Angiotensinogen M235T polymorphism associates with exercise hemodynamics in postmenopausal women

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McCole, Steve D., Michael D. Brown, Geoffrey E. Moore, Robert E. Ferrell, Kenneth R. Wilund, Andrea Huberty, Larry W. Douglass, and James M. Hagberg. Angiotensinogen M235T polymorphism associates with exercise hemodynamics in postmenopausal women. Physiol Genomics 10: 63–69, 2002; 10.1152/physiolgenomics.00106.2002.—We sought to determine whether the M235T angiotensinogen (AGT) polymorphism, either interacting with habitual physical activity (PA) levels or independently, was associated with cardiovascular (CV) hemodynamics during maximal and submaximal exercise. Sixty-one healthy postmenopausal women (16 sedentary, 21 physically active, and 24 endurance athletes) had heart rate (HR), blood pressure (BP), cardiac output, stroke volume (SV), total peripheral resistance (TPR), and arteriovenous O2 difference (a-vDO2) assessed during 40, 60, 80, and 100% VO2 max treadmill exercise. VO2 max did not differ among AGT genotype groups; however, maximal HR was 14 beats/min higher in AGT TT than MM genotype women (P < 0.05). AGT TT genotype women also had 19 beats/min higher HR during 100% VO2 max exercise than AGT MM genotype women (P = 0.008). AGT genotype also interacted with habitual PA levels to associate with systolic BP and a-vDO2 during 100% VO2 max exercise (both P < 0.01). AGT TT genotype women had 11 beats/min higher HR during submaximal than MM genotype women (P < 0.05). AGT genotype interacted with habitual PA levels to associate with systolic BP during submaximal exercise (P = 0.009). AGT genotype, independently or interacting with habitual PA levels, did not associate significantly with diastolic BP, cardiac output, SV, or TPR during maximal or submaximal exercise. Thus this common genetic variant in the renin-angiotensin system appears to interact, both interactively with habitual PA levels and independently, with HR, systolic BP, and a-vDO2 responses to maximal and submaximal exercise in postmenopausal women.

THE RENIN-ANGIOTENSIN-ALDOSTERONE system is intimately involved in the regulation of blood pressure (BP) and cardiovascular (CV) hemodynamics through its effects on angiotensin II, a potent vasoconstrictor, and fluid and electrolyte balance. A common genetic polymorphism within the renin-angiotensin-aldosterone system, a T-for-C substitution at nucleotide 704 of the angiotensinogen (AGT) gene leading to a methionine-to-threonine substitution at codon 235 in some but not all studies, has been associated with hypertension and BP (2, 5, 10, 13, 23). This variant is especially appealing as a candidate locus that might affect CV hemodynamics because it is relatively common (15). Furthermore, it has been shown to affect plasma angiotensinogen levels (11), which are normally close to the Km for the conversion of angiotensinogen to angiotensin I by renin (6). Therefore, changes in angiotensinogen levels could potentially alter angiotensin I and II levels. Thus the AGT locus would appear to be a putative candidate locus that might affect CV hemodynamics.

Previously Krizanova and coworkers (14) assessed the association of BP and heart rate (HR) during rest and submaximal exercise with AGT M235T polymorphisms. The primary aim of a second study, by Rankinen et al. (22), was to examine the association between this common genetic polymorphism and changes in submaximal exercise BP and cardiac output with exercise training. Both of these studies reported significant associations between BP and AGT genotype. Beyond this, very little is known about the association of this potentially important polymorphism with CV hemodynamic responses to maximal and submaximal exercise, including HR, BP, cardiac output, stroke volume (SV), total peripheral resistance (TPR), and arteriovenous O2 difference (a-vDO2). We hypothesized that this common AGT genetic variation would be associated with maximal and submaximal exercise CV hemodynamics in postmenopausal women. Because of the previous findings of Rankinen and coworkers (22), we also hypothesized that AGT genotype would interact with
METHODS

Sixty-one healthy postmenopausal women were recruited to participate in this study. All women had elevated follicle stimulating hormone and luteinizing hormone levels and reported a lack of menses for >2 yr. Women were grouped into three PA categories (16 sedentary, 21 physically active, and 24 endurance athlete) based on their habitual PA history as defined previously (8). Approximately half of the women in each group were on hormone replacement therapy (HRT). The PA and HRT status of all subjects had been constant for >2 yr prior to the study. This study was approved by the University of Pittsburgh Institutional Review Board, and all subjects provided their written informed consent prior to testing.

Sedentary and physically active subjects underwent a screening graded maximal exercise test to exclude those with evidence of CV disease (18). Women with no evidence of CV disease performed a second maximal treadmill exercise test to measure VO2 max (18). Women athletes completed a single maximal treadmill exercise test for both screening and VO2 max measurement. BP, HR, and ECG were monitored before, during, and after these tests. VO2 was measured continuously during all exercise tests. Exercise continued until the subject reached exhaustion or signs or symptoms of CV decompensation occurred. Only subjects with no evidence of CV disease were included in this study. Body composition was determined with dual energy X-ray absorptiometry (DPX-L; Lunar, Madison, WI).

Cardiac output was measured by acetylene rebreathing after ~6 min of treadmill exercise at 40, 60, and 80% of VO2 max and during the last minute of an exercise bout designed to elicit VO2 max in ~6 min (18). During the first 1–2 min of the trial designed to assess hemodynamics at ~100% VO2 max, the treadmill was initially set to the speed and grade that elicited 80% of VO2 max. At ~2 min the grade was increased in an effort to raise VO2 to ~90% over the next 2 min. At minute 4 the grade was increased again a final time. As VO2 levels during this trial were >90% VO2 max for all groups, this trial is denoted as exercise at ~100% VO2 max. SV was determined by dividing cardiac output by HR measured via ECG just prior to the rebreathing maneuver. VO2 was monitored throughout each exercise bout, and a-vDO2 was calculated by dividing VO2 by cardiac output. TPR was calculated as mean arterial pressure (MAP) divided by cardiac output, with MAP estimated as diastolic BP + 1/3(systolic BP – diastolic BP) based on BP measured by auscultation immediately preceding each cardiac output determination. In this study, the hemodynamic data relative to habitual PA levels are not presented, as they have been published previously (17, 18). In these previous studies, HRT was not associated with CV hemodynamic responses to exercise; thus the data of the women on and not on HRT are pooled in the present study.

DNA was isolated from peripheral venous blood samples (19). A 354-bp fragment containing the variable site was amplified following the method of Jeunemaitre et al. (11). The T-C transition was genotyped by the oligonucleotide ligation assay (20) using the biotin-labeled capture oligonucleotides 5’-(biotin)AAGACTGTCFTGCCTCCGTAT-3’ and 5’-(biotin)GAGACTGTCFTGCCTCCGTAC-3’ and an alkaline phosphatase-conjugated detection oligonucleotide 5’-(AP)GGAGCCAGTGTGGACAGCA-3’.

Table 1. AGT allele and genotype distributions in the present study and a large meta-analysis study (15)

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Frequency</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Population</td>
<td>0.45</td>
<td>0.20</td>
</tr>
<tr>
<td>Sedentary</td>
<td>0.45</td>
<td>0.20</td>
</tr>
<tr>
<td>Physically-active</td>
<td>0.45</td>
<td>0.20</td>
</tr>
<tr>
<td>Athletes</td>
<td>0.45</td>
<td>0.20</td>
</tr>
<tr>
<td>General population</td>
<td>0.45</td>
<td>0.20</td>
</tr>
</tbody>
</table>

AGT, angiotensinogen.
Interaction probabilities are for AGT genotype groups. However, maximal HR during the VO\textsubscript{2} max test was significantly different among AGT genotype groups. VO\textsubscript{2} max expressed in absolute terms or relative to body weight also did not differ among AGT genotype groups (Table 2). VO\textsubscript{2} max expressed in absolute terms or relative to body weight also did not differ among AGT genotype groups.

CV hemodynamics at ~100\% VO\textsubscript{2} max exercise. Tests of the interaction between AGT genotype and PA levels were not significant for HR during ~100\% VO\textsubscript{2} max exercise (Table 3). However, AGT genotype was also independently and significantly associated with HR during ~100\% VO\textsubscript{2} max exercise. As during the VO\textsubscript{2} max test, AGT TT genotype women had higher HR during ~100\% VO\textsubscript{2} max exercise than AGT MM genotype women, respectively (P < 0.01).

Tests of the interaction between AGT genotype and PA levels were significant for systolic BP during ~100\% VO\textsubscript{2} max exercise (P < 0.001) (Table 3). AGT TT and MT genotype women had higher systolic BP during ~100\% VO\textsubscript{2} max exercise than AGT MM genotype women in the sedentary group, whereas AGT genotype effects were not significant for systolic BP during ~100\% VO\textsubscript{2} max exercise among the physically active or athletic women. Neither the AGT genotype by habitual PA level interaction nor the AGT main effect for diastolic BP during ~100\% VO\textsubscript{2} max exercise reached the level of significance (both P = 0.07).

AGT genotype, either interactively with habitual PA levels or independently, did not associate significantly with cardiac output, SV, or TPR during exercise at ~100\% VO\textsubscript{2} max. AGT genotype interacted with habitual PA levels to associate significantly (P = 0.01) with a-vDO\textsubscript{2} during ~100\% VO\textsubscript{2} max exercise (Fig. 2). AGT TT and MT genotype women had higher a-vDO\textsubscript{2} during ~100\% VO\textsubscript{2} max exercise than AGT MM genotype women in the sedentary group. There were no significant differences among AGT genotype groups for a-vDO\textsubscript{2} during ~100\% VO\textsubscript{2} max exercise among physically active women; however, AGT MM genotype women had marginally higher a-vDO\textsubscript{2} during ~100\% VO\textsubscript{2} max exercise than AGT MT genotype women (P = 0.06) among the athletes. There was no AGT genotype effect for a-vDO\textsubscript{2} during ~100\% VO\textsubscript{2} max exercise when averaged across all habitual PA groups.

Submaximal exercise CV hemodynamics. Tests of the interaction between AGT genotype and submaximal exercise intensity were significant (P < 0.01) for diastolic BP such that diastolic BP was significantly lower at 40\% VO\textsubscript{2} max than at either 60 or 80\% VO\textsubscript{2} max in the AGT TT genotype group, whereas no such relationship was evident in the other AGT genotype groups. No other AGT genotype by submaximal exercise intensity interactions were significant for any of the other hemodynamic variables.

Tests for the interaction between AGT genotype and habitual PA levels were not significant for submaximal exercise HR (Table 4). AGT genotype associated independently and significantly with HR during submaximal exercise, with AGT TT genotype women again having an 11 beats/min higher HR than AGT MM genotype women (P < 0.05) and with AGT MT genotype women having values between the two AGT homozygote groups.
AGT genotype and habitual PA level interacted to associate significantly ($P < 0.01$) with systolic BP during submaximal exercise (Fig. 3), with systolic BP during submaximal exercise being higher in AGT TT and MT than MM genotype carriers among sedentary women and with there being no AGT genotype-dependent submaximal exercise systolic BP differences among physically active and athletic women. AGT genotype did not independently associate with systolic BP during submaximal exercise across all habitual PA groups. AGT genotype, either interactively with habitual PA levels or independently, did not associate significantly with diastolic BP, cardiac output, SV, TPR, or a-vDO$_2$ during submaximal exercise.
DISCUSSION

The findings of the present study indicate that a common polymorphic variation at the AGT M235T locus is associated with a number of CV hemodynamic responses to maximal and submaximal exercise in postmenopausal women. The most consistent of these associations was with maximal and submaximal exercise HR, with the genotype-dependent associations evident for HR during the VO₂ max test, during exercise at 100% VO₂ max, and during submaximal exercise, with AGT TT genotype women having the highest HR and AGT MM genotype women having the lowest HR at all exercise intensities. These genotype-dependent associations with maximal and submaximal exercise HR were independent of habitual PA levels. Despite these genotype-dependent differences in HR, AGT M235T genotype was not independently associated with BP, SV, TPR, or cardiac output during maximal or submaximal exercise or VO₂ max.

AGT genotype in the present study also interacted significantly with habitual PA levels to associate with maximal and submaximal exercise systolic BP and maximal exercise a-vO₂. These relationships indicated that AGT genotype associated significantly with maximal and submaximal exercise systolic BP among sedentary women, whereas there were no significant AGT genotype-systolic BP associations during maximal or submaximal exercise among physically active or athletic women. AGT TT and MT genotype women also had higher maximal exercise a-vO₂ levels than MM genotype women in the sedentary group, whereas, again, there were no AGT genotype-dependent maximal exercise a-vO₂ relationships among the physically active or athletic women. In all of these interaction relationships, values are means ± SE. Means within a variable with different letters are significantly different at P < 0.05. The n values below the “AGT Genotype” heading indicate the range of sample sizes for the different variables for the group (maximum of 3 per subject, 1 per exercise intensity). Interaction probabilities are for AGT genotype × habitual PA level. Statistical methods as outlined in text. Absent P values indicate P > 0.10.

Table 4. Submaximal exercise CV hemodynamics as a function of AGT genotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>TT</th>
<th>MT</th>
<th>MM</th>
<th>AGT genotype Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>36</td>
<td>78–79</td>
<td>47–54</td>
<td></td>
</tr>
<tr>
<td>VO₂, l/min</td>
<td>1.04 ± 0.05</td>
<td>1.03 ± 0.03</td>
<td>1.03 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>VO₂, ml·kg⁻¹·min⁻¹</td>
<td>17 ± 1</td>
<td>18 ± 1</td>
<td>17 ± 1</td>
<td>0.04</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>122 ± 4a</td>
<td>117 ± 2b</td>
<td>111 ± 3c</td>
<td>0.009</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>156 ± 4</td>
<td>156 ± 2</td>
<td>151 ± 3</td>
<td></td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>80 ± 2</td>
<td>79 ± 1</td>
<td>75 ± 2</td>
<td></td>
</tr>
<tr>
<td>CO, l/min</td>
<td>8.7 ± 0.5</td>
<td>8.7 ± 0.3</td>
<td>8.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>SV, ml</td>
<td>73 ± 4</td>
<td>75 ± 2</td>
<td>78 ± 3</td>
<td></td>
</tr>
<tr>
<td>TPR, dyn·s⁻¹·cm⁻⁵</td>
<td>1,020 ± 64</td>
<td>1,038 ± 37</td>
<td>985 ± 51</td>
<td></td>
</tr>
<tr>
<td>a-vO₂ diff, ml/100 ml</td>
<td>11.7 ± 0.4</td>
<td>11.7 ± 0.2</td>
<td>11.7 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Means within a variable with different letters are significantly different at P < 0.05. The n values below the “AGT Genotype” heading indicate the range of sample sizes for the different variables for the group (maximum of 3 per subject, 1 per exercise intensity). Interaction probabilities are for AGT genotype × habitual PA level. Statistical methods as outlined in text. Absent P values indicate P > 0.10.
it appears that some level of habitual PA, either being physically active or an endurance athlete, eliminates or, in one case, reverses the relationships between AGT genotype and these CV hemodynamic responses to exercise evident in sedentary women.

Bouchard et al. (4) previously concluded that genetics contributed to determining CV hemodynamics at rest and during submaximal exercise. Bielen and co-workers (3) reported that the heritabilities of a number of exercise hemodynamic parameters ranged from 24 to 47%. Prior to studies of specific genetic markers, Landry et al. (16) reported that exercise hemodynamic changes with exercise training were more similar in monozygotic than dizygotic twins. Recently An et al. (1) reported from the HERITAGE Study that the heritabilities of submaximal exercise SV and cardiac output were 40–45% in 438 individuals from 99 two-generation Caucasian families. This same study also indicated that the heritabilities of submaximal exercise SV and cardiac output changes with endurance exercise training were in the range of 24–38% (1).

Krizanova and coworkers (14) appear to be the first to assess the possibility that the AGT M235T polymorphism was associated with CV hemodynamic responses to exercise. They measured BP and HR responses to three submaximal cycle ergometer work rates and found that the increases in diastolic BP from rest to exercise were significantly greater in AGT MM compared with AGT TT genotype individuals. The systolic BP and HR responses to these same work rates were not associated with AGT genotype, nor were the plasma epinephrine, norepinephrine, or angiotensin I and II responses to exercise.

Our results are opposite to those of Krizanova et al. (14), as we found no significant differences in the diastolic BP of individuals with different AGT genotypes during submaximal or maximal exercise. Although these failed to achieve statistical significance, it may be important to note that both systolic BP (P = 0.06) and diastolic BP (P = 0.07) tended to be higher in the women with the AGT TT genotype. One potential reason underlying these different results may be the use of absolute work rates in the previous study (14) vs. the design of the present study, which used a number of submaximal work rates normalized for each individual's VO2_max. The use of absolute work rates would increase the interindividual variation in HR responses, compared with at the same relative work rates, which could make it more difficult or potentially impossible to detect significant genotype-dependent HR responses. Another potential reason for the different results is that Krizanova et al. (14) studied young to middle-aged men, whereas in the present study the associations were assessed in postmenopausal women.

Rankinen and coworkers (22) recently reported data relating maximal and submaximal exercise BP responses to AGT M235T genotype for Caucasians (n = ~475) in the HERITAGE Study. They found no association between AGT genotype and systolic or diastolic BP during 50 W submaximal exercise prior to exercise training. However, as in the study by Krizanova and coworkers (14), this submaximal exercise was at the same absolute work rate for all subjects and was not normalized for each subject's CV fitness. Since relative exercise intensity clearly affects both systolic and diastolic BP, the use of an absolute work rate without normalizing for CV fitness may have obscured any potential association between AGT genotype and submaximal exercise BP responses. Thus it is unclear whether the differences between the studies are the result of differences in relative intensity or other factors. Rankinen and colleagues (22) did find an association in men between diastolic BP during maximal exercise prior to training and AGT genotype (89.7, 83.4, and 80.8 mmHg for the TT, MT, and MM genotype, respectively). Rankinen and coworkers (22) also reported significant associations between AGT M235T genotype and exercise training-induced decreases in submaximal exercise BP. However, the association was only evident in men and only for diastolic BP (~3.7, ~3.3, and ~0.4 mmHg for the MM, MT, and TT genotype, respectively). The exercise training-induced reduction in diastolic BP during maximal exercise tended to be greater in the AGT TT genotype men (~7.8 mmHg) compared with the MT and MM genotype men (~3.3 and ~0.7 mmHg, respectively) (P = 0.045), but the differences were not statistically significant when baseline maximal exercise diastolic BP was included as a covariate.

Our results were consistent with those of Rankinen et al. (22) for BP responses to submaximal exercise. They found no significant associations between AGT M235T genotype and systolic or diastolic BP responses to an absolute submaximal exercise stimulus (22). Although we recognize that they did not reach the level of statistical significance, it may be important to note that during maximal exercise both systolic BP and diastolic BP were somewhat higher in those women with the TT genotype. The present results are somewhat consistent with those of Rankinen and coworkers (22) for BP responses during maximal exercise, as in both studies maximal exercise diastolic BP was, or tended to be, significantly higher in AGT TT genotype individuals.

The most dramatic and consistent of the independent AGT associations in the present study was with HR during maximal and submaximal exercise. These differences between the two homozygote AGT genotype groups were substantial, amounting to 11 beats/min (~9%) during submaximal exercise, 19 beats/min (~11%) during ~100% VO2_max exercise, and 14 beats/min (~8%) during the VO2max test. It is difficult to ascribe these differential HR responses in mechanistic terms either directly or indirectly to the AGT M235T polymorphic variation. HR during maximal and submaximal exercise is primarily regulated by the parasympathetic and sympathetic nervous systems. Although the renin-angiotensin system does interact with the sympathetic nervous system, this control is primarily involved in the regulation of peripheral vascular smooth muscle tone (9) and not central CV chronotropic responses. In fact, angiotensin I infusions

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have, at best, only a minimal impact on HR (7, 21). Furthermore, angiotensin converting enzyme (ACE) inhibitors are believed to be an optimal medication for hypertensives who want to exercise, because they have negligible effects on exercise HR responses and maximal exercise capacities (12, 21). Thus it is entirely possible that the AGT M235T locus is simply a marker for another adjacent genetic variant that may be within the sympathetic nervous system and which would be better linked, in mechanistic terms, to our findings of altered exercise HR responses associated with AGT M235T genotypes. No obvious candidate genes for influencing sympathetic nervous system function have been mapped near the AGT chromosomal locus.

On the other hand, the findings in the present study indicating independent and interactive associations, or trends for associations, between AGT M235T genotype and maximal and submaximal exercise BP can be more easily postulated to be related directly to the AGT M235T variant. The altered renin-angiotensin system function that occurs with the elevated plasma and tissue angiotensinogen levels associated with the AGT TT genotype (11) clearly could affect peripheral vascular smooth muscle tone, thereby directly altering systemic and diastolic BP.

A limitation of this study is the small number of subjects in some of the groups, particularly the sedentary women with the TT genotype. However, analysis of the data indicates that the significant interactions between AGT genotype and habitual PA level were affected by differences between the MT and MM genotypes and not just due to the small number of sedentary women with the TT genotype. Furthermore, the study sample was in Hardy-Weinberg equilibrium.

In summary, a number of CV hemodynamic responses to maximal and submaximal exercise were independently and interactively associated with AGT M235T genotype. Primary among these were the HR responses to maximal and submaximal exercise, which were substantially higher in the AGT TT genotype women. AGT M235T genotype also interacted with habitual PA levels to associate with maximal and submaximal exercise systolic BP and a-vDO2. Thus the AGT M235T locus appears to be a “candidate” locus that, independently and interacting with habitual PA levels, affects CV hemodynamic responses to maximal and submaximal exercise.

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