ORIGINAL ARTICLE

Relationship between ace genotype and short duration aerobic performance development

Mesut Cerit · Muzaffer Colakoglu · Murat Erdogan · Afig Berdeli · Fethi Sirri Cam

Abstract We have previously demonstrated that, *ACE* D allele may be related with a better performance in short duration aerobic endurance in a homogeneous cohort with similar training backgrounds. We aimed to study the variation in the short-duration aerobic performance development amongst *ACE* genotypes in response to identical training programs in homogeneous populations. The study group consisted of 186 male Caucasian non-elite Turkish army recruits. All subjects had undergone an identical training program with double training session per day and 6 days a week for 6 months. Performances for middle distance runs (2,400 m) were evaluated on an athletics track before and after the training period. *ACE* gene polymorphisms were studied by PCR analysis. The distribution of genotypes in the whole group was 16.7% II, $n = 31$; 46.2% ID, $n = 86$; 37.1% DD, $n = 69$. Subjects with *ACE* DD genotype had significantly higher enhancement than the ID ($P < 0.01$) and II ($P < 0.05$) genotype groups. Around 2,400 m performance enhancement ratios showed a linear trend as *ACE* DD $> ACE$ ID $> ACE$ II (P value for Pearson $\chi^2 = 0.461$ and P value for linear by linear association $= 0.001$). *ACE* DD genotype seems to have an advantage in development in short-duration aerobic performance. This data in unison with the data that we have obtained from homogenous cohorts previously is considered as an existence of threshold for initiation of *ACE* I allele effectiveness in endurance performance. This threshold may be anywhere between 10 and 30 min with lasting maximal exercises.

Keywords Genetics · Endurance · Insertion/deletion · Training · *ACE*

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Introduction

High performance in short duration aerobic performance (2–8 min) demands high power output and increased tissue oxygenation. It requires higher VO_{2max} and strength endurance levels. Angiotensin I-converting enzyme (*ACE*) cleaves vasodilator kinins while promoting formation of the vasoconstrictor angiotensin II. Increased plasma angiotensin II levels restrict blood flow to tissues. The human *ACE* gene contains a polymorphism consisting of the presence (insertion, I) or absence (deletion, D) of a 287 base pair sequence in

intron 16 (Rigat et al. 1990). This polymorphism seems to have an important role on *ACE* at a cellular level (Davis et al. 2000; Mizuiri et al. 1997) and may effect angiotensin II production.

The present data on the *ACE* I/D polymorphism and exercise performance are somewhat controversial. The *ACE* I-allele usually seems to be associated with enhanced aerobic endurance performance (Alvarez et al. 2000; Gayagay et al. 1998; Montgomery et al. 1998; Myerson et al. 1999; Nazarov et al. 2001). However, in some studies higher VO_{2max} levels, which indicate an improved oxidative capacity, found to be related with *ACE* D-allele (Rankinen et al. 2000a; Zhao et al. 2003). On the other hand, *ACE* D-allele is related with higher fast-twitch (FT) muscle fiber ratio (Zhang et al. 2003), greater strength gain in the quadriceps muscle in response to training (Folland et al. 2000), and better anaerobic performance (Woods et al. 2001). In contrast, some researchers have not found a relationship between *ACE* genotype and athletic performance in elite athletes (Rankinen et al. 2000b; Taylor et al. 1999), and sedentary subjects (Rankinen et al. 2000a).

Such associations with athletic performance and *ACE* I/D polymorphism have been replicated across different races, geographical locations, athletic status and sporting disciplines (Alvarez et al. 2000; Myerson et al. 1999; Woods et al. 2001). Studies of those of mixed ability and mixed sporting disciplines have thus tended to be negative (Woods et al. 2001) as have those confounded by a mixture of those of different race and sex or training regimen (Nazarov et al. 2001; Taylor et al. 1999).

We have previously demonstrated that *ACE* D allele may be related with a better performance in short duration aerobic endurance in a homogeneous cohort (Cam et al. 2005). However, the study was cross-sectional and the group was small ($n = 88$).

We postulated that *ACE* D allele is associated with a better short-duration aerobic performance development in response to identical training programs in homogeneous populations. To clarify this hypothesis, we aimed to study the variation in the performance as a result of 6 months endurance training in the army recruits.

Methods

Subjects

The study group consisted of 186 male Caucasian non-elite Turkish army recruits. The study had appropriate

ethics committee approval. Written informed consent was obtained from all participants.

Training program

All subjects had undergone an identical training program with double training session per day and 6 days a week for 6 months. The program consists of flexibility exercises, circuit trainings, 2,400 and/or 3,000 m runs, 1,000–3,000 m runs with military equipment, hurdling course, aerobic threshold and anaerobic threshold trainings. The circuit trainings were consisted of gallows, sit-ups, push-ups and rope-climbs, bomb throws, hurdling course. In initial 2 weeks, there were approximately 30 min whole body flexibility exercises and circuit trainings every weekday, 30–45 min anaerobic threshold runs and 45–60 min aerobic threshold runs alternately except Sundays. From third week onwards, one hurdling course training, and one or two of the 1,000 –3,000 m run with military equipment and/or the 2,400 or 3,000 m running were replaced with one of the aerobic or anaerobic threshold training.

Exercise tests

Performances for middle distance runs (2,400 m) were evaluated on an athletics track before and after the training period. Performance times were determined with digital timers in 0.01 s accuracy by three referees. The time in the middle was recorded.

Genetic analysis

Genomic DNA was extracted from 200 μl of EDTA-anticoagulated peripheral blood leucocytes using the QIAmp Blood Kit (QIAGEN, Ontario, Canada, Cat. no:51,106). Amplification of DNA for genotyping the *ACE* I/D polymorphism was carried out by polymerase chain reaction (PCR) in a final volume of 15 μl containing 200 μM dNTP mix, 1.5 mM $MgCl_2$, 1× Buffer, 1 unit of AmpliTaq® polymerase (PE Applied Biosystems) and 10 pmol of each primer. The primers used to encompass the polymorphic region of the *ACE* were 5′-CTGG AGACCACTCCCATCCTTTCT-3′ and 5′-ATGTGG CCATCACATTCGTCAGAT-3′ (Rigat et al. 1992). DNA is amplified for 35 cycles, each cycle comprising denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 1 min with final extension time of 7 min. The initial denaturizing stage was carried out at 95°C for 5 min. The PCR products were separated on 2.5% agarose gel and identified by ethidium-bromide staining. Each DD genotype was confirmed through a second PCR with primers specific for the insertion

sequence (Shanmugam et al. 1993). The samples with II and DD homozygote genotypes and ID heterozygote genotype were selected at random. These samples were then purified by PCR products purified system (Genomics, Montage PCR, Millipore) and directly sequenced by the ABI 310 Genetic Analyzer (ABI Prisma PE Applied Biosystems).

Statistical analysis

Statistical analyses were performed using SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL, USA). Methods applied were frequencies, cross-tabulations, descriptive statistics, and means. Statistical significance was set at the $P < 0.05$ level. A χ^2 test with the data read from Finetti statistics program was used to confirm that the observed genotype frequencies were in Hardy–Weinberg equilibrium. Differences amongst ACE genotype groups in endurance performance were tested with analysis of variance (ANOVA) and post-hoc Bonferroni test. Genotype distribution across performance levels was compared by chi-square for linear trend. Differences between baseline and post-training values of each ACE genotype group were analyzed by t-test.

Results

The distribution of genotypes in the whole group (16.7% II, $n = 31$; 46.2% ID, $n = 86$; 37.1% DD, $n = 69$) did not deviate significantly from those predicted by the Hardy–Weinberg equilibrium. The allele frequencies of the subjects were 0.398 and 0.602 for the I and D alleles, respectively. Baseline 2,400 m performance levels were not different amongst ACE genotype groups (Table 1).

All ACE genotype groups showed significant improvements in 2,400 m performance after training period as compared to baseline levels ($P < 0.001$ for all). However, subjects with ACE DD genotype had significantly higher enhancement than the ID ($P < 0.01$) and II ($P < 0.05$) genotype groups (Table 1, 2). Around 2,400 m performance enhancement ratios (variation %)

Table 2 Differences in 2,400 m performance improvements amongst ACE genotype groups

	ACE II, ($n = 31$)	ACE ID, ($n = 86$)	ACE DD, ($n = 69$)
ACE II	–	1.000	0.013*
ACE ID	1.000	–	0.004**

*$P < 0.05$; **$P < 0.01$ (Bonferroni test)

showed a linear trend as ACE DD > ACE ID > ACE II (P value for Pearson $\chi^2 = 0.461$ and P value for linear by linear association = 0.001).

Discussion

We have previously reported that ACE D allele may be related with a better performance in short-duration aerobic endurance (2,000 m) in a homogeneous cohort (Cam et al. 2005) and, also found that I allele responses better to medium-duration (30 min) aerobic endurance training (Cam et al. 2006).

In this study, we demonstrated that ACE DD genotype has an advantage in short-duration aerobic endurance (2,400 m) development in response to training. Thus, it seems that the initiation of the effectiveness of ACE I allele in better performances or responses to training in endurance events is somewhere between approximately 10–30 min.

High level of power production, VO_{2max} and anaerobic capacity is necessary for success in middle distance running performances. VO_{2max} levels can be sustained 10–12 min (Martin 1990). Since our subjects baseline performances are close to 10 min and post-training performances are better, it suggests that their exertion is at least equal or even higher than VO_{2max}. Running performances corresponding to VO_{2max} resulted in 8–12 mM blood lactate concentrations (Noakes 1988). Ohkuwa et al. (1984) had shown that mean peak blood lactate levels were 12 mM after an exhaustive 3,000 m running in track and field athletes. Thus, it may be postulated a high anaerobic energy contribution exists in 2,400 m maximal running performance.

Table 1 Differences amongst ACE genotype groups in 2,400 m performance

*$P < 0.05$; **$P < 0.01$

[a] {1-[last value/previous value]} ×100

	ACE II ($n = 31$)	ACE ID ($n = 86$)	ACE DD ($n = 69$)	ANOVA χ^2	P
Baseline (s)	599.1 ± 33.0	591.9 ± 33.9	601.0 ± 40.3	1.305	0.274
Post-training (s)	541.0 ± 25.4	532.5 ± 28.0	529.6 ± 28.7	1.809	0.167
P (t-test)	0.0001**	0.0001**	0.0001**		
Variation[a] (%)	9.59 ± 3.64	9.94 ± 3.7	11.6 ± 3.4	6.669	0.02*

ACE D allele seems related with a higher VO_{2max} (Rankinen et al. 2000a; Zhao et al. 2003) and superior performances in middle and long distance swimming (Tsianos 2004). *ACE* DD genotype may be associated with a greater skeletal muscle strength gain in response to training (Colakoglu et al. 2005; Folland et al. 2000; Hopkinson et al. 2004) and a higher anaerobic capacity (Woods et al. 2001). This genotype is found to be related to a higher percentage of type-II muscle fibers (Zhang et al. 2003). Middle distance runners (800–3,000 m) have a relatively high percentage (48–55%) of fast-twitch fibers (Noakes 1991). Therefore, *ACE* DD genotype subjects may have an advantage in short-duration aerobic performances that requires high level VO_{2max}.

Indeed, recent data have some conflictions on the effectiveness of *ACE* I/D polymorphism and exercise performance. Besides many research projects revealing that there may be an association between *ACE* I/D polymorphism and athletic performance, Rankinen et al. (2000b) concluded that there was no relationship between *ACE* I/D polymorphism and elite athlete status in 192 athletes whose VO_{2max} was at least 75 ml kg^{-1} min^{-1}. Likewise, Taylor et al. (1999) did not find any association between *ACE* I/D polymorphism and athletic performance in a cohort, composed with both genders. However, they found a trend toward the DD genotype in males but the trend was inconsistent in females. Also, Sonna et al. (2001) have reported that *ACE* genotype was not strongly related to physical performance in their studies on the effect of training on aerobic power and muscular endurance in 147 healthy US Army recruits of different ethnicity.

Conclusion

ACE DD genotype seems to have an advantage in development in shortduration aerobic performance. There was also a linear trend in performance enhancement as *ACE* DD > ID > II. This data in unison with the data that we have obtained from homogenous cohorts previously is considered as an existence of threshold for initiation of *ACE* I allele effectiveness in endurance performance. This threshold may be anywhere between 10 and 30 min lasting maximal exercises.

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