Lack of association of the ACE genotype with the muscle strength response to resistance training

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Abstract
Introduction: Previous studies have attempted to link the insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene with the variability in muscle strength responses to resistance training (RT); however, the literature is inconclusive. The purpose of the present study was to investigate the association between the ACE I/D genotype and muscle strength response to a RT program in young men.

Methods: 124 men (22 ± 2.6 years; 174.8 ± 6.5 cm; 71.5 ± 13.8 kg) without resistance training experience were tested before and after 11 weeks of five whole-body RT exercises (bench press, seated row, knee extension, knee flexion and sit ups). The bench press 1RM test was used to assess upper-body muscle strength and the isokinetic knee extensor peak torque (PT) was used as a measure of lower-body strength.

Results: At baseline, there were no differences among ACE genotype for 1RM load (54 ± 11.7 kg for II, 58.5 ± 8.9 kg for ID and 52.3 ± 12.2 kg for DD) or knee extensor peak torque (PT) (220.1 ± 36.8 N m for II, 209.4 ± 44.4 N m for ID and 199.7 ± 32.4 N m for DD). Moreover, ACE genotype was not associated with lower-body strength gains (7.1 ± 10.5%, 15.7 ± 10.4% and 14.1 ± 22.7% for II, ID and DD, respectively) or upper-body strength gains (16.2 ± 8.9%, 14.5 ± 11.3% and 21.9 ± 17.1% for II, ID and DD, respectively) in response to RT.

Conclusion: The ACE I/D genotype was not associated with the muscle strength responses to RT.

Keywords: Genetics, exercise, candidate gene, muscle strength, peak torque, strength training

Introduction
Although environmental factors are important in determining muscle strength, it is well recognized that genetic factors have an important influence on this phenotype (Bray et al., 2009; Maes et al., 1996; Reed, Fabritz, Selby, & Carmelli, 1991; Stewart & Rittweger, 2006). One of the most studied candidate genes is the angiotensin-converting enzyme (ACE) gene. ACE is a key enzyme in the renin-angiotensin system which converts angiotensin I to angiotensin II. A functional polymorphism of the ACE gene is defined as the presence (insertion, I-allele) or absence (deletion, D-allele) of a 287 base pair (bp) Alu repeat sequence within intron 16 (Rigat et al., 1990). This polymorphism has been found to be responsible for half of the variation in ACE enzyme activity (Rigat et al., 1990; Woods, D.R., Humphries, & Montgomery, 2000), with those carrying the D-allele (D) presenting an increased ACE enzyme activity (Danner et al., 1995; McCauley, Mastana, Hossack, Macdonald, & Folland, 2009; Tiet et al., 1992; Williams et al., 2005). The renin-angiotensin system also exists in diverse tissues; in particular, the ACE gene is expressed in skeletal muscle (Jones & Woods, 2003). The description of ACE activity in skeletal muscle (Reneland & Lithell, 1994) and the increased muscle tension development after angiotensin II infusion in rats (Rattigan, Dora, Tong, & Clark, 1996) introduced the ACE gene as candidate in association studies with muscle-related phenotypes.

A higher proportion of the DD genotype has been reported in elite sprint athletes when compared to...
endurance athletes and controls (Jones, Montgomery, & Woods, 2002; Myerson et al., 1999; Nazarov et al., 2001; Woods, D. et al., 2001). This genotype has been associated with a greater proportion of fast twitch fibres (Zhang et al., 2003), suggesting that this polymorphism may influence skeletal muscle function, although contradictory evidence also exists (Akhmetov et al., 2006). However, studies investigating the association between ACE I/D genotype and skeletal muscle strength phenotypes have yielded inconsistent results. Although the D-allele has been previously associated with greater isometric and isokinetic strength (Williams, et al., 2005), most studies have found no association of this allelic variant with muscle strength (Folland et al., 2000; McCauley, et al., 2009; Pescatello et al., 2006; Thomis, M.A. et al., 2004; Woods, D., et al., 2001).

Resistance training (RT) is regarded as an effective mean of increasing muscle strength (ACSM, 2009); however, adaptations to RT are highly variable between individuals. Neuromuscular adaptations to RT have been shown to be partially determined by genetic factors (Beunen & Thomis, 2004; Brutsaert & Parra, 2006; Thomis, M. et al., 1998; Thomis, M.A., et al., 2004). Some candidate genes have been investigated but agreement on important contributors has not been reached. In this regard, the ACE gene has been pointed as a potential candidate.

Folland et al. (2000) and Giaccaglia et al. (2008) showed ACE I/D genotype × RT interaction in young and older persons, respectively, with the D-allele carriers presenting higher training-induced increases in muscle strength compared with the II genotype. In contrast, Lima et al. (2011), Charbonneau et al. (2008), Thomis, M.A. et al. (2004) and Williams et al. (2005) failed to show significant association between ACE I/D genotype and the response of muscle strength to RT, while Pescatello et al. (2006) observed that the I-allele carriers showed greater gains. Therefore, although the biological rationale suggests an advantage for the D-allele with regard to muscle strength, the literature is inconclusive. The purpose of the present study was to investigate the association between the ACE I/D genotype and muscle strength response to an RT program in young men. The hypothesis is that the ACE I/D genotype would not influence the response to RT.

Methods

Experiment overview

Participants undertook two days per week of whole-body resistance training, with a minimum of 48 hours between sessions, during 11 weeks. All volunteers performed the same exercises and were instructed to complete 8–12 repetitions until volitional fatigue at a speed of four seconds per repetition (two seconds for the concentric phase and two seconds for the eccentric phase). The RT program characteristics were selected based on literature recommendations (ACSM, 2009; Kraemer et al., 2002).

Participants were initially required to attend three to four sessions in order to become familiarized with the RT program. Maximal knee extensor peak torque (PT) in an isokinetic dynamometer as well as bench press one repetition maximum (1RM) were measured before and after the 11 weeks of training. The initial tests were performed between the third and fourth familiarization sessions and the final tests were performed five to seven days after the last training session. For genotype analyses, 4 ml of venous blood were collected from all the volunteers at the end of the study period in the fed state.

Participants

One hundred and sixty college non-resistance trained men volunteered to participate in the study. Participants were selected at random from respondents to fliers distributed over the university campus, and by word of mouth. The training classes were part of college activities. The criteria for entering the analysis included being at least 18 years of age, no previous resistance training experience and being free of clinical problems that could be aggravated by the study procedures. To be included in the statistical analysis, participants were permitted to miss only four training sessions during the 12-week program. Final training adherence was 89%. The volunteers were oriented not to change their nutritional habits during the study period; if a relevant change were detected (i.e. becoming a vegetarian, taking nutritional supplements or ergogenic aids, etc.) the participant's data were excluded from the analysis. Although they were untrained in a resistance training sense, all were physically active, with involvement in other activities such as walking, jogging, martial arts and team sports. At the end of the study, 124 participants met the criterions to be included in the analysis (21.98±2.63 years; 174.84±6.51cm; 71.49±13.81 kg).

All participants were notified of the research procedures, requirements, benefits and risks before providing informed consent. The Institutional Research Ethics Committee granted approval for the study.

Procedures

One repetition maximum test. In the week before the experiment and 5–7 days after the last training session, the load for 1RM was determined for each
Measurement of isokinetic peak torque (PT). Subjects warmed up on a cycle ergometer at 25–50 Watts for 5 min. After the cycle warm-up, they were seated on the isokinetic dynamometer and actively warmed up the involved quadriceps muscles by performing 10–12 submaximal knee extension repetitions at 300°·s⁻¹ (Bottaro, Russo, & Oliveira, 2005). For familiarization with isokinetic exercise, subjects performed two sets of four maximal repetitions at 60°·s⁻¹ with 1-min rest between sets (Parcell, Sawyer, Tricoli, & Chinevere, 2002). The familiarization session was performed between 48 and 72 hours before the first isokinetic training protocol.

Knee extensor isokinetic PT was measured on the Biodex System 3 Isokinetic Dynamometer (Biodex Medical, Inc., Shirley, NY, USA). Calibration of the dynamometer was performed according to the manufacturer’s specifications before every testing session. The participants sat upright with the axis of rotation of the dynamometer arm oriented with the lateral femoral condyle of the right knee. Belts were used to secure the thigh, pelvis and trunk to the dynamometer chair to prevent additional body movement. The chair and dynamometer settings were recorded to ensure the same positioning for all tests. The flexor torque produced by the relaxed segment was used for gravity correction. Subjects were instructed to fully extend and flex the knee and to work maximally during each set of exercises. Verbal encouragement was given throughout the testing session. The tests comprised two sets of four maximal repetitions at 60°·s⁻¹ (Bottaro, et al., 2005; Parcell, et al., 2002). Participants were instructed to fully extend and flex the knee and to work maximally during each set. After each set, participants were required to take 60 s of rest before the onset of the next set (Bottaro, et al., 2005; Parcell, et al., 2002). The knee strap was released during each rest period to ensure unrestricted blood flow to the lower limb. The procedures were administered to all participants by the same investigator. Knee extensor PT baseline test and retest ICC and standard error of the mean were 0.98 and 2.3% respectively.

Resistance training intervention. A whole-body multiple-set resistance training program was implemented using a combination of free weights and machines. The sessions consisted of five exercises, two for the upper body (bench press and seated row), two for the lower body (one for the knee extensors and one for the knee flexors) and one for the midsection (sit ups). To improve external validity, and follow literature recommendations (ACSM, 2009; Kraemer et al., 2002), participants performed two sets of 8–12 repetitions. Participants were instructed to adjust training loads carefully; if participants could not perform eight repetitions or could lift the load more than 12 times, they were instructed to adjust the load in order to ensure the completion of the required number of repetitions.

Training was conducted two days a week, with a minimum of 48 hours between sessions, for 11 weeks. Twice-weekly training sessions were chosen because the current physical activity guidelines state that adults should do at least 150 minutes per week of moderate intensity physical activity and also two or more days per week of muscle-strengthening activities (USDHHS, 2008). The sets started every three minutes, leading to a rest interval of approximately two minutes. During the training sessions, music tracks with 120 bpm were played in order to facilitate control of movement speed. Each participant kept a training log where the loads used and the numbers of repetitions performed in each exercise were recorded. Training sessions were closely supervised, in a ratio of five volunteers per supervisor, because previous research has demonstrated greater gains in supervised versus unsupervised training (Gentil & Bottaro, 2010).

Genotypes. For genotype analysis, blood samples were obtained using EDTA vacutainer tubes from each volunteer in the fed state. High molecular weight DNA was extracted from peripheral venous blood leukocytes using a salting out protocol. The ACE I/D polymorphism was identified by polymerase chain reaction (PCR) using the forward (5’-CTGGAGAC-CACTCCCATCCTTTCT-3’) and reverse (5’-GA TGGGCCATCACATTCGTCAGAT-3’) primers described by Zhao et al. (2003). Amplicons were electrophoresed on 1% agarose gel and fragments were visualized by ethidium bromide staining and ultraviolet transillumination. The PCR product is a 190 bp fragment in the presence of the deletion (D) allele and a 490 bp fragment in the presence of the insertion (I) allele. Therefore, three ACE I/D genotypes were possible: II – a 490 bp band; DD – a 190 bp band; and heterozygote ID – the presence of both 490 and 190 bp bands. As an attempt to avoid mistyping of ID as DD, all samples classified as homozygous DD were subjected to a second amplification reaction using forward (5’-TGGGACCA-CAGCGCCCGCCACTAC-3’) and reverse (5’-TGGGACCA-CAGCGCCCGCCACTAC-3’) primers that anneal to an insertion-specific sequence as described by Gonzalez et al. (2006). Negative and positive controls were included in this second PCR and the presence of a 335 bp fragment demonstrates
misting whereas a real DD genotype shows no amplification. All genotypes were determined by two independent investigators.

Statistical analyses

Distribution of ACE I/D genotypes was analysed by Pearson chi-square test to verify agreement with the Hardy–Weinberg equilibrium. The data were in normal distribution, according to the Kolmogorov–Smirnov test. To test for differences in age, height, weight, initial bench press 1RM and knee extensor PT, one-way analysis of variance (ANOVA) was performed. To examine the association between the ACE I/D genotypes and the RT-induced adaptations, a repeated measures ANOVA (genotype x time) was performed, in which the within-subject factors were pre and post values of the phenotypes under study and the between-subject factors were the genotypes (DD, ID and II). Relative percentage change was calculated for the knee extensor PT and bench press 1RM using the following equation: [(post values – pre values)/pre values x 100]. The two-step cluster analysis was used to create homogenous groups according to increases in bench press 1RM and knee extensor PT. Cluster analysis was performed because it seeks to identify homogeneous subgroups of cases in a population, minimizing within-group and maximizing between-group variations. Whereas the use of fixed values for classifying groups may create heterogeneous groups, with relatively large within-group variations Pearson chi-square was used to analyse association between the distribution of muscle strength gains and ACE I/D genotype. A sample size of 114 subjects (23 for II, 68 for ID, and 33 for DD groups) provided > 0.85% statistical power at an α level of 0.05 (2-tailed) for both outcomes (PT and 1RM). All statistical analyses were performed using the Statistical Package for the Social Sciences 16.0 software (SPSS, Chicago, IL, USA). Data are expressed as means ± standard deviation.

Results

Participants’ characteristics according to ACE I/D genotype are shown in Table I. The genotype distribution was in expected Hardy–Weinberg equilibrium (P > 0.05). At baseline, no significant differences were found for any variable (P > 0.05).

There were no genotype by time interactions for knee extensor PT or bench press 1RM (P > 0.05). According to the results, all genotypes significantly increased bench press 1RM and knee extensor isokinetic PT, with no difference between groups (P < 0.05). Additional comparisons between carriers of the D allele (ID+DD) and the II genotype revealed no difference between groups for baseline values or changes in knee extensors PT and bench press 1RM (P > 0.05).

Two clusters were formed for knee extensor PT: (1) HPT – high response (25.8 ± 14.9%; 22 participants); and (2) LPT – low response (3.3 ± 7.1%; 94 participants). The analysis of bench press 1RM data led to the formation of three clusters: (1) HBP – high response (30.1 ± 9.6%; 21 participants); (2) IBP – intermediate response (16.1 ± 3.3 g·cm⁻²; 51 participants); and (3) – low response (3.3 ± 4.8%; 42 participants). According to the Pearson chi-square results, there was no significant association between the distribution of ACE I/D genotypes and knee extensor PT (Figure 1) or bench press 1RM response (Figure 2).

Discussion

Genotype distribution was in expected Hardy–Weinberg equilibrium in the present study sample. ACE genotype frequencies have been shown to differ among race groups. Commonly, the allele frequency among Caucasian individuals is approximately 50% for both the I and D alleles, compared with 41% and 59% for the I and D alleles, respectively, in black individuals (Mathew, Basheeruddin, & Prabhakar, 2001). In the USA, racial groups are relatively well

<table>
<thead>
<tr>
<th>Variable</th>
<th>II</th>
<th>ID</th>
<th>DD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>68</td>
<td>33</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.9 ± 1.9</td>
<td>21.9 ± 3.2</td>
<td>20.9 ± 1.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.7 ± 9.6</td>
<td>175.6 ± 6.8</td>
<td>174.2 ± 6.6</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>68.6 ± 17</td>
<td>71.6 ± 14.4</td>
<td>67.3 ± 6.9</td>
</tr>
<tr>
<td>Knee extensor PT pre (N m)</td>
<td>220.1 ± 36.8</td>
<td>209.4 ± 44.4</td>
<td>199.7 ± 32.4</td>
</tr>
<tr>
<td>Knee extensor PT post (N m)</td>
<td>235.1 ± 40.6*</td>
<td>239.3 ± 40.8*</td>
<td>223.3 ± 29.2*</td>
</tr>
<tr>
<td>ΔKnee extensor PT (%)</td>
<td>7.1 ± 10.5</td>
<td>15.7 ± 10.4</td>
<td>14.1 ± 22.7</td>
</tr>
<tr>
<td>Bench press 1RM pre (kg)</td>
<td>54 ± 11.7</td>
<td>58.5 ± 8.9</td>
<td>52.3 ± 12.2</td>
</tr>
<tr>
<td>Bench press 1RM post (kg)</td>
<td>62 ± 10.1*</td>
<td>66.4 ± 8.9*</td>
<td>62.7 ± 10.5*</td>
</tr>
<tr>
<td>ΔBench press 1RM (%)</td>
<td>16.7 ± 8.9</td>
<td>14.5 ± 11.3</td>
<td>21.9 ± 17.1</td>
</tr>
</tbody>
</table>

PT – peak torque, 1RM – one repetition maximum

*p < 0.05 – pre versus post
defined and easily identified. In Brazil, however, such subgroups are much less well defined. Most Brazilians are of mixed European, African and Amerindian ancestry. Despite that, the $ACE\ I/D$ genotype distribution of the present study was in Hardy–Weinberg equilibrium, with frequencies of 18.6, 54.8 and 26.6% for the $ACE\ II$, $ID$, and $DD$ genotypes, respectively. Pescatello et al. (2006) reported similar frequencies for the white population for the $ACE\ II$ (23.1%), $ID$ (46.1%), and $DD$ (30.8%) genotypes.

The results of the present study provide no evidence of an association between $ACE\ I/D$ genotype and baseline upper- and lower-body muscle strength. These results are in agreement with previous cross-sectional results (Charbonneau, et al., 2008; Folland, et al., 2000; Giaccaglia, et al., 2008; McCauley, et al., 2009; Pescatello, et al., 2006; Thomis, M. A., et al., 2004; Williams, et al., 2005) and, in conjunction with them, indicate that the genetic variant under investigation does not play a pivotal role in determining muscle strength phenotypes.

The results of experimental designed studies analysing the effects of the $ACE\ I/D$ genotype in resistance training adaptations are controversial. Giacca-glia et al. (2008) studied a population of older adults before and after 18 months of walking and light RT, and reported that the DD homozygous presented greater gains in knee extensor isokinetic strength than the II homozygous. Earlier, Folland et al. (2000) found that the knee extensor isometric strength gains was associated with $ACE\ I/D$ genotype, with young men that carry the D-allele presenting greater strength increases than II homozygous after 9 weeks of RT. However the baseline results for 1RM and isokinetic strength were similar among genotypes.

Williams et al. (2005) examined the $ACE\ I/D$ genotype associations with quadriceps muscle strength in 81 young Caucasian men. Baseline isometric strength was significantly associated with the $ACE\ I/D$ genotype, with I-allele homozygous showing the lowest strength values. On the other hand, no association was found with changes in strength in 44 men who completed an 8-week RT program. The lack of association between strength gains and $ACE\ I/D$ genotype in the Williams study could be due to small statistical power. However, with regard to training adaptations, this issue is not likely for the present study that reported the same results. In agreement with the present study, Charbonneau et al. (2008) found no difference in knee extensor 1RM response between different $ACE\ I/D$ genotypes after 10 weeks of unilateral knee extensor RT. Similarly, a recent study by Lima et al. (2011) did not find a pivotal role for the $ACE\ I/D$ polymorphism in determining muscle strength response to RT in older women.

In contrast to the present study, Pescatello et al. (2006) reported greater increases in maximal isometric voluntary (MVC) contraction after 12 weeks (twice a week) of unilateral RT in young people carrying the I allele than in DD homozygous, but interestingly found no difference for 1RM gains. Likewise, Thomis, M.A. et al. (2004) studied 57 young male twins who underwent 10 weeks of elbow flexors RT and showed no association between $ACE\ I/D$ genotype and 1RM gains, but reported a borderline significance for larger isokinetic knee flexion PT in II homozygous. According to Pescatello et al. (2006), it seems plausible that the exercise-induced ACE signalling effects are greater with the $ACE\ I/D$ allele due to its associations with increased bradykinin activity compared with the $ACE\ D$ allele. The authors suggested that the bradykinin is involved in the exercise pressor reflex, being released by high-intensity, static muscle contractions in direct proportion to lactate production and inversely related to pH. Also it appears that differences in association could be in some way related to the specificity of the
strength testing mode (1RM versus MVC) or muscle group studied (upper versus lower body). However, in our study we assessed strength by 1RM upper-body isoinertial test and PT lower-body isokinetic test, and also found no relation between ACE I/D genotype and strength gains.

Conclusions
In summary, the present results do not suggest a pivotal influence of the ACE I/D polymorphism in determining dynamic muscle strength response to RT in young men. It is true that different methodological approaches make comparisons between studies difficult; however, despite these differences, if the ACE gene were a robust contributor of muscle strength some agreement would be noted in the literature. One possible explanation for the lack of congruent results is that the ACE I/D polymorphism is one of many genetic variants contributing to the variance in the muscle strength response to RT, and that only a small part of the variability in these phenotype may be attributable to the ACE I/D genotype (Pescatello et al., 2006). According to this assumption, future investigators should not focus their effort in designing studies to examine the ACE gene individually in association with muscle strength. It is important that upcoming studies investigate the interaction between different genes, which we believe will lead to a better understanding of the genetic contribution of muscle strength in response to RT.

References


