

RENIN-ANGIOTENSIN SYSTEM GENES AND EXERCISE TRAINING-INDUCED CHANGES IN SODIUM EXCRETION IN AFRICAN AMERICAN HYPERTENSIVES

Objective: To determine whether angiotensin-converting enzyme (ACE) and angiotensinogen (AGT) genotypes could predict changes in urinary sodium excretion in response to short-term aerobic exercise training (AEX).

Design: Longitudinal intervention.

Setting: The study was conducted at the University of Maryland at College Park and at Baltimore, and the University of Pittsburgh General Clinical Research Center.

Participants: 31 (age 53 ± 2 years) sedentary, hypertensive ($146 \pm 2/88 \pm 2$ mm Hg) African Americans.

Intervention: Aerobic exercise training (AEX) consisted of seven or eight consecutive days, 50 minutes per day, at 65% of heart rate reserve. Participants underwent a 24-hour period of ambulatory blood pressure (BP) monitoring and urine collection at baseline and 14–18 hours after the last exercise session.

Main Outcome Measures: Angiotensinogen (AGT) M235T and ACE I/D genotype and sodium excretion and ambulatory BP.

Results: Average sodium excretion for the entire group independent of genotype increased after AEX (108 ± 9 vs 143 ± 12 mEq/day, $P=.003$). Sodium excretion significantly increased after exercise training in the ACE II (114 ± 22 vs 169 ± 39 mEq/day, $P=.04$), but not in the ID (100 ± 8 vs 133 ± 17 mEq/day, $P=.12$) or DD (113 ± 18 vs 138 ± 11 mEq/day, $P=.13$) genotype groups. In the II genotype group, the increase in sodium excretion was significantly and inversely correlated with decreases in 24-hour diastolic ($r=-.88$, $P=.02$) and mean ($r=-.95$, $P=.004$) BP. The AGT TT and MT+MM genotype groups similarly increased their sodium excretion by 34 ± 16 ($P=.05$) and 37 ± 17 ($P=.05$) mEq/day respectively.

Conclusions: These results suggest that African American hypertensives with the ACE II genotype may be more susceptible to sodium balance and BP changes with exercise training compared with those with the ID and DD genotypes. (*Ethn Dis.* 2006;16:666–674)

Key Words: Blood Pressure, Exercise, Genetics, Sodium

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INTRODUCTION

The renin-angiotensin system (RAS) is associated with long-term blood pressure (BP) regulation through its influence on the cardiovascular and renal systems. Angiotensinogen (AGT) is the substrate for the RAS and is produced in many tissues involved in BP regulation, including the kidney.¹ Angiotensinogen (AGT) is cleaved by renin to form angiotensin I, which is converted to biologically active angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II causes vasoconstriction of the vasculature and sodium reabsorption in the kidney. Because of the roles that ACE and AGT play in sodium handling, genes that encode for ACE and AGT can contribute to sodium handling.

Much research has focused on the ACE insertion/deletion (I/D) gene polymorphism, defined by the presence or

absence of a 287-bp DNA sequence in intron 16 at the ACE locus² and its association with cardiovascular disease (CVD) risk factors.^{3,4} A number of studies have demonstrated that the ACE I/D gene polymorphism accounts for a substantial portion of plasma and tissue ACE-level variability.^{5,6} Evidence suggests that hypertension among African Americans may be related to RAS function and renal sodium handling.^{7,8} When the ACE I/D allele frequency was assessed in those of African descent, the D allele frequency was significantly higher than in Caucasians,⁹ but evidence conflicts on whether the ACE I/D polymorphism is associated with ACE activity in those of African descent.^{10,11}

The methionine-to-threonine amino acid substitution at codon 235 (M235T) is caused by a thymine-to-cytosine transition at nucleotide 704 in exon 2 at the AGT locus. Studies have reported a higher frequency of T alleles in both Caucasian and Japanese hypertensives.^{12–14} The M235T polymorphism has also been associated with AGT levels, in which those with the T allele demonstrated greater plasma AGT levels.^{13,15} Studies have also investigated the M235T polymorphism's association with hypertension in those of African descent. The T allele is more frequent among those of African descent compared to Caucasians, but reports have shown no association between the M235T polymorphism and hypertension among those of African descent.^{16,17}

A relationship between dietary sodium and BP has been described in ecologic, epidemiologic, and experimental human studies.¹⁸ Research has also shown a relationship between di-

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Evidence suggests that hypertension among African Americans may be related to RAS function and renal sodium handling.^{7,8}

etary sodium-induced changes in BP and the ACE I/D polymorphism, but the results have been mixed as to whether the II or DD genotype is more likely to be associated with dietary sodium-induced changes in BP.¹⁹⁻²¹ The findings have also been mixed with regards to the association of the AGT M235T polymorphism with dietary sodium-induced changes in BP.^{20,22,23}

The relationship between the ACE I/D polymorphism and exercise training-induced changes in BP has also been investigated.^{24,25} Individuals with the II genotype demonstrate greater reductions in blood pressure after long-term exercise training compared to those with the DD genotype. Some research has been conducted on the association between the AGT M235T polymorphism and exercise-induced changes in exercise BP. The MM genotype has been associated with a reduction in submaximal exercise diastolic BP after exercise training and a reduction in diastolic BP in those exercising over an extended period of time.^{26,27} Possibly, ACE and/or AGT genotype-dependent changes in BP with exercise training are associated with differences in sodium handling among the ACE and AGT genotypes; however, this possibility has not been investigated.

Even when diet was controlled, sodium excretion responses to exercise training were heterogeneous.^{28,29} However, rarely do studies on the effects of exercise training report on changes in sodium excretion. ACE and AGT are integral components of the RAS and contribute to the regulation of sodium

balance. Because of the association between the ACE I/D polymorphism and plasma and tissue ACE levels and the association between the AGT M235T polymorphism and plasma AGT levels, these RAS gene polymorphisms may indirectly affect the intermediate phenotype, sodium handling, and may interact with exercise training to affect sodium excretion responses. We hypothesized that the ACE I/D and the AGT M235T gene polymorphisms would be independently associated with exercise training-induced changes in 24-hour urinary sodium excretion and 24-hour ambulatory BP in African American hypertensives.

METHODS

We studied 31 (23 women and 8 men) hypertensive African Americans between 50 and 65 years of age. The institutional review boards of the University of Maryland and the University of Pittsburgh Medical Center approved the study. All subjects signed an informed consent form before beginning the screening process. Subjects were determined to be hypertensive by a physician's diagnosis and by casual blood pressure measurements obtained during the screening phase of the study. If a subject was using antihypertensive medications known to affect sodium handling, they were tapered off this medication before the beginning of baseline testing. All subjects had their height and weight measured to calculate body mass index (BMI, kg/m²), followed by a physical examination, assessment of fasting blood chemistries, and a urinalysis. Creatinine clearance was calculated at baseline using the Cockcroft-Gault equation³⁰ to ensure that subjects had normal kidney function.

A graded maximal exercise test (Bruce protocol) was performed to screen for coronary artery disease and to assess maximal oxygen consumption (VO₂max) as an index of cardiovascular fitness. VO₂max was measured contin-

uously throughout the graded exercise test, and standard criteria were used to determine if VO₂max was achieved.³¹ Heart rate and electrocardiogram (ECG) were recorded before, continuously during, and for six minutes after the exercise test. Blood pressure was recorded before and at the end of each stage of the exercise test. The exercise test was terminated when subjects could no longer continue or when CVD signs or symptoms occurred.³¹ The maximal heart rate achieved during the graded exercise test was used to prescribe each subject's exercise training intensity. The subjects must have had <2-mV ST segment and no signs or symptoms of cardiovascular decompensation during the exercise test to be included in the study. An additional study inclusion criterion was that for those not on antihypertensive medications and those tapered from antihypertensive medications, casual blood pressure was required to remain between 135-159/85-99 mm Hg.

Subjects were excluded from further participation in the study if they had a history of signs and symptoms of CVD, kidney or liver disease, diabetes, a form of arthritis that limited physical activity, or a secondary form of hypertension. All subjects were community dwelling, nonsmokers, and sedentary (participated in aerobic exercise <20 minutes per day and less than two days per week for the prior year).

Baseline Testing

After subjects were tapered off of antihypertensive medications known to affect sodium handling, they underwent a dietary recording and stabilization period. Fourteen subjects maintained a strictly controlled dietary intake during the course of the study. These subjects met with a General Clinical Research Center (GCRC) research dietitian to select food items that were typical of their usual diet. The food selection was used to develop a diet for each subject. The diets were then

prepared by the GCRC kitchen so that each subject consumed a diet that was identical in composition, calories, and electrolytes to their usual diet. This diet was consumed before and during both testing periods. The remaining subjects completed a three-day dietary recording period to record their usual dietary intake at baseline. These subjects were given a food log and were instructed to record all foods and liquids that they ingested for the two days prior and the day of urine collection. For the final testing period, 48 hours prior to the 24-hour ambulatory BP monitoring and urine collection period, subjects repeated the three-day diet that they consumed at baseline.

24-Hour Ambulatory BP Monitoring and Urine Collection

On the third day of their dietary recording or stabilization period, both before and after exercise training, all subjects began a 24-hour ambulatory BP monitoring and urine collection period. The subjects were fitted with the ambulatory BP monitor (Spacelabs, Miami, Fla., model #90219/90207), which was programmed to measure BP every 30 minutes between the hours of 6:00 AM and 10:00 PM and every 60 minutes from 10:00 PM to 6:00 AM. Subjects were given an activity diary to record their daily activity each time that the monitor measured their blood pressure. Subjects were asked to not exercise during any of the 24-hour ambulatory BP monitoring periods and to pause momentarily and maintain their body position during each BP measurement. The final 24-hour urine collection and ambulatory BP monitoring period occurred 14–20 hours after the last exercise training session to avoid the acute effects of exercise on BP. Blood pressure data were downloaded to a computer and analyzed by using Spacelabs analysis software package (Version 1.03.15). The software automatically edited BP and heart rate values

that were outside the default limits established by the manufacturer (BP >260/150 mm Hg, pulse pressure >150 mm Hg, and heart rate >200 beats/minute).

During the same 24-hour period that ambulatory BP was monitored, before and after exercise training, subjects began a urine collection after their first void in the morning. The collection ended after their first void the following morning. The urine collection containers were kept on ice, and the urine volume was measured to the nearest 1.0 mL by using a graduated cylinder. A 10-mL aliquot sample was obtained from the 24-hour urine collection and used to determine electrolyte concentrations.

Gene Polymorphism Determination

DNA was isolated from peripheral venous blood samples. The ACE I/D polymorphism in intron 16 at the ACE gene locus was determined by using standard polymerase chain reaction (PCR) techniques according to the methods of Hagberg et al.³² A 354-bp fragment containing the AGT M235T site was amplified according to the methods of Jeunemaitre et al.¹³ The T-C transition was genotyped by oligonucleotide ligation assay.³³

Exercise Intervention

In order to assess the effects of exercise, independent of potentially confounding variables, all subjects completed a short-term aerobic exercise training protocol (seven or eight consecutive days). This short-term exercise training protocol was previously shown not to alter cardiovascular fitness (VO₂-max), body weight, or body composition,²⁸ all of which may influence sodium handling. Exercise consisted of supervised treadmill walking at 65% of each subject's heart rate reserve. Each exercise session began with a 10-minute warm-up consisting of walking and stretching. After the warm-up session

was completed, the subjects performed treadmill walking for 30 minutes, followed by a five-minute rest period. The subjects resumed treadmill walking for an additional 20 minutes followed by a 10-minute cool-down period. To ensure that subjects remained at 65% of their heart rate reserve, they wore a heart rate monitor (Model 6124, Polar Electro Canada, Quebec) during each exercise session, and the investigators recorded heart rate every 10 minutes. Resting heart rate and BP were measured before the warm-up and after the cool-down periods of each exercise training session.

Data and Statistical Analyses

Statistical analyses were performed by using SPSS (version 10, SPSS Inc., Chicago, Ill, USA). A chi-square test was used to test if the genotype distribution of the ACE I/D and the AGT M235T gene polymorphisms were in Hardy-Weinberg equilibrium. Subject characteristics among the ACE and among the AGT genotype groups were compared by one-way ANOVA. Paired sample *t* tests were used to determine whether the average 24-hour values for sodium and potassium excretion and systolic, diastolic, and mean BP were different after the short-term exercise training intervention in the entire group of subjects. One-way repeated measures analysis of variance was conducted to compare the changes in these variables after the intervention among the ACE and among the AGT genotype groups. If differences in subject characteristics that could have influenced the outcome variables were detected among the ACE and among the AGT genotype groups, these variables were entered into analysis of covariance models as covariates. The changes in the outcome variables within each genotype group were also tested by using paired-samples *t* tests. Correlation analyses between the changes in the outcome variables were conducted in the entire group and within each ACE

Table 1. Baseline subject characteristics in the ACE I/D genotype groups

Variable	Genotype			P value
	II (n=7)	ID (n=14)	DD (n=10)	
Age, years	53 ± 2	58 ± 2	52 ± 2	.06
Body weight, kg	91.2 ± 4	88 ± 4	92.1 ± 5	.74
BMI, kg/m ²	33 ± 0.8	30 ± 6	33 ± 2	.42
24-hour SBP, mm Hg	141 ± 2	141 ± 2	135 ± 3	.18
24-hour DBP, mm Hg	90 ± 1	83 ± 2	82 ± 3	.10
24-hour MBP, mm Hg	107 ± 1	103 ± 2	101 ± 3	.20
VO ₂ max, mL/kg/min	21 ± 2	22 ± 2	20 ± 2	.90
Creatinine clearance, mL/min	89 ± 10	95 ± 10	114 ± 11	.29
24-hour sodium, mEq/day	123 ± 21	101 ± 8	117 ± 17	.52
24-hour potassium, mEq/day	52 ± 8	48 ± 7	53 ± 7	.90

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; MBP=mean blood pressure; VO₂max=maximal oxygen consumption. Values are means ± standard error.

and AGT genotype group. All values are reported as means ± standard error. A *P* value <.05 was considered statistically significant.

RESULTS

Subjects

Characteristics of the 31 subjects who completed the study are provided in Table 1. The subjects were moderately obese, hypertensive, and sedentary based on their VO₂max values at baseline. All subjects had normal renal function based on their creatinine clearance values. Thirteen of the 31 subjects were using antihypertensive medications, and six of these subjects had their antihypertensive medications completely withdrawn. The remaining subjects continued using minimal doses of antihypertensive medications that do not directly affect sodium handling. We were unable to determine AGT genotypes in three subjects. Each subject completed the exercise intervention with 100% compliance. The subjects exercised at an average training intensity of 65% ± 2% of their heart rate reserve for an average of 51 ± 1 minute/session.

Genotype and Allele Frequencies

The ACE I and D allele frequencies were .45 and .55, respectively, which are

similar to the allele frequencies observed in previous studies in African American populations.^{9-11,34} The ACE genotype frequencies were .23, .45, and .32, for the II, ID, and DD genotype groups, respectively. The AGT T and M allele frequencies were .73 and .27, respectively. The AGT genotype frequencies were .50, .46, and .07 for the TT, MT, and MM genotype groups, respectively. The distributions of the ACE ($\chi^2=.53$, $P=.77$) and AGT ($\chi^2=.93$, $P=.30$) genotype frequencies did not deviate significantly from the Hardy-Weinberg expectation. Because of the expected low frequency of the MM genotype in African Americans, the MM and MT genotype groups were combined into an M allele carrier group (MT+MM).

Baseline

No significant differences in subject characteristics were seen between the ACE genotype groups, although the II and DD genotype groups tended to be younger than the ID genotype group (Table 1). In the AGT genotype groups, the TT genotype group had a significantly lower 24-hour potassium excretion compared to the MT+MM genotype group (37 ± 6 vs 63 ± 5 mEq/day, $P=.002$). Also, the TT genotype group tended to be heavier than the MT+MM genotype group (Table 2). No other differences in subject char-

acteristics were seen between AGT genotype groups.

After Short-Term Exercise Training

Consistent with short-term aerobic exercise interventions, we saw no significant changes in body weight or VO₂max. In response to the standardized short-term exercise training protocol, average 24-hour sodium excretion significantly increased in the entire group ($N=31$) independent of genotype from 108 ± 9 mEq/day to 143 ± 12 mEq/day ($P=.003$) (Table 3). This finding was true for subjects who consumed the prepared diets (100 ± 13 vs 137 ± 7, $P=.03$) and for those who completed three-day dietary records at baseline and repeated these diets during the final testing period (105 ± 11 vs 126 ± 15, $P=.04$). After short-term exercise training, we saw no significant change in average 24-hour potassium excretion in the entire group. In a separate analysis, we combined those subjects who continued on minimal doses of antihypertensive medications with those who had their medication completely withdrawn and compared them to subjects who were not being treated with antihypertensive medication. This separate analysis showed that both groups similarly increased their sodium excretion rate after exercise training, and the change in

Table 2. Baseline subject characteristics in the AGT M235T genotype groups

Variable	Genotype		P value
	TT (n=14)	MT+MM (n=14)	
Age, years	56 ± 2	56 ± 2	.81
Body weight, kg	95 ± 4	84 ± 4	.05
BMI, kg/m ²	32 ± 1	30 ± 1	.37
24-hour SBP, mm Hg	141 ± 2	137 ± 2	.24
24-hour DBP, mm Hg	85 ± 2	83 ± 2	.31
24-hour MBP, mm Hg	105 ± 2	102 ± 2	.23
VO ₂ max, mL/kg/min	21 ± 2	21 ± 1	.85
Creatinine clearance, mL/min	93 ± 14	88 ± 9	.73
24-hour sodium, mEq/day	119 ± 12	101 ± 11	.30
24-hour potassium, mEq/day	37 ± 6	63 ± 5	.002

BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; MBP=mean blood pressure; VO₂max=maximal oxygen consumption.

Values are means ± standard error.

sodium excretion did not significantly differ between the two groups (*P*=.12).

Changes in 24-hour sodium excretion after exercise training were different among the ACE genotype groups (Table 4). In the II genotype group, average 24-hour sodium excretion significantly increased after short-term exercise training (114 ± 22 vs 169 ± 39 mEq/day, *P*=.04). No significant increase in sodium excretion was seen in the ID (100 ± 8 vs 133 ± 17 mEq/day, *P*=.12) and the DD (113 ± 18 vs 138 ± 11 mEq/day, *P*=.13) genotype groups. The DD, ID, and II genotype groups showed a 21%, 33%, and 48% increase in sodium excretion, respectively, which suggests an I allele dosage effect. Three subjects (two with the ID and one with the DD genotype) did not complete the 24-hour ambulatory BP monitoring after short-term exercise training. We saw a consistent but small

decrease in 24-hour systolic (141 ± 2 vs 139 ± 2 mm Hg), diastolic (90 ± 1 vs 87 ± 1 mm Hg), and mean BP (107 ± 2 vs 105 ± 2 mm Hg) in the II genotype group. We also saw a 2-mm Hg reduction in mean blood pressure in the DD genotype group after exercise training. Twenty-four hour ambulatory BP values after exercise training were virtually identical to the baseline values in the ID genotype group.

Both AGT genotype groups similarly increased their 24-hour sodium excretion with short-term exercise training. The TT genotype group increased their 24-hour sodium excretion from 109 ± 14 to 143 ± 22 mEq/day (*P*=.05). The MT+MM genotype group increased their 24-hour sodium excretion from 103 ± 12 to 141 ± 15 mEq/day (*P*=.05). We found no significant changes in 24-hour systolic, diastolic, and mean BP in the AGT

genotype groups after short-term exercise training.

For the entire group, the correlation between the change in 24-hour sodium excretion and the changes in 24-hour systolic, diastolic, and mean BP were not significant. However, in the ACE II genotype group, the change in sodium excretion was significantly and negatively correlated with the change in 24-hour mean (*r*=-.95, *P*=.004) and diastolic (*r*=-.88, *P*=.02) BP. In the ACE ID and DD genotype groups, we saw no significant correlation between the change in sodium excretion and the changes in systolic, diastolic, and mean BP. The correlation between the change in sodium excretion and the changes in BP were not significant in any of the AGT genotype groups.

DISCUSSION

The present study was hypothesis-driven. We selected gene variants that affect protein levels in the blood, which could affect the intermediate phenotype, sodium excretion, and the endpoint phenotype, ambulatory BP. We found that in response to a standardized short-term exercise training protocol, and independent of genotype group, 24-hour sodium excretion was increased in hypertensive African Americans. However, we found substantial interindividual variability of the changes in sodium excretion in response to our standardized exercise training protocol. When the data were analyzed based on ACE I/D genotype, only the II genotype group significantly increased sodium excretion. We found a significant inverse correlation between the change in sodium excretion and the change in 24-hour diastolic and mean BP only in the II genotype group. Systolic, diastolic, and mean 24-hour ambulatory BP and potassium excretion did not change significantly after training in the entire group or in any ACE genotype group. Both AGT genotype groups increased

Table 3. Average values for 24-hour sodium and potassium excretion rates and ambulatory blood pressure in the entire group

Variable	Before	After	P value
Sodium, mEq/day	108 ± 9	143 ± 12	.003
Potassium, mEq/day	56 ± 7	71 ± 13	.43
Systolic BP, mm Hg	141 ± 2	139 ± 2	.14
Diastolic BP, mm Hg	90 ± 1	87 ± 1	.21
Mean BP, mm Hg	103 ± 1	102 ± 2	.27

BP=blood pressure.

Values are means ± standard error.

Table 4. Change values and P values for average 24-hour systolic, diastolic, and mean blood pressure and sodium excretion in ACE and AGT genotype groups

ACE Genotype	Change in 24-hour SBP (mm Hg)	Change in 24-hour DBP (mm Hg)	Change in 24-hour MBP (mm Hg)	Change in 24-hour Sodium Excretion (mEq/day)	Change in 24-hour Potassium Excretion (mEq/day)
II (n=7)	-2 ± 1 P=.14	-3 ± 2 P=.21	-2 ± 2.0 P=.17	55 ± 21 P=.04	4 ± 4 P=.32
ID (n=12)	-2 ± 2 P=.22	1 ± 2 P=.74	0 ± 2.0 P=.89	32 ± 19 P=.13	1 ± 8 P=.92
DD (n=9)	1 ± 3 P=.67	1 ± 2 P=.78	-2 ± 2.7 P=.43	24 ± 15 P=.13	-15 ± 18 P=.43
AGT Genotype					
TT (n=14)	2 ± 2 P=.29	.9 ± 2 P=.64	1 ± 2 P=.69	34 ± 16 P=.05	12 ± 15 P=.45
MT+MM (n=14)	1 ± 2 P=.50	-.3 ± 1 P=.78	-2 ± 2 P=.21	37 ± 17 P=.05	-3 ± 5 P=.62

SBP=systolic blood pressure; DBP=diastolic blood pressure; MBP=mean blood pressure. Values are means ± standard error.

their 24-hour sodium excretion with no significant difference between their responses.

The genotype and allele frequencies were similar to those of previous studies that have investigated the ACE I/D gene polymorphism^{9-11,34} and the AGT M235T gene polymorphism¹⁶ in African Americans and those of African descent. None of these previous studies in African Americans or in individuals of African descent determined whether the ACE I/D gene or the AGT M235T gene polymorphisms were associated with interindividual responses to an exercise intervention.

To our knowledge, the present study is the first to show that the ACE I/D gene polymorphism is associated with 24-hour sodium excretion responses to aerobic exercise training in African Americans. Differences in dietary sodium intake before, compared to after, exercise training did not likely contribute to the change in sodium excretion. Fourteen of the 31 subjects consumed identical diets during the testing periods. The remaining 17 subjects completed three-day dietary records at baseline, beginning two days before the collection of urine. Two days before the final testing, subjects were given their three-day dietary records that they completed at baseline and repeated them during the final testing period. Inspection of the dietary records did not indicate that sodium intake was differ-

ent from the baseline testing period. Furthermore, when we analyzed these groups separately, both groups similarly increased their sodium excretion after exercise training. The short-term exercise training program was vigorous for the subjects, not in terms of exercise intensity, but in terms of the daily exercise training. Thus, sodium balance may have been transiently altered because of the sudden change in habitual levels of physical activity (ie, going from being sedentary to performing daily exercise).

At baseline, 24-hour sodium excretion was similar for all three ACE genotype groups. However, after short-term aerobic exercise training, only the II genotype group significantly increased sodium excretion. In addition, the increase in sodium excreted during the 24-hour period was greater, though not significantly greater, in the ID compared to DD genotype groups after exercise training, which suggests a possible I allele dosage effect. Giner et al found an allele dosage effect of the ACE I/D polymorphism. These investigators used 24-hour ambulatory BP to assess BP sensitivity to dietary sodium in hypertensive subjects.²⁰ These investigators found that the II genotype had the greatest BP sensitivity to sodium, with the DD genotype group demonstrating the smallest BP response and the ID genotype group demonstrating an intermediate BP response to dietary sodium.

To our knowledge, the present study is the first to show that the ACE I/D gene polymorphism is associated with 24-hour sodium excretion responses to aerobic exercise training in African Americans.

One explanation for the ACE genotype-dependent differences in sodium excretion responses to exercise training could be ACE genotype-dependent differences in plasma ACE levels. ID and DD genotype individuals have 25%–50% higher plasma ACE levels than II genotype individuals.^{5,6} Other studies have also found an association between higher ACE activity and the DD genotype.^{11,35-37} ACE affects sodium reabsorption in the kidney. Therefore, a better explanation may be related to ACE genotype-dependent renal mRNA ACE levels. Mizuiri et al found that glomerular and tubular ACE mRNA expression was lowest in the II genotype group, highest in the DD genotype group, and intermediate in the ID genotype group.³⁸ Although the

effects of exercise training on plasma and tissue ACE levels are unclear, ACE genotype-dependent differences in glomerular and tubular ACE levels may have contributed to the ACE genotype-dependent differences in sodium excretion responses to short-term aerobic exercise training in the present study. However, the specific mechanism for this ACE gene-exercise interaction is presently unclear.

Another possible explanation for the differential sodium excretion response to a standardized short-term exercise training protocol may be related to ACE genotype-dependent changes in plasma insulin levels, which directly affect sodium reabsorption by the kidneys.^{39,40} Specifically, insulin reduces urinary sodium excretion through increases in tubular sodium reabsorption. Using an identical exercise training protocol as that used in the present study, we previously showed that fasting and glucose-stimulated plasma insulin levels were significantly reduced in a similar group of African American hypertensives.²⁸ Moreover, in a separate study, we showed that the changes in plasma insulin levels with exercise training were ACE genotype-dependent.⁴¹ We found that the II genotype group exhibited the greatest reduction in plasma insulin levels with exercise training compared to the ID and DD genotype groups. Based on our two previous studies, the II genotype group may have increased their sodium excretion more than subjects with a D allele because they reduced their plasma insulin levels to the greatest degree, thereby leading to reduced sodium reabsorption and enhanced sodium excretion compared to the ID and DD genotype groups.

Recent studies have investigated whether the BP response to exercise training in hypertensive individuals was ACE genotype-dependent. Zhang et al showed that the reduction in diastolic and mean BP with exercise training was greater in the II genotype group com-

pared to the DD genotype group.²⁵ Similarly, Hagberg et al demonstrated that the II and ID genotype groups had a greater reduction in systolic and diastolic BP compared to the DD genotype group after long-term endurance exercise training.²⁴ Thus, the changes in BP response to long-term aerobic exercise training may also depend, in part, on an individual's ACE genotype.

Only a few studies have investigated AGT M235T genotype-dependent changes in BP with exercise training. Recently, Rankinen et al reported that after 20 weeks of exercise training, the MM and MT genotype groups reduced their submaximal exercise diastolic BP.²⁶ Recently, Rauramaa et al reported that older men with the MM genotype who exercised over a six-year period increased their resting systolic BP by only 1 mm Hg, but those who had the MM genotype and did not exercise increased their resting systolic BP by 14 mm Hg.²⁷ Rauramaa et al also found that resting diastolic BP was reduced by 6.2 mm Hg in subjects with the MM genotype group who exercised over a six-year period, but in those in the MM genotype group who did not exercise, diastolic BP increased by 2.8 mm Hg. The findings from these two studies may indicate a possible AGT gene-exercise interactive effect on BP, but because the study populations were primarily normotensive and Caucasian, additional research is needed to determine if such an interaction exists for hypertensives of different ethnicities.

In these previous exercise training studies, changes in body weight and/or body composition may have contributed to the exercise training-induced changes in blood pressure. In the present study, we used a short-term exercise training protocol because it has been shown to not alter body weight or body composition,²⁸ both of which may affect renal sodium handling and blood pressure. We did not observe a signifi-

cant change in average 24-hour ambulatory BP in the entire group probably because the exercise training protocol was not of sufficient duration for changes in BP-regulating mechanisms to affect an endpoint phenotype such as the level of BP.

We found that the change in 24-hour sodium excretion was inversely related with the change in 24-hour diastolic and mean BP only in the II genotype group. Two previous studies reported that hypertensive subjects with the ACE II genotype had BP that was more sensitive to changes in sodium intake than hypertensive subjects with the ID and DD genotypes.^{20,21} Therefore, in the present study, the significant increase in sodium excretion after short-term exercise training may have contributed to the change in BP in the II genotype group. Although a correlation does not indicate causality, the significant correlation between the change in sodium excretion and the change in 24-hour ambulatory BP that was only observed in the II genotype group supports this contention. This finding suggests that individuals with the II genotype may be more sensitive to exercise-induced changes in sodium handling. The nature of the ACE gene interaction with an environmental factor is different in these previous studies compared to the present study. These previous studies assessed the interaction between the ACE I/D polymorphism and dietary sodium on BP. The present study assessed the interaction of the ACE I/D polymorphism and exercise training on 24-hour sodium excretion.

The AGT TT and MT+MM genotype group similarly increased their 24-hour sodium excretion by 34 and 37 mEq/day, respectively. Angiotensinogen (AGT), though inactive, is one of the precursors of angiotensin II, which causes sodium reabsorption. Polymorphisms of the AGT gene have been associated with plasma AGT levels. Jeunemaitre et al showed that TT homozygotes had \approx 20% higher plasma

AGT concentrations compared to MM homozygotes.¹³ An explanation for the lack of an association between the AGT M235T gene polymorphism and sodium excretion responses to short-term aerobic exercise training may be that the substitution of threonine for methionine at codon 235 of the AGT gene is not functional.⁴² However, the AGT G-6A polymorphism, located in the proximal 5'-flanking region of the gene, is a functional mutation in humans that affects transcription, but the transcription differences between the G and A alleles is small.⁴³ Even though the AGT G-6A and the M235T polymorphisms are in complete linkage disequilibrium,⁴³ we did not observe any AGT M235T genotype-dependent differences in sodium excretion responses to short-term exercise training.

In summary, the present study demonstrates that exercise training may act as a stimulus to perturb physiological systems and uncover responses that are partly genotype dependent. Herein, we report on the potential interaction of the ACE I/D and the AGT M235T gene polymorphisms and exercise training on changes in 24-hour urinary sodium and potassium excretion and changes in ambulatory BP in African American hypertensives. Only subjects with the ACE II genotype group demonstrated a significant increase in sodium excretion, which was inversely related with the change in 24-hour diastolic and mean BP. Our findings suggest that hypertensive African Americans with the ACE II genotype may be more likely to alter their sodium handling with short-term exercise training, and that their BP may be more responsive to exercise training-induced changes in sodium excretion. This finding does not prove a causal effect of the ACE I/D polymorphism because a previous study has shown that the ACE I/D polymorphism is in linkage disequilibrium with 17 other variants at the ACE locus.⁴⁴ Further research is needed to assess the mechanism by

which short-term exercise training alters renal sodium handling responses, a potentially important hypertension-related phenotype, in hypertensive subjects.

ACKNOWLEDGMENTS

This work was supported by NIH/NCRR/GCRC Grant #5M01 RR00056, NIA Fellowship Grant #R03 AG12781 to MDB, and Mid-Atlantic American Heart Association BGIA #99603164 to MDB NIA grant AG009802T0, and the University of Maryland General Clinical Research Center Grant M01 RR 16500, General Clinical Research Programs, National Center for Research Resources (NCCR), NIH.

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