ACE insertion/deletion polymorphism and submaximal exercise hemodynamics in postmenopausal women

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Hagberg, James M., Steve D. McCole, Michael D. Brown, Robert E. Ferrell, Kenneth R. Wilund, Andrea Huberty, Larry W. Douglass, and Geoffrey E. Moore. ACE insertion/deletion polymorphism and submaximal exercise hemodynamics in postmenopausal women. J Appl Physiol 92: 1083–1088, 2002; 10.1152/japplphysiol.00135.2001.—We sought to determine whether the angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) polymorphism is associated with submaximal exercise cardiovascular hemodynamics. Postmenopausal healthy women (20 sedentary, 20 physically active, 22 endurance athletes) had cardiac output (acetylene rebreathing) measured during 40, 60, and 80% VO2max exercise. The interaction of ACE genotype and habitual physical activity (PA) level was significantly associated with submaximal exercise systolic blood pressure, with only sedentary women exhibiting differences among genotypes. No significant effects of ACE genotype or its interaction with PA levels was observed for submaximal exercise diastolic blood pressure. ACE II genotype or its interaction with PA levels was observed for tertile differences among genotypes. No significant effects of ACE genotype or interaction with PA levels was observed for tertile differences among genotypes. No significant effects of ACE genotype or interaction with PA levels was observed for tertile differences among genotypes. No significant effects of ACE genotype or interaction with PA levels was observed for tertile differences among genotypes. No significant effects of ACE genotype or interaction with PA levels was observed for tertile differences among genotypes. No significant effects of ACE genotype or interaction with PA levels was observed for tertile differences among genotypes. No significant effects of ACE genotype or interaction with PA levels was observed for tertile differences among genotypes. No significant effects of ACE genotype or interaction with PA levels was observed for tertile differences among genotypes. No significant effects of ACE genotype or interaction with PA levels was observed for tertile differences among genotypes. No significant effects of ACE genotype or interaction with PA levels was observed for tertile differences among genotypes. No significant effects of ACE genotype or interaction with PA levels was observed for tertile differences among genotypes.

A common insertion (I)/deletion (D) polymorphism at the angiotensin-converting enzyme (ACE) locus has been found to be related to a number of physiological and pathological phenotypes in the central and peripheral cardiovascular (CV) system (5, 15). Our group (8) and others have previously shown that this common genetic variant is associated with endurance performance and maximal O2 consumption (VO2max). In our previous study, VO2max was related to the presence of the I allele in a gene-dosage-dependent fashion as a result of differences in maximal arteriovenous oxygen difference (a-v)O2 (7). Maximal cardiac output was virtually identical in the ACE II, ID, and DD genotype groups, and maximal stroke volume (SV) was somewhat higher in the ACE ID and DD genotype women, although the differences were not significant. This slight difference in maximal SV was completely offset by a significantly higher maximal heart rate (HR) in the ACE II compared with the ID and DD genotype women. Thus ACE genotype clearly associates with a number of CV hemodynamic responses during maximal exercise. However, it is not known whether these CV hemodynamic response differences among ACE genotype groups during maximal exercise are evident during submaximal exercise.

To our knowledge, our group’s study (7) and only three others have assessed the association between the ACE I/D polymorphism and CV hemodynamics during exercise (6, 12, 13). The first study assessed only blood pressure (BP) and HR during submaximal and maximal exercise (6). The other two studies found significant ACE genotype-exercise training interactive associations with submaximal exercise HR and diastolic BP (12, 13). Therefore, in the present study, we hypothesized that the ACE I/D polymorphism would be associated with CV hemodynamics, including HR, BP, cardiac output, SV, total peripheral resistance (TPR), and (a-v)O2 during submaximal exercise in postmeno-

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pausal women. Because of the recent results of Rankinen et al. (12, 13), we also hypothesized that there would be significant interactions between ACE genotype and habitual physical activity (PA) level to associate with CV hemodynamic responses to submaximal exercise. We addressed this hypothesis by studying subjects with substantially different levels of habitual PA.

METHODS

Sixty-two healthy postmenopausal women were recruited to participate in this study. Women were considered postmenopausal if they had elevated levels of follicle-stimulating and luteinizing hormones and reported a lack of menses for >2 yr. Women were recruited into three PA categories (sedentary, physically active, endurance athlete) on the basis of their habitual PA history as defined previously (7). 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 before the study. The Institutional Review Board of the University of Pittsburgh approved this study, and all subjects provided their written, informed consent before testing.

Sedentary and physically active subjects underwent a screening graded maximal exercise test to exclude those with evidence of CV disease (11). They then performed a second maximal treadmill exercise test to measure \( V\dot{O}_2^{\text{max}} \) (11). Women athletes completed a single maximal treadmill exercise test for both screening and \( V\dot{O}_2^{\text{max}} \) measurement (11). BP, HR, and electrocardiogram were monitored before, during, and after all tests. \( O_2 \) uptake (\( V\dot{O}_2 \)) was measured continuously during all exercise tests. Exercise continued until the subject reached exhaustion or signs or symptoms of CV decompensation occurred. Body composition was determined with dual energy X-ray absorptiometry (DPX-L, Lunar, Madison, WI). DNA was isolated from peripheral venous blood samples and was typed for the ACE I/D variant by using the procedures of Hagberg et al. (7).

Cardiac output was measured by acetylene rebreathing after ~6 min of treadmill exercise at 40, 60, and 80% of \( V\dot{O}_2^{\text{max}} \) (11). SV was determined by dividing cardiac output by HR measured via electrocardiogram just before the rebreathing maneuver. \( V\dot{O}_2 \) was monitored throughout each exercise bout, and \( (a-v)O_2 \) was calculated by dividing \( VO_2 \) by cardiac output. TPR was calculated as mean arterial pressure (MAP) divided by cardiac output with MAP estimated as diastolic BP + 1/3 \( \times \) systolic BP-diastolic BP on the basis of BP measured by auscultation immediately preceding each cardiac output determination. Data on the effect of habitual PA levels and HRT status on submaximal and maximal exercise hemodynamics have been published previously (10, 11). In these studies, HRT was not associated with different CV hemodynamic responses to exercise, and the data of the women on and not on HRT are pooled in the present study.

For each dependent variable (systolic and diastolic BP, HR, cardiac output, SV, TPR, and \( (a-v)O_2 \)), we conducted a mixed-model repeated-measures factorial ANOVA (SAS Online version 8.1.1, 1999). The three levels of physical activity and three genotypes at the ACE locus form the factorial, and each subject was measured for each dependent variable at 40, 60, and 80% \( V\dot{O}_2^{\text{max}} \), resulting in three repeated measures. Random effects included variation among subjects, covariance among repeated measures, and the residual variation within subjects. Different covariance structures were used to fit the correlation between repeated measures (submaximal exercise intensity) within subject, and the best-fitting variance-covariance structure was chosen by using the Bayesian Information Criterion. If the ANOVA model was significant for an ACE genotype or ACE genotype by habitual PA level interaction effect, contrasts were conducted for appropriate means comparisons among genotypes using \( t \) probabilities to identify significant differences. The degrees of freedom method used was Kenward-Roger. Each model and dependent variable also adequately met the assumptions of variance homogeneity and normality. Because not all trials yielded technically valid results, the sample sizes for the different CV hemodynamic variables during submaximal exercise vary somewhat; these sample sizes are included in Table 3, where these results are presented. \( P < 0.05 \) was considered statistically significant. Least squares means ± SE are reported, with correction for any unequal replication and averaging across the three submaximal exercise intensities (40, 60, and 80% \( V\dot{O}_2^{\text{max}} \)).

RESULTS

As our group has shown previously in these women (7), ACE allele and genotype frequencies in our total study population were similar to those in larger population studies and did not differ significantly from Hardy-Weinberg expectations (Table 1). Furthermore, the distributions in each habitual PA group were not different from each other and did not differ significantly from Hardy-Weinberg expectations (data not shown). Age, body weight, height, and percent body fat were similar in the three ACE genotype groups (Table 2). However, as shown previously in these women (7), \( V\dot{O}_2^{\text{max}} \) (in ml·kg\(^{-1}\)·min\(^{-1}\)) was significantly different among ACE genotype groups, with the ACE II genotype women having the highest \( V\dot{O}_2^{\text{max}} \) values. Also, as shown previously in these women (7), maximal HR differed significantly among ACE genotype groups with the ACE II genotype women having an ~10 beats/min higher maximal HR than otherwise similar ACE ID or DD genotype women.

**Submaximal exercise hemodynamics.** There was a significant interactive effect between ACE genotype and habitual PA levels on submaximal exercise systolic BP (\( P < 0.01 \)) (Table 3), with ACE ID genotype having the lowest and ACE II genotype women the greatest submaximal exercise systolic BP among sedentary women, whereas no ACE genotype differences were observed for submaximal exercise systolic BP among physically active women or women athletes (Fig. 1). ACE genotype did not significantly affect submaximal exercise systolic BP averaged across habitual PA.
Table 2. Subject characteristics and $\dot{V}O_2\text{max}$ as a function of ACE genotype

<table>
<thead>
<tr>
<th>ACE Genotype</th>
<th>II (n = 12)</th>
<th>ID (n = 33)</th>
<th>DD (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>62 ± 1</td>
<td>63 ± 1</td>
<td>66 ± 1</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>60 ± 3</td>
<td>60 ± 2</td>
<td>60 ± 2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>160 ± 2</td>
<td>160 ± 1</td>
<td>159 ± 2</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>33 ± 2</td>
<td>33 ± 1</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>$\dot{V}O_2\text{max}, \text{mlkg}^{-1}\cdot\text{min}^{-1}$</td>
<td>32 ± 9</td>
<td>29 ± 1b</td>
<td>27 ± 1b</td>
</tr>
<tr>
<td>Maximal HR, beats/min</td>
<td>172 ± 4a</td>
<td>162 ± 2b</td>
<td>162 ± 3b</td>
</tr>
</tbody>
</table>

Values are means ± SE. $\dot{V}O_2\text{max}$, maximal O$_2$ uptake. Means within a variable with different letters are significantly different at $P < 0.05$.

Table 3. Submaximal exercise CV hemodynamics as a function of ACE genotype

<table>
<thead>
<tr>
<th>ACE Genotype</th>
<th>II (n = 30–33)</th>
<th>ID (n = 86–96)</th>
<th>DD (n = 36–41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mmHg</td>
<td>160 ± 4</td>
<td>151 ± 2</td>
<td>156 ± 3</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>82 ± 2</td>
<td>78 ± 1</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>124 ± 4a</td>
<td>113 ± 2b</td>
<td>116 ± 3b</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>9.1 ± 0.4</td>
<td>8.7 ± 0.3</td>
<td>8.3 ± 0.4</td>
</tr>
<tr>
<td>Stroke volume, ml</td>
<td>74 ± 3</td>
<td>77 ± 2</td>
<td>72 ± 3</td>
</tr>
<tr>
<td>Total peripheral resistance, dyn·s·cm$^{-5}$</td>
<td>1,030 ± 60</td>
<td>990 ± 40</td>
<td>1,060 ± 50</td>
</tr>
<tr>
<td>(a-v)O$_2$, ml/100 ml</td>
<td>12.2 ± 0.3</td>
<td>11.5 ± 0.2</td>
<td>11.7 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. BP, blood pressure; (a-v)O$_2$, arteriovenous O$_2$ difference. Means within a variable with different letters are significantly different at $P < 0.05$. Numbers in parentheses below the ACE 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 ACE genotype × habitual PA level. Statistical methods are as outlined in text.

ACE polymorphism and exercise hemodynamics

Submaximal exercise CV hemodynamics as a function of ACE genotype

Dissection of the data for maximal exercise in the present study.

DISCUSSION

The present results indicate that ACE genotype is significantly, independently, and interactively associated with the responses of a number of critical CV hemodynamic variables to submaximal exercise in postmenopausal women. HR during submaximal exercise ranging in intensity from 40–80% $\dot{V}O_2\text{max}$ was independently and significantly associated with ACE genotype. In addition, the effect of ACE genotype on systolic BP, cardiac output, and SV during submaximal exercise was dependent on habitual PA level in the present study.
Bouchard et al. (3) previously established, via heritability and family studies, that genetics contributed significantly and substantially to determining CV hemodynamics at rest and in response to submaximal exercise. Bielen and co-workers (2) reported that the heritabilities of some exercise hemodynamic parameters were in the range of 24–47%. Although no specific markers were investigated at the time, Landry et al. (9) also found that exercise hemodynamic changes with exercise training were significantly more similar in monozygotic than dizygotic twins. Expanding on these results, a recent paper by An et al. (1) from the HERITAGE Study indicated that the heritabilities of SV and cardiac output during submaximal exercise were on the order of 40–45% in a relatively large population (n = 438) from 99 two-generation Caucasian families. This same study also indicated that the heritabilities of the change in submaximal exercise SV and cardiac output with a highly standardized endurance exercise training intervention were in the range of 24–38% (1).

To our knowledge, the first study to assess potential relationships between specific genetic markers, in fact ACE genotype, and exercise CV hemodynamics was that of Friedl et al. in 1996 (6). They found a trend for higher HR in ACE homozygotes compared with heterozygotes during submaximal exercise at the same absolute work rate (P = 0.086). There also was a trend for higher submaximal exercise diastolic BP in ACE DD compared with ACE ID and II genotype men (P = 0.094). At maximal exercise, ACE DD genotype men had a significantly higher diastolic BP than ACE ID and II genotype men (93, 85, and 82 mmHg, respectively; P = 0.01). Rankinen and co-workers in 2000 (12, 13) previously provided evidence from the exercise training intervention phase of the HERITAGE Study that HR and BP

and some baseline CV hemodynamic measures, as well as their changes with exercise training, in the HERITAGE study. They found that before exercise training BP and HR responses to 50 W exercise were not independently associated with ACE genotype. Relative to the changes in submaximal exercise CV hemodynamics, only the exercise training-induced change in diastolic BP during 50 W exercise was associated with ACE genotype (P = 0.05) whereas the changes in submaximal exercise systolic BP, HR, cardiac output, and SV responses with exercise training were not independently associated with ACE genotype.

It was previously reported in the same women as in the present study that VO2 max was ~20% higher in ACE II than DD genotype women (7). The higher VO2 max in the ACE II genotype women was associated with a significantly higher maximal HR. However, the ACE II genotype women had a somewhat lower maximal SV, and maximal cardiac index was virtually identical in the three ACE genotype groups. Thus the higher VO2 max in the ACE II genotype women was the result of a significantly higher (a-v)O2 during maximal exercise. The present study extends these results by indicating that the ACE genotype-dependent differences in HR during maximal exercise are already evident at submaximal work rates in these same women. The ACE II genotype women in the present study had higher submaximal exercise HR than ACE ID genotype women. However, all three ACE genotype groups again had similar submaximal exercise cardiac output values.

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changes with exercise training were associated with ACE genotype in some subsets of the overall population. Our cross-sectional data indicate that the effect of ACE genotype on many of CV hemodynamics measured in the present study, including systolic BP, cardiac output, and SV, was dependent on habitual PA levels. Thus the habitual PA level of an individual appears to be an important consideration when attempting to understand CV hemodynamic responses to submaximal exercise as a function of ACE genotype.

It is difficult to ascribe the ACE genotype-dependent differences in submaximal and maximal exercise HR directly to the I/D variant at the ACE gene locus. ACE inhibition is generally believed to be an optimal medication for hypertensive individuals choosing to exercise because it has minimal to no impact on HR responses to exercise or maximal exercise capacity. Furthermore, angiotensin I infusions have minimal effects on restoring HR. Thus it is unlikely that the minimal alterations in plasma and tissue ACE activity associated with the different ACE genotypes could have a substantial direct or indirect effect on exercise HR responses. Therefore, it is entirely possible that the ACE polymorphic variation may simply be a marker for another variation in the same chromosomal region that affects the sympathetic nervous system, which would have a more direct role in regulating HR responses to exercise. No obvious candidate genes for sympathetic nervous system response have been localized to chromosome 17q23, which contains the ACE gene. However, a number of calcium channel subunits, known to be expressed in the heart and vasculature (voltage-gated calcium channel γ-1 and β-1 subunits and T-type voltage-dependent calcium channel α-1G subunit) have been mapped to this general chromosomal region and may be candidate genes for these HR responses.

On the other hand, the significant interaction between ACE genotype and habitual PA levels for systolic BP, cardiac output, and SV during submaximal exercise could potentially be the direct or indirect result of ACE genotype. ACE genotype has been found to associate with vascular smooth muscle function at rest, and the present results would imply that ACE genotype interacting with habitual PA levels could also affect vascular smooth muscle function during exercise. Such alterations in vascular smooth muscle function during exercise clearly could directly and indirectly affect BP, SV, and cardiac output.

It is important to bear in mind that both the ACE I and D alleles are common in all ethnic groups examined to date (4). For example, in Caucasians the frequencies for both the I and D alleles are ~0.50, giving rise to population genotype frequencies of 0.25 for the two ACE homozygote groups and 0.50 for ACE heterozygotes. Therefore, even the least frequent ACE homozygote genotypes are present in 25% of a Caucasian population. However, the allele frequencies are substantially different in other ethnic groups, with the D and I alleles having frequencies of 0.65 and 0.35 in African Americans, respectively, and 0.30 and 0.70 in Chinese, respectively (4). These allele frequencies result in expected DD, ID, and II genotype frequencies of 0.42, 0.46, and 0.12 in African Americans, respectively, and 0.09, 0.42, 0.49 in Chinese, respectively. Thus the ACE genotype-dependent CV hemodynamic responses to submaximal exercise evident in this study could have an impact on the submaximal exercise CV hemodynamic responses on an individual basis and could well also affect CV hemodynamic responses to submaximal exercise on a population-wide basis, although perhaps differently when compared among different ethnic groups.

In conclusion, the present results indicate that the association previously found between HR responses to maximal exercise and ACE genotype is already evident during submaximal exercise. ACE genotype also interacts with the habitual PA levels of these postmenopausal women to affect a number of CV hemodynamic responses during submaximal exercise, including systolic BP, cardiac output, and SV.

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