ORIGINAL ARTICLE

No correlation between circulating ACE activity and VO_{2max} or mechanical efficiency in women

Stephen H. Day · Peter Gohlke · Sukhbir S. Dhamrait · Alun G. Williams

Abstract The insertion (I) variant of the angiotensin-1 converting enzyme (ACE) I/D genetic polymorphism is associated with lower circulating and tissue ACE activity. Some studies have also suggested associations of ACE I/D genotype with endurance phenotypes. This study assessed the relationships between circulating ACE activity, ACE I/D genotype, mechanical efficiency and the maximal rate of oxygen uptake in sedentary individuals. Sixty-two untrained women were tested for mechanical efficiency during submaximal cycle ergometry (delta and gross efficiencies during exercise between 40 and 80 W) and the maximal rate of oxygen uptake during incremental treadmill running. Respiratory variables were measured using indirect calorimetry. Venous blood was obtained for direct assay of circulating ACE activity, allowing for the assessment of correlations between two continuous variables, rather than a categorical analysis of endurance phenotype by genotype alone. ACE I/D genotype was also determined, and was strongly associated with circulating ACE activity ($P < 0.0005$). Neither circulating ACE activity (27.4 ± 8.4 nM His-Leu-ml^{-1}) nor ACE genotype showed a statistically significant association with any of the endurance phenotypes measured. The weak correlations observed included $r = -0.122$ ($P = 0.229$) for the relationship between delta efficiency ($23.9 \pm 2.5\%$) and circulating ACE activity and $r = 0.134$ ($P > 0.6$) for the relationship between maximal aerobic power (149.1 ± 22.9 ml kg$^{-2/3}$ min^{-1}) and circulating ACE activity. The data do not support a role for systemic ACE activity in the regulation of endurance performance in sedentary individuals, extending this observation to a large female cohort.

Keywords Angiotensin-1 converting enzyme · Aerobic power · Endurance · Genetics

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Introduction

Angiotensin converting enzyme (ACE, kininase II) is a key component of the circulating human renin–angiotensin system (RAS), generating vasopressor angiotensin II (ANG II), and degrading vasodilator kinins (Dzau 1988). Local RAS also exist in diverse tissues (Dzau et al. 2001) including human muscle (Reneland and Lithell 1994). ACE appears to influence tissue growth (Geisterfer et al. 1988; Ishigai et al. 1997; Gordon et al. 2001) and higher levels of ACE activity have been linked to muscle strength in humans (Williams et al. 2005). Conversely, low ACE activity (as marked by a common genetic variation)

has been associated with an increased proportion of type I muscle fibres (Zhang et al. 2003), whilst ACE inhibition can stimulate angiogenesis (Silvestre et al. 2001) and decrease the local vascular resistance of arterioles and large conductance vessels leading to a redistribution of blood flow towards skeletal muscle (Giudicelli et al. 1991). However, no study has yet evaluated directly the role of ACE in the regulation of endurance parameters including aerobic power and mechanical efficiency in humans.

A polymorphism of the ACE gene has been identified in humans, with the absence (deletion allele, D) rather than the presence (insertion allele, I) of a 287 base pair fragment being associated with higher tissue (Danser et al. 1995) and serum (Rigat et al. 1990) ACE activity. Although not directly functional (Cox et al. 2002), the ACE I/D polymorphism is a convenient marker of ACE activity, accounting for up to 40% of the interindividual variation in circulating ACE activity in European populations (Rigat et al. 1990). It has been reported that the I allele is associated with elite endurance performance (Gayagay et al. 1998; Montgomery et al. 1998; Myerson et al. 1999), aerobic capacity in postmenopausal women (Hagberg et al. 1998) and the training response of mechanical efficiency (Williams et al. 2000), although considerable conflicting evidence also exists (Rankinen et al. 2000a, b). Studies to date have related cardiovascular or fitness phenotypes to variations in the ACE gene, but this can be considered a surrogate marker for ACE activity. Given that the influence of the ACE I/D polymorphism on endurance exercise performance is currently equivocal, relating the gene product (ACE) directly to functional exercise measures becomes highly desirable (Montgomery et al. 2002). There is considerable conjecture that the observed gene/performance interaction attributed to this polymorphism may be due to linkage disequilibrium with other genes close to the ACE locus (Jeunemaitre et al. 1992; Rankinen et al. 2000a, b; Tiret et al. 2002). Investigations of ACE activity in relation to endurance exercise performance would therefore be a useful step in helping to address this. The direct influence of ACE activity on endurance capacity has only been examined using ACE inhibition in clinical populations (Zannad et al. 1992), in healthy populations with respect to haemodynamic response (Fagard et al. 1982), with regards to some thermoregulatory, cardiovascular and renal exercise responses (Mittleman 1996) and in an animal model with respect to endurance capacity (Bahi et al. 2004).

A greater understanding of the role of ACE (and the ACE gene) in the regulation of endurance capacity would be useful for scientists and practitioners working in various fields such as ageing, cardiovascular rehabilitation and sports performance. Thus, the aim of this study was to examine the relationships between circulating ACE activity (as indicated by direct measurement and ACE genotype), aerobic power and mechanical efficiency in women. Women were chosen as the subject cohort in this study as previous studies have demonstrated associations of VO_{2max} (Hagberg et al. 1998) and mechanical efficiency (Williams et al. 2004) with ACE genotype in a female population. As already stated, some evidence associates the ACE I allele with endurance phenotypes (Gayagay et al. 1998; Hagberg et al. 1998; Montgomery et al. 1998; Myerson et al. 1999; Williams et al. 2000). Potential physiologic mechanisms that could explain these associations include an increased proportion of type I muscle fibres (Zhang et al. 2003), enhanced capillary density in muscle via increased angiogenesis and/or an increased production of nitric oxide (Silvestre et al. 2001) [itself related to enhanced metabolic efficiency in skeletal muscle (Shen et al. 2000)] in the presence of a low level of ACE. Such mechanisms could produce associations between low ACE activity and high levels of performance in tests of endurance phenotypes. Consequently, in this study, it was hypothesized that lower circulating ACE activity would be related to greater aerobic power and mechanical efficiency.

Methods

The study had appropriate ethics committee approval (the Sport, Health and Exercise Ethics Committee at Staffordshire University). Written informed consent was obtained from all participants. The study was conducted with both assessors and subjects blind to the subjects' ACE genotype and activity.

Subjects

Subjects were female Caucasian sedentary volunteers drawn from the student and staff populations of Staffordshire University. Only subjects who had not been involved in any structured training program during the previous 6 months were eligible to participate. The volunteers were screened to ensure that they were clinically asymptomatic for any medical condition that could have affected their participation.

We aimed to have at least 80% power to detect a significant ($P < 0.05$) negative one-tailed correlation of magnitude $r = 0.35$ (which would require a sample size of 50) between ACE activity and the endurance

parameters we measured. We recruited 62 subjects, of whom most but varying numbers completed successful tests of the various endurance parameters. There was also a problem with one blood sample so ACE activity was not available for that subject. Therefore, in the worst case [delta efficiency (DL)], we had 46 sets of usable data, giving us 80% power to detect a significant correlation of $r = -0.365$. In the best cases (gross efficiency at 40 W and the VO_{2max} measures), we had 58 sets of usable data, giving us 80% power to detect a significant correlation of $r = -0.326$.

The mean ± SD characteristics of the 62 subjects who participated in the study were: age 24 ± 7 years, body mass 65.6 ± 9.8 kg, stature 1.70 ± 0.10 m, body mass index (BMI) 23.8 ± 3.1 kg m^{-2}. For each of the subgroups that successfully completed tests of the various endurance parameters, there were no notable differences in characteristics from the whole group of 62. Prior to testing, each volunteer was familiarized with all procedures during a full practice session. For 24 h prior to the tests, subjects abstained from exercise and alcohol and fasted overnight.

Gross and delta efficiency during cycle ergometry

Subjects performed a test of mechanical efficiency of muscle contraction during cycle ergometry. Subjects cycled on an electrically braked cycle ergometer (Lode Rehcor^{TM}, Lode, Netherlands) at 60 r.p.m. at external power outputs of 40, 60 and 80 W for 4 min per stage. The ergometer was calibrated in accordance with the manufacturer's instructions, and seat and handlebar height and angle were adjusted appropriately for individual subjects. Expired air was analysed breath-by-breath using a Quark B2 analyser (Cosmed, Rome, Italy) calibrated before each test according to the manufacturer's instructions. Heart rate was monitored telemetrically (Polar Electro^{TM}, Kempele, Finland). The rate of oxygen uptake (VO_2) and respiratory exchange ratio (RER) data were assessed by two reviewers independently, who checked that each subject was in steady state during the fourth minute of exercise at each stage. Some subjects displayed physiological responses suggesting that they may have been exercising above lactate threshold when exercising at 80 W and even 60 W in some cases (e.g. RER approaching 1, marked rise in minute ventilation); the VO_2 and RER data in those cases were not used in any analyses. The data from the acceptable tests were then averaged for each 30 s period. For the group as a whole, there were no significant differences between the VO_2 and RER data for the seventh and eighth 30-s periods at each stage (all $P > 0.05$, paired t tests).

Consequently, the mean VO_2 and RER of the seventh and eighth 30 s periods (i.e., from 3–4 min) at each stage for each subject were calculated. A conversion factor dependent on RER was applied to the oxygen uptake measured, to give rate of energy expenditure (Brouwer 1957). Gross efficiency was calculated as the ratio of the work performed per minute to the energy expended per minute at each stage (GE40, GE60 and GE80), expressed as a percentage. DE was calculated as the ratio of the change in work performed per minute to the change in energy expended per minute between 40 W and 80 W, expressed as a percentage (Gaesser and Brooks 1975). The ratio limits of agreement (Nevill and Atkinson 1997) for the measure of DE was 0.97 */÷ 1.22.

Maximal rate of oxygen uptake (VO_{2max})

Subjects underwent a standard incremental treadmill test (BASES 1997), which was chosen as the method to assess VO_{2max} because it produces higher values than those obtained from most alternative test procedures in healthy individuals (McArdle et al. 2001). Subjects began running at a modest speed of 8–10 km h^{-1} at a treadmill grade of 1%. Stages of 4 min duration were used, with running speed increased by 1 km h^{-1} between stages. Expired air and heart rate were monitored as described in the previous paragraph. When heart rate exceeded 90% of the age-predicted maximum heart rate, the running speed was kept constant and the treadmill grade increased by 1% per minute until volitional exhaustion. VO_{2max} was expressed in three ways: in 'absolute' non-scaled units of ml min^{-1} (ABS), in the traditional unit ml kg^{-1} min^{-1} (TRAD) and in the more recently recommended scaling unit of ml kg$^{-2/3}$ min^{-1} (SCA) (Nevill et al. 1992). The ratio limits of agreement for aerobic capacity was 0.97 */÷ 1.17.

Determination of plasma ACE activity

Fasting 10 ml blood samples were obtained from a superficial forearm vein. All subjects abstained from alcohol and caffeine intake, and from exercise, for at least 24 h prior to blood sampling. Plasma was separated immediately from 10 ml of the whole blood by centrifugation at 1,500g for 10 min, and stored at below –20°C until analysis. ACE activity was assayed using a modified fluorometric method using carbo-benzoxy-phenyl-alanyl-histidyl-leucine (Z-phe-his-leu) as a substrate (Depierre and Roth 1975; Unger et al. 1982). The intra-assay coefficient of variation for the ACE measurements lay between 1.9 and 5.6% when

determined on seven different days. The inter-assay coefficient of variation for the ACE measurements was 9.4%.

Determination of ACE genotype

An additional 5 ml EDTA sample of whole blood was drawn, from which leukocyte DNA was extracted by salting out. ACE genotype was determined using a three-primer polymerase chain reaction (PCR) amplification as previously described (Montgomery et al. 1997), with products resolved on a 7.5% polyacrylamide gel by two independent staff blind to all subject data.

Statistical analysis

All data were analysed using SPSS for Windows (release 11.0) statistical software package (SPSS Inc., Chicago, IL) and are expressed as mean ± SD. The accepted level of significance was set at $P = 0.05$ for all statistical tests. One-tailed partial correlations, controlling when appropriate for body mass and/or stature, or BMI, were used to seek negative relationships between circulating ACE activity and the endurance-related parameters. The associations of ACE genotype with VO_{2max} and measures of mechanical efficiency were assessed for linear trend using analysis of covariance (ANCOVA) with body mass and/or stature, or BMI, used as covariates when appropriate.

Results

There were no significant correlations between circulating ACE activity (27.4 ± 8.4 nM His-Leu-ml^{-1}) and any of the efficiency measures (all data shown in Table 1). The (lack of) relationship between circulat-

ing ACE activity and DE is illustrated in Fig. 1 as an example. Similarly, there were no significant correlations between circulating ACE activity and VO_{2max} expressed using any unit (Table 1). The (lack of) relationship between circulating ACE activity and SCA is shown in Fig. 2.

The distribution of the ACE genotypes for the 62 subjects was II 14 (23%), ID 25 (40%) and DD 23 (37%), which is in Hardy–Weinberg equilibrium (the slightly lower numbers of subjects that successfully completed each laboratory test were also in Hardy–Weinberg equilibrium). As expected, there was a very strong and statistically significant association of genotype with circulating ACE activity ($P < 0.0005$, ANCOVA) as has been reported by several authors previously, with DD (33.8 ± 8.9 nM His-Leu-ml^{-1}) showing greater ACE activity than both ID (25.9 ± 5.5 nM His-Leu-ml^{-1}) and II (20.5 ± 4.1 nM His-Leu-ml^{-1}) and ID showing greater ACE activity than II. However, linear trend analysis demonstrated that ACE genotype had no association with any of the measures of efficiency or VO_{2max} (all data shown in Table 2), corresponding with the results for circulating ACE activity. Other subject characteristics such as body mass, stature and BMI were also independent of both ACE genotype and activity.

Discussion

The data from this study demonstrate that no significant correlation exists between mechanical efficiency during cycle ergometry and either circulating ACE activity or ACE I/D genotype in sedentary women. The study was powered to detect correlations of magnitude $r \sim 0.35$ or greater between circulating ACE activity and DE. Previous work has examined mechanical efficiency in relation to ACE genotype, using the same mode of exercise as in this study

Table 1 Pearson correlation analyses between circulating ACE activity and the measures of mechanical efficiency and maximal oxygen uptake

	Sample size (n)	Mean ± SD	Pearson correlation with circulating ACE activity	One-tailed significance of correlation
DE (%)	47	23.9 ± 2.5	$r = -0.122$	$P = 0.229$
GE40 (%)	58	14.4 ± 1.4	$r = -0.073$	$P = 0.297$
GE60 (%)	56	16.7 ± 1.4	$r = 0.136$	$P > 0.6$
GE80 (%)	51	18.0 ± 1.4	$r = 0.139$	$P > 0.6$
ABS VO_{2max} (ml min^{-1})	58	2403 ± 376	$r = 0.141$	$P > 0.6$
TRAD VO_{2max} (ml kg^{-1} min^{-1})	58	37.0 ± 6.2	$r = 0.059$	$P > 0.6$
SCA VO_{2max} (ml kg$^{-2/3}$ min^{-1})	58	149.1 ± 22.9	$r = 0.134$	$P > 0.6$

DE delta efficiency between 40 and 80 W, *GE40* gross efficiency at 40 W, *GE60* gross efficiency at 60 W, *GE80* gross efficiency at 80 W, *ABS VO_{2max}* absolute non-scaled units of ml min^{-1}, *TRADVO_2max* traditional units of ml kg^{-1} min^{-1}, *SCA VO_{2max}* scaling unit of ml kg$^{-2/3}$ min^{-1}
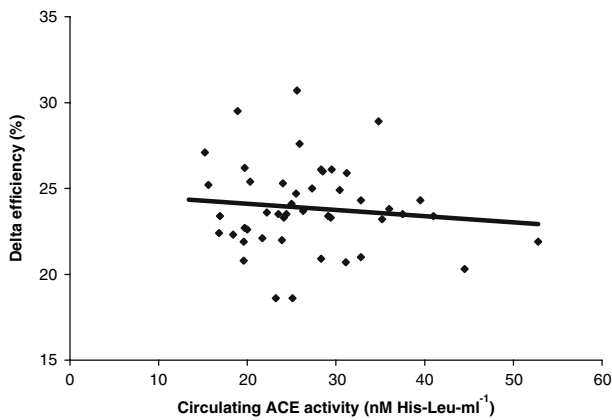
Fig. 1 No significant relationship between circulating ACE activity and delta efficiency ($n = 46$, $r = -0.112$, $P = 0.229$)
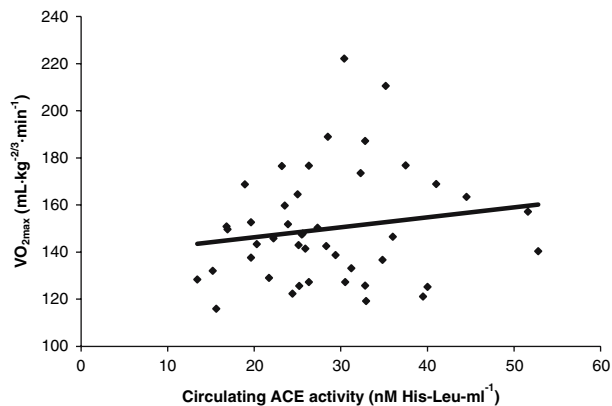

Fig. 2 No significant relationship between circulating ACE activity and VO_{2max} expressed in units of ml kg$^{-2/3}$ min^{-1} ($n = 58$, $r = 0.134$, $P > 0.6$)

Table 2 Analyses of associations of ACE genotype with the measures of mechanical efficiency and VO_{2max}

	Mean ± SD within each genotype group	P value
DE (%)	II 24.4 ± 2.5	0.093
	ID 24.3 ± 2.5	
	DD 22.8 ± 2.5	
GE40 (%)	II 14.5 ± 1.3	0.673
	ID 14.4 ± 1.3	
	DD	
	14.3 ± 1.3	
GE60 (%)	II 16.9 ± 1.4	0.636
	ID 16.7 ± 1.4	
	DD16.7 ± 1.3	
GE80 (%)	II 18.2 ± 1.4	0.523
	ID 18.0 ± 1.4	
	DD 17.9 ± 1.3	
ABS VO_{2max} (ml min^{-1})	II 2320 ± 352	0.298
	ID 2418 ± 355	
	DD 2446 ± 347	
TRAD VO_{2max} (ml kg^{-1} min^{-1})	II 35.5 ± 5.6	0.371
	ID 37.4 ± 5.6	
	DD 37.2 ± 5.5	
SCA VO_{2max} (ml kg$^{-2/3}$ min^{-1})	II 143.8 ± 22.3	0.334
	ID 149.8 ± 22.4	
	DD 151.2 ± 22.1	

Data are mean ± SD, after adjustment for influences of covariates. *P* values are for linear trend from ANCOVA. *DE* delta efficiency between 40 and 80 W, *GE40* gross efficiency at 40 W, *GE60* gross efficiency at 60 W, *GE80* gross efficiency at 80 W, *ABS* VO_{2max} absolute non-scaled units of ml min^{-1}, *TRAD* VO_{2max} traditional units of ml kg^{-1} min^{-1}, *SCA* VO_{2max} scaling unit of ml kg$^{-2/3}$ min^{-1}

(Williams et al. 2000, 2004), but neither study actually assayed ACE activity directly. Nevertheless, these prior studies demonstrated that DE was independent of ACE I/D genotype prior to a training programme in both men and women, which is now confirmed by the lack of association with circulating ACE activity observed in the current study.

ACE activity is a highly heritable trait with the variation at the ACE I/D locus (not itself directly functional) marking up to 40% of the interindividual variation in circulating ACE activity. Therefore, measuring ACE activity would provide greater predictive or statistical power than ACE I/D genotype. It is possible to conduct a power calculation based on the results observed here to predict how many subjects would be needed to establish statistical significance for correlations of the magnitudes we have reported. For example, the strongest negative correlation observed (between DE and circulating ACE activity) was $r = -0.122$. Based on $r = -0.122$, more than 400 subjects

would be required to establish statistical significance, and of course even then the relationship would remain very weak and explain extremely little of the inter-individual variability. Therefore, it is possible that very weak correlations exist between circulating ACE activity and the endurance parameters we assessed here, but our data suggest that a study of substantially larger scale would be required to successfully identify these correlations, and any biological significance is unlikely.

A lack of association between circulating ACE activity and VO_{2max} was also seen in this study, and this is again the first report of a direct analysis between directly assayed circulating ACE activity and endurance-related fitness phenotypes. This lack of association is consistent with the results of Rankinen et al. (2000a) who found no association between ACE genotype and pre-training aerobic fitness phenotypes and Woods et al. (2002) who found no association between ACE genotype and VO_{2max} prior to training in military recruits. It therefore seems very unlikely that prolonged exposure to a genotype-dependent level of circulating ACE activity influences the maximal rate of

oxygen uptake in healthy but relatively sedentary individuals. Recently, Bahi et al. (2004) also observed no difference in endurance capacity in rats treated for at least 10 weeks with an ACE inhibitor compared to a non-inhibited control group, suggesting that a moderate duration of exposure to a lower level of circulating ACE activity also fails to influence endurance in healthy animals.

All participants were pre-menopausal, although they were not standardized with respect to menstrual cycle status or contraceptive pill usage. Current literature suggests that many determinants of VO_{2max} are not influenced by menstrual cycle status (Janse de Jonge 2003) and consequently this factor was not considered with regard to the study design. Additionally it has been found that low dose oral contraceptives do not influence endurance performance (Bryner et al. 1996). However, it is acknowledged that aerobic capacity is reduced in female athletes using triphasic oral contraceptives (Lebrun et al. 2003) and the possibility remains that this may have had an influence on the results of this study.

The present study also investigated any associations of ACE genotype with the endurance parameters measured—thus taking the approach of the previous studies in this field. Similarly to the results regarding the ACE activity data, the ACE genotype results failed to demonstrate an association between ACE genotype and the endurance variables in sedentary subjects, which further supports the findings of the various previous authors (Rankinen et al. 2000a; Williams et al. 2000; Woods et al. 2002). Although the power of this study was not great to detect genotype associations (and was designed with analyses of circulating ACE activity in mind, rather than genotype), the sample was nevertheless easily large enough to detect an association between circulating ACE activity and ACE genotype ($P < 0.0005$) once again (Rigat et al. 1990; Williams et al. 2005).

The only study thus far demonstrating a significant association between ACE genotype and VO_{2max} is that of Hagberg et al. (1998) who observed an association between VO_{2max} and the I allele in habitual exercisers from a specialist group of postmenopausal women. Therefore, the subject characteristics from that study were different to this and other previous studies, which might partly explain that unusual finding. Indeed, it is possible that any associations between endurance parameters and circulating ACE activity or ACE genotype are only unmasked following periods of intense endurance training. For example, an over-representation of the I allele has been observed in elite endurance athlete populations (Gayagay et al. 1998;

Myerson et al. 1999), although this has not been reproduced in all studies (Rankinen et al. 2000b). In addition, Williams et al. (2000) found that DE in military recruits significantly increased in those individuals homozygous for the I allele following an 11-week training programme, despite no association with genotype before training. It would therefore be useful in future work to examine the influence of circulating ACE activity on the magnitude of response to endurance training.

Another possible explanation for the lack of association observed in this study between ACE activity and the endurance phenotypes may be related to the fact that we examined circulating ACE activity rather than local ACE activity in skeletal muscle (Reneland and Lithell 1994). Circulating ACE accounts for only a small proportion of total body ACE (Cushman and Cheung 1971) and local tissue ACE has greater physiological and pathological significance (Dzau et al. 2001). It may be that inter-individual variability in local ACE activity is more important in mediating differential endurance capacity than circulating ACE activity. This aspect requires further examination using studies that combine appropriately large sample sizes with the time-consuming and invasive procedures required to glean the necessary data. Future studies should also consider the role of ACE in kinin metabolism, given that the ACE I allele is associated with increased kinin activity (Murphey et al. 2000) and the recently published evidence suggesting that at least some of any associations between ACE status and endurance phenotypes seems to be mediated through alterations in kinin activity at the bradykinin B2 receptor (Williams et al. 2004).

In conclusion, the results of this study suggest that it is unlikely that long-term exposure to a particular level of circulating ACE activity influences mechanical efficiency or maximal oxygen uptake in sedentary individuals.

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