Angiotensin-converting enzyme genotype affects the response of human skeletal muscle to functional overload

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The response to strength training varies widely between individuals and is considerably influenced by genetic variables, which until now, have remained unidentified. The deletion (D), rather than the insertion (I), variant of the human angiotensin-converting enzyme (ACE) genotype is an important factor in the hypertrophic response of cardiac muscle to exercise and could also be involved in skeletal muscle hypertrophy – an important factor in the response to functional overload. Subjects were 33 healthy male volunteers with no experience of strength training. We examined the effect of ACE genotype upon changes in strength of quadriceps muscles in response to 9 weeks of specific strength training (isometric or dynamic). There was a significant interaction between ACE genotype and isometric training with greater strength gains shown by subjects with the D allele (mean ± s.e.m.: II, 9.0 ± 1.7%; ID, 17.6 ± 2.2%; DD, 14.9 ± 1.3%, ANOVA, P < 0.05). A consistent genotype and training interaction (ID > DD > II) was observed across all of the strength measures, and both types of training. ACE genotype is the first genetic factor to be identified in the response of skeletal muscle to strength training. The association of the ACE I/D polymorphism with the responses of cardiac and skeletal muscle to functional overload indicates that they may share a common mechanism. These findings suggest a novel mechanism, involving the renin–angiotensin system, in the response of skeletal muscle to functional overload and may have implications for the management of conditions such as muscle wasting disorders, prolonged bed rest, ageing and rehabilitation, where muscle weakness may limit function. Experimental Physiology (2000) 85.5, 575–579.

The considerable individual variation in human muscular strength (Hakkinen et al. 1998) reflects the interaction of environmental factors (e.g. specific training and habitual use) with genetic elements. In old age, for example, there is a significant decline in skeletal muscle strength and muscle size (Grimby & Saltin, 1983; Kalman et al. 1990) that contributes to a loss of mobility and can inhibit the essential activities of daily living (Hyatt et al. 1990). Prolonged bed rest (Berg et al. 1997), space flight (Baldwin, 1996) or immobilisation (MacDougall et al. 1980) also cause a loss of muscle strength, largely as a consequence of disuse atrophy. Functional overload, in the form of strength training is regarded as an effective means of increasing strength and restoring functional capacity in these groups (MacDougall et al. 1980, Germaine et al. 1995, Tracy et al. 1999). The increase in strength with training is associated with muscle hypertrophy (Narici et al. 1989, Garfinkel & Cafarelli, 1992; Hakkinen et al. 1998), although the relationship between strength gains and hypertrophy is not always clear (Jones, 1992). The individual response to strength training is highly variable (Folland et al. 1998) and strength gains are significantly affected by inheritance (Thomis et al. 1998), although no specific genetic locus has been identified. The cause of this variation may reveal important information on the regulation of muscular strength.

Angiotensin-converting enzyme (ACE) is a key component of the circulating human rennin–angiotensin system (RAS) generating angiotensin II, a vasoconstritor, and degrading vasodilator kinins. Local ACE expression may also modulate tissue growth processes as both angiotensin II and kinins appear to have growth regulatory effects (Geisterfer et al. 1988, Ishigai et al. 1997, Kai et al. 1998). A functional polymorphism of the ACE gene has been identified, with the absence (deletion, D) rather than the presence (insertion, I) of a 287 base pair fragment associated with higher tissue (Costerousse et al. 1993, Danser et al. 1995) and serum ACE
activity (Rigat et al. 1990). The D allele has been associated with growth of vascular smooth muscle at the site of coronary angioplasty (Ohishi et al. 1993) and human cardiac hypertrophy in response to exercise (Montgomery et al. 1997). There is considerable ACE activity in skeletal muscle (Reneland & Lithell, 1994), which raises the possibility that the ACE D allele might similarly influence the response of skeletal muscle to functional overload. We therefore studied the influence of the ACE deletion allele on the response of the human quadriceps muscle group to specific strength training programmes in healthy, recreationally active young men.

METHODS

Subjects
All the subjects were volunteers from amongst the University staff and students. They were healthy males (18-30 years), recreationally active with no history of knee pathology or leg strength training. Subjects were instructed to maintain their habitual levels of activity throughout the study period. The study protocol was approved by the local Ethics Committee and conformed to the Declaration of Helsinki. Subjects gave their written informed consent to the procedures. Forty-seven subjects (ACE genotypes: 8 II, 24 ID, 15 DD) were recruited.

Fourteen subjects, whose ACE genotype distribution and baseline characteristics did not differ from those who continued, later withdrew from the study because they were unable to adhere to the training schedule. Amongst the 33 subjects who completed the training programme (6 II, 17 ID, 10 DD) baseline physical characteristics (age, 214 ± 0.5 years; mass, 759 ± 2.1 kg; height, 180 ± 1 cm; body mass index, 23.3 ± 0.6 kg m⁻²) and activity levels (2.8 ± 0.3 h exercise per week) were independent of ACE genotype.

Strength training
The training and all of the strength measurements were of the quadriceps muscle group and the training consisted of three training sessions per week for 9 weeks. One leg of each subject performed purely isometric training while the other leg carried out only dynamic training. Legs were randomly assigned to one form of training. All of the training was carried out on identical leg extension machines, one of which was adapted for isometric contractions. In this case a strain gauge facilitated force measurement and visual feedback for each contraction. The isometric training was 10 repetitions of 2 s duration at each of four knee joint angles (1.22, 1.57, 1.92 and 2.27 rad). The dynamic training was four sets of ten repetitions, taking 1 s to lift and 1 s to lower the weight. Each leg worked at 75% of their respective maxima, and had 2 min rest between sets. All of the training sessions were supervised with recording of the training loads and continual verbal encouragement.

Strength testing
A battery of strength measurements was completed on each testing occasion. Baseline measurements were taken on three occasions, each 1 week apart, and twice post-training, 3 days apart. The primary strength measure was unilateral isometric leg extension strength at a joint angle of 1.57 rad assessed with a conventional strength-testing chair (Parker et al. 1990). For the three baseline tests this measure had a coefficient of variation of 3.5%. Each measurement involved four maximum voluntary contractions with 30 s rest between each. On one occasion electrically stimulated switches were superimposed on the maximum voluntary contractions to calculate the level of voluntary muscle activation (Rutherford et al. 1986).

Additional strength measurements were taken with a Cybex Norm isokinetic dynamometer (Lumex Inc., USA). These included peak isometric torque at four angles (1.22, 1.57, 1.92 and 2.27 rad) and peak isokinetic torque at three velocities (0.79, 2.62 and 5.24 rad s⁻¹).

ACE genotyping
A saline mouth rinse was used to collect epithelial cells and ACE genotype was determined by a polymerase chain reaction (O’Dell et al. 1995). The study was double blind with respect to the subjects’ ACE genotype.

Statistical analysis
Pre- and post-training mean values were calculated for each leg of each subject and the difference was expressed as a percentage increase. The contribution of all factors to the variance of the results was examined with multivariate analysis. The group data are expressed as means ± s.e.m. The difference between ACE genotypes was examined with analysis of variance (ANOVA) and Scheffé’s post hoc test. To analyse the importance of the D allele a protected Student’s t test was used (critical P < 0.025).

RESULTS
There were no differences in pre-training strength in relation to ACE genotype (II, 650 ± 50 N; ID, 657 ± 23 N; DD, 640 ± 30 N; ANOVA, P = 0.91) or between the isometric and dynamic training legs (Student’s paired t test, P = 0.97). Prior to the training the level of quadriceps muscle activation during a maximum voluntary contraction was 97.2 ± 2.1% of full activation and this was not associated with ACE genotype. The individual increases in strength as a result of training varied from 3.2 to 29.7% (average of both legs) and was unrelated to baseline strength, or any other physical or activity characteristic.

The response to isometric training was strongly genotype dependant (increase in isometric strength: II, 9.0 ± 17 %; ID, 17.6 ± 22 %; DD, 14.9 ± 13 %; ANOVA, P < 0.05; Scheffé’s post hoc test, P < 0.05 II and ID, Fig. 1). The gains in

Figure 1
ACE genotype and the response to functional overload. Percentage increase in isometric strength after 9 weeks of isometric training for 33 previously untrained healthy young men (means ± s.e.m.; II, n = 6; ID, n = 17; DD, n = 10).
strength were predominantly associated with the presence of the D allele compared to its absence (ID and DD vs. II, protected t test, \( P < 0.025 \)). The ID and DD genotype subjects improved by 97 and 66\% more than the II genotype subjects. All of the strength measures, taken at different joint angles and speeds of contraction, showed the same trend with greater gains for subjects with the D allele genotypes.

The dynamic training was a weaker stimulus to strength gains than isometric training (increase in isometric strength: 11.5 ± 1.0\% vs. 15.2 ± 1.3\%; paired t test, \( P < 0.001 \)) nor did it produce such a strong training-genotype interaction (mean increase in isometric strength after dynamic training was: II, 10.2 ± 1.2\%; ID, 115 ± 1.6\%; DD, 118 ± 1.5\%). The interaction was stronger, but still non-significant for a more specific strength test (mean increase in isokinetic strength at 0.78 rad s\(^{-1}\) after dynamic training was: II, 8.4 ± 3.3\%; ID, 11.2 ± 2.6\%; DD, 14.9 ± 4.1\%).

**DISCUSSION**

The present results indicate a clear involvement of the ACE gene in the response of skeletal muscle to functional overload. This is the first genetic factor to be identified as functionally important in this context. The consistent genotype and training interaction we have observed across all of our strength measures, and both types of training, reinforces the validity of this finding within the study population. The suggestion of a gene-environment interaction is enhanced by its marked dependence on the magnitude of the training stimulus. There was no association of strength with genotype in the untrained (pre-training) state, a weak association in limbs exposed to the lesser (dynamic) training stimulus, and a strong statistically significant association in limbs undergoing the more effective isometric strength training. The dynamic training may have been less effective because it involved lower forces, less uniform loading and possibly also a shorter duration of loading.

Whilst the ACE gene is a close neighbour of the growth hormone (GH) gene, and GH may play a role in the response to functional overload, ACE gene I/D polymorphism is not linked to the GH gene (Jeunemaitre et al., 1992, McKamie et al. 1995). It is more probable that the ACE gene directly modulates the adaptation to functional overload through the pivotal role of ACE within the systemic and/or local skeletal muscle RAS by the generation of angiotensin II or the degradation of kinins.

Local RASs have been found in myocardium (Dzau, 1988), smooth muscle (Gibbons & Dzau, 1990) and skeletal muscle (Renland & Lithell, 1994) and the ACE D allele is associated with higher systemic (Rigat et al. 1990) and cardiac tissue ACE activities (Danser et al. 1995), although this has not been examined in skeletal muscle. ACE is responsible for the production of angiotensin II, which is a potent growth factor in cardiac and vascular tissue (Geisterfer et al. 1988, Kai et al. 1998) and ACE also degrades kinins that inhibit growth in cardiac myocytes (Ishigai et al. 1997). The D allele has recently been associated with left ventricular hypertrophy in response to physical training (Montgomery et al. 1997). Thus the ACE genotype that regulates the hypertrophic process in other tissues may do likewise in skeletal muscle. ACE has been identified as present within the endothelial cells of skeletal muscle (Schauflberger et al. 1998) and both angiotensin II and kinin receptors are expressed in skeletal muscle (Stoll et al. 1995, Rabito et al. 1996). Most notably, though, Schauflberger and colleagues (1998) found a significant correlation between the quantity of ACE mRNA transcripts and skeletal muscle fibre area in a cross-sectional survey of five cardiac patients and eight control subjects.

Skeletal muscle hypertrophy is associated with strength training (Narici et al. 1989, Garfinkel & Cafarelli, 1992, Hakkinen et al. 1998) and is probably the major contributor to the increase in strength seen with medium to long term training. Hence the D allele may promote hypertrophy and greater strength gains in response to functional overload. This suggestion of an effect of the D genotype influencing strength gains via muscle hypertrophy may appear in contrast to an earlier report (Montgomery et al. 1999) where the I (and not D) allele was associated with a greater relative whole-body anabolic response to training. However the two studies are not comparable since the former involved basic army training that consists of varied physical activity (Williams et al. 1999) compared to the present study where specific systematic strength training of a single muscle group was undertaken.

Care should be taken in inferring that hypertrophy is the only mechanism of strength gain as there are consistent reports of discrepancies between the increase in strength and the increase in muscle size, leading to a rise in specific tension (Garfinkel & Cafarelli, 1992; Hakkinen et al. 1998). This discrepancy is typically attributed to morphological and neurological adaptations (Narici et al. 1989, Jones, 1992). Interestingly, there is evidence that angiotensin II can affect both sympathetic (Jonsson et al. 1993) and neuromuscular transmission (Walt, 1986) and could, in theory, influence neural adaptations to strength training.

The systemic RAS has considerable influence on skeletal muscle and whole-body metabolism (Montgomery et al. 1999) which may modulate local tissue responses to overload. Angiotensin II, which may be related to the ACE D allele, stimulates systemic growth hormone release, affects steroid hormone metabolism (Messerli et al. 1977) and, conversely, may decrease plasma IGF1 concentration (Brink et al. 1996). However, a functional link between ACE genotype and systemic (or local) angiotensin II has yet to be demonstrated. In contrast the ACE I allele has been found to enhance the plasma insulin response to a glucose load (Cong et al. 1999). There may be separate and complementary actions of the I and D alleles. The finding of allele dominance, and even a suggestion of heterozygote advantage (Fig. 1) is consistent with this idea. Such an effect could also be due to the lowest ACE levels (the II genotype) having a rate-limiting effect on one or more processes.

Ours is the first report identifying a genetic locus that influences the response to strength training. The association of the ACE I/D polymorphism with the responses of cardiac and skeletal
muscle to functional overload suggests that they may share a common mechanism. Although the observed effect of the D allele is most likely to be due to local hypertrophic actions, other factors may play a role in the response to functional overload. The results could have considerable implications for understanding the genetic basis of muscle wasting diseases and conditions such as ageing, injury rehabilitation, bed rest and space flight, where muscle weakness limits function.


