Interaction between Angiotensin Converting Enzyme Insertion/Deletion Genotype and Exercise Training on Knee Extensor Strength in Older Individuals

Authors
V. Giaccaglia¹, B. Nicklas²–³, S. Kritchevsky², J. Mychalecky²–³, S. Messier¹, E. Bleecker³, M. Pahor⁵

Affiliations
1 Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, United States
2 Department of Internal Medicine, WFUSM, Winston-Salem, United States
3 Genomics Center, WFUSM, Winston-Salem, United States
4 Department of Health and Exercise Science, Wake Forest University, Winston-Salem, United States
5 Department of Aging and Geriatric Research, Univ. of Florida, Winston-Salem, United States

Abstract
Prior data in young individuals suggest that the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism interacts with exercise to affect athletic performance, but the direction of the genotype effect depends on the outcome assessed (endurance vs. strength). The purpose of this study was to determine whether the ACE I/D genotype influences physical function responses to exercise training in older individuals. Physical function (muscle strength, walking distance, and self-reported disability) was measured before and after an 18-month randomized, controlled exercise trial in 213 older (≥ 60 yrs), obese (BMI ≥ 28 kg/m²) men and women. Exercise training consisted of walking and light weight lifting for one hour 3 times/wk. At baseline, there were no associations between ACE I/D genotype and measures of physical function. Following exercise training, individuals with the DD genotype showed greater gains in knee extensor strength compared to II individuals. There was a significant (p = 0.014) interaction between ACE I/D genotype and exercise treatment on percent change in knee strength. In addition, there was a trend towards a greater improvement in physical disability score in DD genotypes (p = 0.13), but changes in 6-minute walk distance were not different between genotype groups. Thus, changes in muscle strength with exercise training in older individuals may be dependent on ACE I/D genotype.

Introduction
Declines in physical function with age are associated with a higher incidence of disability and institutionalization [19,22]. Currently, regular exercise is the only therapy known to improve physical function in the elderly [4,12,17,25]. However, there is considerable heterogeneity in the responsiveness to exercise training and data show that at least part of this variability can be attributed to genetic factors [1]. Previous studies in younger persons show that genetic variation in the angiotensin-converting-enzyme (ACE) gene is associated with variable physical performance and contributes to variability in training responses [10]. ACE catalyzes the conversion of angiotensin I to angiotensin II as well as the breakdown of bradykinin. The ACE gene localizes to chromosome 17q23 and the polymorphism shown to interact with physical exercise is defined by the presence (insertion, I allele) or the absence (deletion, D allele) of a 287 bp fragment in intron 16. The I allele is associated with lower ACE activity [24] and an increased half-life of bradykinin [15]. An excess representation of this allele occurs in elite endurance athletes [3,14,16]. Conversely, the D allele is associated with higher angiotensin II concentrations (a growth factor) [2], a greater percentage of fast twitch muscle fibers [32], and with training-related strength gains [5]. These studies in younger persons suggest that the direction of the ACE I/D genotype effect depends on the type of training performed and the outcome assessed (aerobic endurance vs. strength). We previously showed that, among older individuals who reported engaging in exercise, those with the ACE DD or ID genotypes were less likely to develop mobility limitation than those with the II genotype, and that the strength of this association was greater among those reporting engaging in strength training [11]. However, the effects of this gene variant on physical function responses to exercise training have not been rigorously tested in a randomized controlled trial in an older population. Therefore, the purpose of this study was to determine whether ACE I/D genotype influences physical function.
function at baseline and in response to 18 months of combined aerobic and resistive exercise training in older, sedentary men and women. We hypothesized that the I allele would interact with exercise training to influence changes in walking distance and the D allele would interact with exercise training to influence changes in muscle strength.

**Methods**

The study population consisted of older (≥60 yrs), overweight and obese (BMI ≥ 28 kg/m²), sedentary men and women who had been enrolled in a randomized, controlled trial to determine the effects of exercise and dietary-induced weight loss, alone and in combination, on physical function. All participants provided written, informed consent to participate in the study according to the WFU School of Medicine’s Institutional Review Board. A total of 316 subjects met the study criteria and were assigned to one of four treatments (exercise, dietary weight loss, exercise and dietary weight loss, or control). Specific inclusion/exclusion criteria and primary study results were previously published [12,13,18]. The racial background of the enrolled participants was 75% white/Caucasian based on self-report, 22% black/African-American and 3% representation from Native American, Asian/Pacific Islander, and Hispanic populations. The trial was completed in early 2001, and after study completion all participants were called back (in 2001 –2002) and asked to donate blood for extraction of DNA for genetic analyses. A total of 213 of the original 316 participants agreed to return to the clinic for the blood draw. The current study reports our findings of retrospective analyses among these 213 persons who consented to genetic testing.

**Exercise and no-exercise interventions**

The 3 d wk⁻¹ exercise program consisted of an aerobic phase (15 min), a light resistance-training phase (15 min), a second aerobic phase (15 min), and a cool-down phase (15 min). The first four months of the 18-month intervention was facility-based. After the first four months, participants who wished to exercise at home underwent a two month transition phase in which they alternated between the facility and the home. After the first four months of facility-based exercise, 64% of the subjects in the two exercise groups remained in the facility-based program, 24% opted for the home-based program, and 12% of the subjects chose a combined facility-home-based program. Participants were provided with an aerobic exercise prescription that included walking within a heart rate range of 50 – 65% of heart rate reserve. The resistance training portion of the program consisted of two sets of 12 repetitions of the following exercises: (a) leg extension, (b) leg curl, (c) heel raise, and (d) step up. Cuff weights and weighted vests were used to provide resistance. A 1 – 1.5 minute rest interval separated each exercise. Following two orientation sessions, participants began with the lowest possible resistance. Weight was increased after the participant performed two sets of 12 repetitions for two consecutive days. Exercise and attendance logs were used to gather data and monitor progress.

One-half of the exercise group also underwent a dietary weight loss intervention designed to produce and maintain an average weight loss of 5% for the duration of the 18-month intervention. During the first six months of the study, individuals in the diet groups attended weekly behavior counseling sessions with a registered dietitian consisting of three group sessions and one individual session per month. The diet groups met monthly for the final 12 months of the study. The no-exercise, no-diet control group met monthly for one hour for the first three months during which topics concerning osteoarthritis, obesity, and exercise were discussed. Monthly phone contact was maintained during months 4 – 6 and bi-monthly contact during months 7 – 18.

**Clinical procedures**

Information concerning co-morbid conditions was obtained from a medical history, medication use, and a physical examination. Objective and self-reported measures of physical function, along with body weight and height, were obtained at baseline, and 6- and 18-months post-randomization.

**Physical disability** was measured by the self-reported FAST functional performance inventory [4]. This measure uses 23 questions to assess perceived difficulty with a number of activities including basic activities of daily living (ADL), instrumental ADLs, ambulation, transferring, and upper extremity strength. The scale for each question ranged from 1 (no difficulty) to 5 (unable to do) and the score of each question is averaged to create a composite score with an alpha reliability of 0.79.

**Walking distance**

A six-minute walk test was used as the aerobic endurance outcome. Participants were instructed to walk as far as possible in a 6-minute time period on an established course. Performance was measured by the total distance covered. This test is significantly correlated to treadmill time and symptom-limited maximal oxygen consumption (r = 0.52 and r = 0.53, respectively) and has a 3-month test-retest reliability of 0.86 [23]. General instructions, a demonstration, including a question and answer period, and a habituation period preceded all testing.

**Muscle strength**

Knee concentric extension muscular strength was assessed using a Kin-Com 125E isokinetic dynamometer (Chattanooga Corp, Hixson, TN, USA) in one-half of the study participants who were randomly selected for this outcome. Prior to testing, a warm-up period was provided to habituate the subjects to the testing equipment. Knee extension strength was assessed at a velocity of 30°-second⁻¹. Gravity effect torque was calculated based on the subject’s leg weight at a 45° angle. Two maximal reproducible trials were averaged and the maximum number of trials for each test was six. Peak torque was calculated as the average torque between joint angles of 40 – 80°.

**DNA extraction and genotyping**

DNA was isolated from whole blood and the concentration and purity (Abs260/Abs280) was determined by a UV spectrophotometer. The ACE insertion/deletion polymorphism was determined using polymerase chain reaction (PCR) amplification with subsequent visualization of PCR products on 2% agarose gels by electrophoresis. The sequences of the sense and antisense primers were 5’-CTGGAGACCACCTCCATCTTTC-3’ and 5’CATGTGGCCATCAGATTCGTCAGAT-3’, respectively. The insertion allele (I) is detected as a 490-basepair band, and the deletion allele (D) is visualized as a 190-basepair band. The PCR products were visualized independently by two laboratory technicians and genotypes that were not scored identically were reanalyzed.
Statistical analyses

Statistical analyses were performed using SPSS statistical software (SPSS Inc., Chicago, IL, USA). Baseline differences among genotype groups were tested using analysis of variance. Genotype differences in absolute and relative changes in physical function among the exercisers were tested using a general linear model, adjusting for age, sex, race, and weight loss treatment. To test for a statistically significant interaction between exercise treatment and genotype, a gene X treatment (exercise, no exercise) interaction term was added to the models. All data are presented as mean ± standard error of the mean (SEM), and the level of significance was set at p < 0.05 for all analyses.

Results

Genotype differences at baseline (Table 1)

Overall, 38% of the study population was DD, 39% ID, and 23% II and these genotype frequencies met the criteria for Hardy-Weinberg equilibrium. There were no differences in age, gender, race or baseline BMI between genotypes (Table 1). In addition, there were no associations between ACE I/D genotype and exercise compliance (DD = 61 ± 24%; ID = 66 ± 23%; II = 68 ± 24%; p = 0.65). Changes in body weight among those who exercised were not different among genotypes (II = – 6.6 ± 2.6 kg, ID = – 5.2 ± 1.9 kg, DD = – 6.7 ± 2.2 kg, p = 0.58). Among the exercisers, neither relative nor absolute changes in 6-minute walking distance were different between genotype groups (Table 2). However, in the sub-sample of participants that underwent isokinetic strength testing, there was an effect of ACE genotype on relative changes in concentric knee strength with the DD genotypes showing greater gains in strength compared to II individuals (Table 2). In addition, there was a trend for a significant ACE genotype effect on absolute changes in knee strength. Moreover, there was a trend for improvements in the physical disability score to be greater in the DD genotypes, although the difference was not statistically significant. Fig. 1 shows percent changes in concentric knee strength in exercisers and non-exercisers by ACE I/D genotype. There was a significant interaction between exercise treatment and genotype on changes in knee strength (p = 0.014), with the ID and II genotypes showing a similar response to exercise training as the no-exercise control group.

Genotype differences in responses to exercise (Table 2)

The number of genotyped participants that completed the 18-month intervention was 176 (n = 87 for exercise, n = 89 for no-exercise). Overall adherence to the exercise intervention (calculated as number of sessions attended at the center or reported as performed at home divided by the number of sessions prescribed) was 65 ± 24% among participants who completed the study. There was no association between ACE I/D genotype and exercise compliance (DD = 61 ± 24%; ID = 66 ± 23%; II = 68 ± 24%; p = 0.65). Changes in body weight among those who exercised were not different among genotypes (II = – 6.6 ± 2.6 kg, ID = – 5.2 ± 1.9 kg, DD = – 6.7 ± 2.2 kg, p = 0.58). Among the exercisers, neither relative nor absolute changes in 6-minute walking distance were different between genotype groups (Table 2). However, in the sub-sample of participants that underwent isokinetic strength testing, there was an effect of ACE genotype on relative changes in concentric knee strength with the DD genotypes showing greater gains in strength compared to II individuals (Table 2). In addition, there was a trend for a significant ACE genotype effect on absolute changes in knee strength. Moreover, there was a trend for improvements in the physical disability score to be greater in the DD genotypes, although the difference was not statistically significant. Fig. 1 shows percent changes in concentric knee strength in exercisers and non-exercisers by ACE I/D genotype. There was a significant interaction between exercise treatment and genotype on changes in knee strength (p = 0.014), with the ID and II genotypes showing a similar response to exercise training as the no-exercise control group.

Discussion

The findings indicate that the ACE I/D genotype interacts with exercise training to affect muscle strength in older men and women. Specifically, individuals homozygous for the D allele...
showed dramatic improvements in isokinetic knee strength following an 18 month exercise training intervention, while the ID and II genotypes showed no strength improvement relative to the no-exercise controls. In addition, self-reported disability improved more in DD individuals in response to the exercise training. Therefore, changes in physical function with exercise training in older individuals may be dependent on genetic variation in the ACE gene.

Most of the data regarding the effects of ACE gene variation on physical performance come from young individuals. As reviewed previously [10], this genotype is a promising marker for athletic performance, as well as a predictor of the physiological response to exercise training in younger persons. Studies in young people showing that the DD genotype is more frequent in successful power/sprint athletes [16,28,30]. On the other hand, the finding of a similar genotype response in walking distance in our study is not consistent with data in younger persons which show an association between frequency of the I allele and aerobic endurance [3,16,31]. However, this association is not present in all studies [21], and all of the positive associations are seen in highly trained/elite athletes, suggesting that genetic variation in the ACE gene may influence performance only under a relatively intense training stimulus. One explanation as to why improvements in walking distance were not greater in individuals homozygous for the I allele in the current study is that the aerobic training stimulus (walking at 50–65% of heart rate reserve) was not intense enough to interact with this gene in altering aerobic endurance. Another reason may be that the six-minute walk is not a solely aerobic task and involves muscle strength. Since strength improved more in DD individuals this may have masked smaller improvements in aerobic capacity, resulting in similar improvements between genotypes in walking speed.

To date, most studies examining the effects of this gene variant on physical function in the elderly are cross-sectional analyses. In a study of elderly Danish twins, neither physical performance at baseline, nor longitudinal change in function, were associated with ACE I/D [7]. On the other hand, there are data which suggest an association with this genotype and longevity, with a lower mortality and greater longevity seen in persons with the D allele [7,20]. A recent paper reports combined, retrospective data from 4 exercise trials in elderly Danish men and women which showed no effect of the ACE I/D variant on responses to the physical training [6]. However, all outcomes were not assessed in every study, resulting in small sample sizes for most outcomes. In addition, the intensities of the training stimuli are unclear and may not have been strong enough to uncover an effect of genotype on responses.

The present study did not assess the possible mechanisms by which ACE I/D may modulate physical function responses to exercise, but, most likely, the greater strength performance in DD individuals is due to the effects of an increased angiotensin II level [2], a growth factor that augments overload-induced hypertrophy of muscle [8,26]. This mechanistic possibility is confirmed by data showing a greater frequency of the D allele in athletes with exercise-induced left ventricular hypertrophy [9] and a greater left ventricular mass growth in men with the DD genotype following exercise training [14]. In rats, inhibition of angiotensin II production via ACE inhibition (akin to the I allele) attenuated skeletal muscle hypertrophy in response to a 28-day overloading protocol, but, perfusion of exogenous angiotensin II did result in muscle hypertrophy in ACE-inhibited animals [8]. In addition, a recent study showed a positive relationship between quadriceps muscle strength and circulating ACE activity in young men [29]. These data clearly show that ACE activity (and therefore genetic variation in the ACE gene) is likely to modulate changes in skeletal muscle strength. On the other hand, there may also be behavioral factors underlying the propensity for greater strength gains in the DD genotype. A recent paper showed there was greater adherence to an aerobic training program in I carriers compared to DD, suggesting this may be an underlying factor in the greater endurance of persons with the I allele [27]. Although we did not observe a genotype difference in exercise session frequency, we did not measure increases in exercise volume or intensity throughout the trial. It is quite likely that the DD genotypes progressed more rapidly in the amount of weight lifted, but further studies are needed to document this.

Although our study is the first to use data from a randomized, controlled trial to test whether the ACE I/D variant modulates physical function responses to exercise training in the elderly, there are some limitations that need to be mentioned. First, the retrospective call-back to obtain DNA resulted in smaller sample sizes and may have biased the sample, although there were no baseline differences between genotypes in demographic or functional characteristics. In addition, there was not total compliance to the frequency of the exercise training sessions, but, there were no genotype differences in compliance. Moreover, while the 6-min walk test is a good measure of endurance in this population, it does not provide a direct measure of maximal aerobic capacity. Finally, the training regimen was a combination of two exercise modes of light- to moderate-intensity and it may be that geno-

**Fig. 1** Percent changes in knee strength in exercisers and non-exercisers by ACE I/D genotype. There was a significant effect of the interaction between exercise treatment and genotype ($p = 0.014$).
type differences would be exaggerated if the exercise stimulus and compliance to exercise were greater or if the intervention consisted of only one type of exercise at a time. Thus, further study is required to confirm or refute our findings and to understand their physiological basis.

References


