatively regulate renin expression in mice; however, human studies linking genetic variation in the VDR with renin are lacking. We evaluated: 1) whether genetic variation in the VDR at the *FokI* polymorphism was associated with plasma renin activity (PRA) in a population of hypertensives and a separate population of normotensives; and 2) whether the association between *FokI* genotype and PRA was independent of 25(OH)D levels.

Design/Patients/Measurements—Genetic association study, assuming an additive model of inheritance, of 375 hypertensive and 146 normotensive individuals from the HyperPATH cohort, who had PRA assessments after 1 week of high dietary sodium balance (HS) and 1 week of low dietary sodium balance (LS).

Results—The minor allele (T) at the *FokI* polymorphism was significantly associated with lower PRA in hypertensives (LS: $\beta = -0.22$, $P < 0.01$; HS: $\beta = -0.19$, $P < 0.01$); when repeated in normotensives, a similar relationship was observed (LS: $\beta = -0.17$, $P < 0.05$; HS: $\beta = -0.18$, $P = 0.14$). In multivariable analyses, both higher 25(OH)D levels and the T allele at *FokI* were independently associated with lower PRA in hypertensives, however, 25(OH)D was not associated with PRA in normotensives.

Conclusions—Genetic variation at the *FokI* polymorphism of the VDR gene, in combination with 25(OH)D levels, was associated with PRA in hypertension. These findings support the vitamin D-VDR complex as a potential regulator of renin activity in humans.

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KEY TERMS

vitamin D; renin; polymorphism; FokI; VDR

INTRODUCTION

Vitamin D deficiency and excess activity of the renin-angiotensin system (RAS) have both been associated with cardiovascular diseases^{1–4}. Vitamin D negatively regulates renin expression via its interactions with the vitamin D receptor (VDR), linking vitamin D physiology with activity of the RAS^{5, 6}.

Li et al. showed that VDR knock-out mice displayed a phenotype of high plasma renin activity (PRA) and hypertension that was ameliorated with RAS antagonism⁵. Inhibiting the production of active 1,25-dihydroxyvitamin D (1,25(OH)₂D) by inactivating the 1 α -hydroxylase enzyme also resulted in high PRA, which was reduced with administration of 1,25(OH)₂D⁷. In contrast, over-expression of the VDR in juxtaglomerular cells of mice suppressed renin expression independently of parathyroid hormone and calcium⁸. These findings have been consolidated by implicating vitamin D as an inhibitor of renin gene expression via its interaction with the VDR^{2, 6, 9}.

Cross-sectional human studies, including our own, have observed similar relationships between vitamin D and the RAS; vitamin D metabolites have been inversely associated with circulating renin, PRA^{10–13}, and vascular tissue RAS activity^{14, 15}. However, to our knowledge, these phenotypic relationships have not been correlated with genetic variation of the VDR gene in humans.

The *FokI* single nucleotide polymorphism (SNP) is arguably the most studied SNP within the VDR gene^{16, 17}; it represents a missense mutation in the translation initiation site which modifies the length and functional activity of the VDR protein^{16, 17}. The clinical significance of this SNP is highlighted by its associations with several adenocarcinomas^{18, 19}, bone mineralization^{16, 20, 21}, fracture risk^{22, 23}, and 25-hydroxyvitamin D (25(OH)D) levels^{17, 20}.

Given the previously identified importance of the *FokI* SNP in the biology of the VDR, and the lack of studies evaluating the link between VDR genetics and the RAS, we assessed the association of genetic variation at the *FokI* SNP with PRA in a Caucasian hypertensive population that underwent a rigorous phenotyping protocol for PRA, including dietary sodium and body posture control. Subsequently, we evaluated the same association in a separate population of Caucasian normotensives who completed the same study procedures; thus, representing a distinct study population with reliable phenotyping of PRA. Since we have previously observed an inverse association between 25(OH)D and PRA¹³, we also investigated the inter-relationship between *FokI* genotype and 25(OH)D in predicting PRA. Demonstrating a relationship between PRA and variation in the VDR would further support the biologic roles of 1,25(OH)₂D and the VDR in regulating renin.

METHODS

Study Population

This cross-sectional analysis was performed on data gathered from subjects studied in the International Hypertensive Pathotype (HyperPATH) Consortium. The HyperPATH study is an on-going, multi-site study, aimed at investigating the pathophysiologic and genotypic mechanisms involved in hypertension and cardiovascular diseases. A particular strength of

the HyperPATH study design is its rigorous study protocol (described below), conducted in Clinical Research Centers, and designed to minimize notable confounders of PRA (dietary sodium intake, body posture, diurnal variation, and medications); these approaches reduce variation in PRA measurements and enhance the power to detect real differences with relatively small sample sizes. Participants in this analysis were studied at four collaborating centers: Brigham and Women's Hospital (Boston, MA), University of Utah Medical Center (Salt Lake City, UT), Vanderbilt University Hospital (Nashville, TN), and Hôpital Européen Georges Pompidou (Paris, France). The Brigham and Women's Hospital served as the central laboratory for all lab processing. Although results from the HyperPATH cohort have been previously reported, the current described analyses are original and have not been published.

Subjects with chronic kidney disease, coronary heart disease, heart failure, suggested or known causes of secondary hypertension, and active malignancy were not enrolled in the HyperPATH study. Enrolled subjects were classified as having hypertension if they had an untreated seated diastolic blood pressure (DBP) > 100 mmHg, a DBP > 90 mmHg with one or more antihypertensive medications, measured as the average of three readings with standard manual sphygmomanometer, or the use of two or more antihypertensive medications. Enrolled subjects were classified as normotensive if the average of three consecutive seated blood pressure readings was less than 140/90 mmHg, and they had no first-degree relatives diagnosed with hypertension prior to the age of 60 years. Study procedures included dietary sodium modulation to maintain high-sodium (HS) and low-sodium balance (LS) in sequence (below).

The association between *FokI* and PRA was assessed in the hypertensive population first, and then subsequently evaluated in the normotensive population. This approach facilitated our effort to evaluate this relationship in more than one independent population with reliable phenotyping of the outcome variable PRA. Since the *FokI* polymorphism, RAS activity, and 25(OH)D levels are known to be confounded by race and ethnicity^{16, 24, 25}, the final study population for this analysis was restricted to Caucasian subjects who were successfully maintained in sodium balance per study protocols (below) and had successful genotyping of *FokI* from available frozen blood (n=375 subjects with hypertension, n=146 subjects with normotension). From this total study population, 25(OH)D concentrations were measured at a later date on the subset of subjects who had remaining frozen blood available for assay (n=223 subjects with hypertension, n=111 subjects with normotension).

The HyperPATH Study Protocol to Phenotype PRA

To avoid interference with PRA assessment, hypertensive participants taking angiotensin converting enzyme inhibitors, angiotensin receptor blockers, or mineralocorticoid receptor antagonists, were withdrawn from these medications three months before study initiation. Beta-blockers and diuretics were withdrawn one month before study initiation. If needed for blood pressure control, hypertensive subjects were treated with amlodipine and/or hydrochlorothiazide temporarily; however, these medications were stopped three weeks prior to laboratory evaluation of PRA.

Subjects were maintained in HS (≥ 200 mmol Na/24h) and then LS (≤ 10 mmol Na/24h), for 5–7 days each. Both study diets also included fixed quantities of potassium (80 mmol/day) and calcium (1000mg/day). After each diet phase, participants were admitted to the institutional Clinical Research Center and maintained in a supine position overnight. For each diet phase, baseline blood sampling was obtained in the morning and used to measure PRA, and then frozen without preservatives until assayed for future use. Baseline blood pressure was determined while supine between the hours of 8:00 AM and 10:00 AM, following 10 hours of overnight rest using the average of five readings from a Dinamap

automated device (Critikon, Tampa, FL). Sodium balance and diet compliance were confirmed on admission to the Clinical Research Center with a 24-hr urine sodium excretion of ≥ 150 mmol for HS, and ≤ 30 mmol for LS. Study protocols were approved by the Human Subjects Committees/Institutional Review Boards at each location, and informed written consent was obtained from each subject.

Biochemical Assessments

All subjects had PRA measured from the baseline morning supine blood obtained on the first day of study on each diet using the Diasorin assay (Stillwater, MN). At a later date, a majority subset of subjects (above) also had a single 25(OH)D level measured from the remaining baseline frozen blood samples obtained on the first day of study using the Diasorin assay. Since 25(OH)D was measured from stored frozen samples, we thawed, aliquoted, and measured 25(OH)D levels from 19 participants who also had 25(OH)D levels measured on fresh samples from the original day of study. The correlation coefficient comparing levels from fresh and frozen samples was 0.97¹⁴.

VDR genotyping

The VDR gene is a large gene (> 100 Kb) located on chromosome 12q13. The *FokI* SNP (rs2228570, formerly known as rs10735810) lies within exon 2 and is characterized by a cytosine or thymidine nucleotide variant. DNA from each subject was extracted from peripheral leukocytes obtained from frozen blood samples²⁶. Genotyping of the *FokI* SNP was conducted using the Applied Biosystems 3100 genetic analyzer with a completion rate of 98.5%. The genotype frequencies in the hypertensive population were 119 for cytosine homozygotes (CC), 195 for heterozygotes (CT), and 61 for thymidine homozygotes (TT) and were in Hardy-Weinberg equilibrium by χ^2 testing ($P=0.20$). The genotype frequencies in the normotensive population were 62 for CC, 66 for CT, and 18 for TT and were also in Hardy-Weinberg equilibrium by χ^2 testing ($P=0.95$).

Statistical Methods

Analyses were performed to first evaluate the association of PRA and *FokI* genotype assuming an additive model in the hypertensive population. In efforts to substantiate the associations seen in hypertensives, the same analyses were subsequently evaluated in a normotensive population. Though the normotensive population was smaller in size, it represented a distinctly unique phenotypic population with PRA measures. To contextualize our previously reported inverse association between 25(OH)D and PRA¹³ with the VDR, we investigated whether the association between *FokI* genotype and PRA was independent of, or modified by, 25(OH)D levels.

Linear regression was employed to test the associations between PRA and frequency of the T allele at *FokI* (categorized as CC, CT, or TT). All linear regression models included adjustments for sibling relatedness using a mixed effects model and are reported with effect estimates (β), the 95% confidence intervals for β , and the corresponding p-value. The level for significance for all tests conducted was set at $\alpha=0.05$ and reported as two-tailed P -values. Data analyses were performed using SAS v9.1 (Cary, NC) statistical software. Because PRA was not normally distributed, it was transformed using the natural logarithm to normalize its distribution. For graphical representation, PRA values were untransformed using the natural exponential and plotted as mean values with the 95% confidence intervals for the mean. Because age is a known independent predictor of PRA, we adjusted for age in all multivariable linear regression models. Since 25(OH)D has been inversely associated with PRA in our hypertensive population¹³, we also adjusted for 25(OH)D (categorized as <37.5 , 37.5–75, and ≥ 75 nmol/L; divide by 2.5 to convert to ng/mL) in the subset of subjects who had it measured, to test whether *FokI* genotype and 25(OH)D were both independently

associated with PRA when evaluated together. We also explored whether a potential interaction between *FokI* genotype and 25(OH)D was associated with PRA using an adjusted interaction model that included age, 25(OH)D, *FokI* genotype, and an interaction term between 25(OH)D and *FokI* genotype.

RESULTS

Study Populations

Consistent with prior reports from the HyperPATH cohort²⁷, the hypertensive study population was older, male-predominant, and had a higher BMI than the normotensive population (Table 1). Both populations were vitamin D insufficient according to current consensus²⁸. As previously observed²⁷, although both populations demonstrated appropriate PRA responses to dietary sodium manipulation, physiologic suppression and stimulation of PRA was blunted in hypertensives when compared to normotensives (Table 1); further illustrating the differences in RAS physiology between the two populations.

FokI genotype and PRA in Hypertensives

The relationship between *FokI* genotype and PRA was evaluated under controlled dietary sodium conditions in hypertensives. Under both dietary conditions, the T allele was associated with lower PRA (LS: $\beta = -0.219$, $[-0.362, -0.076]$, $P\text{-trend}=0.003$; HS: $\beta = -0.197$, $[-0.334, -0.059]$, $P\text{-trend}=0.005$) (Figure 1).

FokI genotype and PRA in Normotensives

The relationship between *FokI* genotype and PRA was subsequently evaluated in the smaller normotensive cohort. Similar inverse associations were observed between the T allele and PRA; this was particularly evident in LS conditions where the variation in PRA was maximal, but did not achieve statistical significance in HS conditions where PRA was suppressed (LS: $\beta = -0.165$, $[-0.313, -0.016]$, $P\text{-trend}=0.030$; HS: $\beta = -0.181$, $[-0.419, -0.057]$, $P\text{-trend}=0.135$) (Figure 2).

The Inter-relationship Between *FokI* genotype and 25(OH)D

Since 25(OH)D and 1,25(OH)₂D concentrations have previously been inversely associated with PRA^{10–12}, including in this hypertensive HyperPATH population¹³, and the VDR is hypothesized to mediate the association between 1,25(OH)₂D and PRA, we investigated the inter-relationship between *FokI* genotype and 25(OH)D in predicting PRA. When *FokI* genotype and 25(OH)D status (categorized as: <37.5 , $37.5\text{--}75$, and ≥ 75 nmol/L) were evaluated together in the hypertensive population, they both remained independently associated with PRA irrespective of dietary sodium balance (Table 2). However, we found no interaction between *FokI* genotype and 25(OH)D in predicting PRA in hypertensives (HS: $P\text{-interaction}=0.58$; LS: $P\text{-interaction}=0.74$), suggesting that the frequency of the T allele at *FokI* and higher 25(OH)D concentrations may have additive, rather than dependent, associations with PRA.

We subsequently evaluated whether *FokI* genotype and 25(OH)D were independent predictors of PRA in the smaller normotensive population. The T allele at *FokI* remained significantly associated with PRA, in LS balance where variability of PRA was maximal, but was a non-significant trend in HS conditions (Table 3). Vitamin D status was not significantly associated with PRA in normotensives. As was the case in hypertensives, we observed no significant interaction between *FokI* genotype and 25(OH)D in predicting PRA (HS: $P\text{-interaction}=0.39$; LS: $P\text{-interaction}=0.83$).

***FokI* genotype and 25(OH)D concentration**

Prior studies have reported an association between *FokI* genotype and concentrations of 25(OH)D¹⁷. In contrast, we did not observe any relationship between 25(OH)D concentrations and *FokI* genotype in hypertensives ($\beta = -0.03$, $[-4.70, 4.64]$, P -trend=0.99), or normotensives ($\beta = -1.14$, $[-7.14, 4.87]$, P -trend=0.71).

DISCUSSION

Vitamin D deficiency is an epidemic^{28, 29} that has been associated with heightened RAS activity and cardiovascular disease^{1, 2}. Animal experiments have demonstrated that 1,25(OH)₂D is a negative regulator of renin expression via its interactions with the VDR^{5, 8}. These findings have been translated to human cross-sectional studies that have established an inverse association between circulating vitamin D metabolites and PRA^{10–13}; however, human genetic studies implicating the VDR gene in this relationship have not been reported. This paucity of human genetic association studies may be related to the difficulty in acquiring reliable phenotyping of PRA in a sufficiently large human population. In this study, we observed that increasing frequency of the T allele at the *FokI* VDR gene polymorphism was independently associated with lower PRA in a large population of meticulously characterized hypertensives. Similar trends were observed in a smaller independent population of normotensives, especially when maintained in LS balance. We also demonstrated that both vitamin D and VDR genotype were *independently* associated with PRA in hypertensives. These findings may represent further evidence that the relationship between vitamin D and PRA is mediated by the VDR, and to our knowledge, is the first report linking VDR polymorphisms to PRA in humans.

The *FokI* SNP is arguably the most studied polymorphism in the VDR gene, and has been previously associated with several cancers, bone density, fracture risk, and 25(OH)D levels^{17–23}; thus, it represents a logical and attractive polymorphism with which to evaluate the role of genetic variation in the VDR with PRA. The *FokI* SNP is also considered to be a functional polymorphism of the VDR, and because it is not in linkage disequilibrium with other VDR SNPs, associations with *FokI* genotype are considered independent markers of the VDR gene¹⁶. Some *in vitro* studies in cell lines (HeLa and fibroblast) have suggested that the C allele results in a shorter VDR protein variant (424 amino acid) that is more active than the longer VDR protein variant associated with the T allele (427 amino acids)^{16, 17, 30–32}. These studies assessed “functionality” of the VDR using transcriptional activation with reporter constructs under the control of vitamin D responsive elements. In contrast, similar *in vitro* experiments by other investigators failed to show a difference in functional activity between these VDR protein variants³³. *In vivo* human studies have shown mixed results; the T allele at *FokI* has been associated with decreased bone mineralization, but also higher 25(OH)D levels^{17, 20}. We observed lower PRA with the TT genotype at *FokI*, suggesting that if genetic variation at the *FokI* SNP influences the activity of the VDR, then the T allele would confer increased activity using PRA as a functional read-out. This is in contrast to prior *in vitro* studies suggesting increased VDR activity with the C allele at *FokI*, but may be consistent with *in vivo* findings suggesting increased 25(OH)D production with the TT genotype²⁰. Our observations may further support the notion that different vitamin D target genes could have varying sensitivities and interactions with VDR protein variants resulting from *FokI* genotypes. Since we did not characterize the VDR protein variants in our subjects, nor perform functional assays on them, we are limited in making further conclusions in reference to VDR protein activity.

Our findings support the hypothesis that the negative regulation of renin by 1,25(OH)₂D is mediated in part by the VDR⁶. Our study population was maintained free of all blood pressure medications and in strict dietary sodium balance; we expect that both of these study

procedures substantially increased the reliability and validity of our outcome variable, PRA. We observed the same significant findings under both dietary conditions in hypertension; thus, despite the variation in PRA amplitudes induced by dietary sodium manipulation, the T allele at *FokI* was associated with lower PRA. The confidence in this genetic association would be enhanced if it could be replicated in an independent, and ideally larger, population of hypertensives. A major challenge in conducting this replication experiment, however, is the lack of another known cohort of hypertensive individuals who have undergone appropriate control of known contributors of variability in PRA (salt intake, body posture, medication washout) and genotyping of *FokI*. Realizing this limitation, we tested the association between *FokI* genotype and PRA within the normotensive population of the HyperPATH cohort. Despite the smaller size of this population, we observed that the T allele at *FokI* was again associated with lower PRA; although this was only statistically significant in LS balance, where the larger variability in unsuppressed PRA gave us greater power to detect the trend. These corroborative trends in normotensives may serve as a practical replication of our findings in hypertensives, albeit in a less than ideal sample size and with marginal statistical significance.

Consistent with studies by Resnick et al. and Tomaschitz et al. showing an inverse relationship between vitamin D metabolites and PRA, we also previously demonstrated that increasing 25(OH)D status was associated with lower PRA in this population of hypertensives¹³. Since we speculate that the mechanism of this association between 25(OH)D and PRA is ultimately mediated via the VDR, we investigated whether there was an interaction between 25(OH)D and *FokI* in relation to PRA. We found that both frequency of the T allele at the *FokI* SNP and higher 25(OH)D concentrations were independently associated with lower PRA in hypertensives, and did not interact with each other in predicting PRA, supporting an additive relationship between vitamin D levels and variation in the VDR with PRA. However, we may have been underpowered to conclusively exclude a statistical interaction between *FokI* genotype and 25(OH)D due to a combination of small sample size and effect sizes. Since only 15% of hypertensives with a TT genotype had 25(OH)D levels ≥ 75 nmol/L, a larger sample size would likely be needed to adequately assess for interaction, particularly a sample with a broader distribution of 25(OH)D levels. In normotensives, 25(OH)D was not associated with PRA; this may suggest that the effect size of vitamin D concentrations in predicting PRA in normotensives was small when compared to *FokI* genotype, and/or that the sample size of normotensives was too small to conclusively determine this relationship.

Our results must be interpreted within the context of our study design. First, this analysis was cross-sectional, and thus cannot prove causality or directionality of associations. Though our population size was small for a genetic association study, our meticulous phenotyping protocol enhanced the ability to detect differences in PRA. Furthermore, the consistent direction of association in each dietary condition among both the hypertensive and normotensive populations support our conclusions. Parathyroid hormone has been associated with PRA³⁴; however, whether its role is independent of vitamin D metabolites remains unresolved⁸. Our study design controlled for dietary sodium and calcium intake, but we did not have ionized calcium, parathyroid hormone, or 1,25(OH)₂D measurements that could have shed further insight on potential interacting mechanisms. The generalizability of our findings is limited to the population we analyzed, a relatively homogenous group of Caucasians; this demographic restriction limited possible confounding by race and ethnicity^{16, 24}. Though our study design controlled for many major confounders of PRA, several other factors that are known (estrogen status, stress, sympathetic nervous system activity) or not known to affect PRA may have influenced our measurements³⁵. Recent studies have suggested that VDR agonists may reduce renal injury³⁶ and that VDR genotypes (including *FokI*) associate with end-stage renal disease³⁷. Though our entire

study population had normal renal function on enrollment (calculated glomerular filtration rate [GFR] > 60 mL/min), the majority of them did not have further assessments of their GFR under each dietary condition. Thus, we are limited in evaluating whether diet-induced changes in GFR influence the relationship between *FokI* and PRA.

Emerging evidence continues to implicate vitamin D deficiency with cardiovascular diseases and implicates the RAS as a likely contributor². Herein, we demonstrate that in addition to the independent association between vitamin D metabolites and PRA, genetic variation of the VDR protein may also independently influence PRA. These findings further support the biologic role of 1,25(OH)₂D as an inhibitor of renin expression in humans, and suggest that the combination of 25(OH)D status and genetic variation at the *FokI* SNP may enhance prediction of PRA and RAS activity, which may in turn be relevant for cardiovascular and metabolic risk. Future studies to confirm these associations and correlate functional studies on the VDR protein are needed.

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Figure 1.

The adjusted relationships between *Fok1* genotype and PRA in hypertensives in LS balance (**A**) and HS balance (**B**). Bars represent the untransformed PRA and whiskers represent the 95% confidence interval. The population by genotype was n=119 for CC, n=195 for CT, and n=61 for TT.

Figure 2.

The adjusted relationships between *FokI* genotype and PRA in normotensives in LS balance (A) and HS balance (B). Bars represent the untransformed PRA and whiskers represent the 95% confidence interval. The population by genotype was n=62 for CC, n=66 for CT, and n=18 for TT.

Table 1
Characteristics of the hypertensive and normotensive study populations

Values are represented as means with standard deviations for all variables except PRA, which was not normally distributed, and is represented by the median and the interquartile range (25th–75th percentile). (25[OH]D=25-hydroxyvitamin D; LS=low dietary sodium balance; HS=high dietary sodium balance; SBP=systolic blood pressure; DBP=diastolic blood pressure).

		HYPERTENSIVE Study Population	NORMOTENSIVE Study Population
N		375	146
Age (years)		48.3 (8.2)	39.9 (10.9)
Gender (%female)		39.0	56.2
BMI (kg/m ²)		27.3 (3.9)	24.4 (3.7)
25(OH)D (nmol/L)		56.0 (23.1)	60.8 (20.5)
Frequency of T (minor) allele		0.42	0.35
SBP (mmHg)	LS	132.2(18.4)	105.3(10.3)
	HS	146.8(20.3)	109.8(11.4)
DBP (mmHg)	LS	79.9(11.0)	63.1(7.0)
	HS	87.5(11.4)	66.1(8.1)
PRA (μg/L/h)	LS	1.90 (1.00, 3.40)	2.48 (1.60, 4.20)
	HS	0.48 (0.20, 0.80)	0.34 (0.20, 0.59)
24hr urine sodium (mmol)	LS	16.1(19.8)	11.4(17.6)
	HS	216.2(74.9)	226.1(78.0)

Multivariable linear regressions results evaluating the independent associations of *FokI* genotype and 25(OH)D status on PRA in hypertensives maintained in dietary sodium balance. (LS=low dietary sodium balance; HS=high dietary sodium balance; 25(OH)D status categorized as <37.5, 37.5–75, and ≥ 75 nmol/L)

Table 2

Variable	LS			HS		
	Effect Estimate (β)	95% Confidence Intervals for β	P	Effect Estimate (β)	95% Confidence Intervals for β	P
Age (years)	−0.038	[−0.054, −0.023]	<0.0001	−0.004	[−0.020, 0.012]	0.65
25(OH)D status	−0.242	[−0.432, −0.052]	0.013	−0.229	[−0.413, −0.044]	0.015
<i>FokI</i> genotype (T allele)	−0.267	[−0.454, −0.082]	0.005	−0.278	[−0.455, −0.100]	0.002

Table 3

Multivariable linear regressions results evaluating the independent associations of *FokI* genotype and 25(OH)D status on PRA in normotensives maintained in dietary sodium balance. (LS=low dietary sodium balance; HS=high dietary sodium balance; 25(OH)D status categorized as <37.5, 37.5–75, and ≥ 75 nmol/L)

Variable	LS			HS		
	Effect Estimate (β)	95% Confidence Intervals for β	P	Effect Estimate (β)	95% Confidence Intervals for β	P
Age (years)	−0.019	[−0.038, −0.001]	0.036	−0.024	[−0.045, −0.003]	0.025
25(OH)D status	−0.087	[−0.334, 0.160]	0.485	0.086	[−0.246, 0.417]	0.610
<i>FokI</i> genotype (T allele)	−0.219	[−0.425, −0.013]	0.038	−0.250	[−0.548, 0.047]	0.099