Title: RELATİONSHIP BETWEEN ACE GENOTYPE AND SHORT DURATION AEROBİC PERFORMANCE DEVELOPMENT

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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 six days a week for six 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. 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 minutes with lasting maximal exercises.

Key words: Genetics, Endurance- Insertion/deletion -Training-*ACE*

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 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 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 anaaerobic 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. 2000b; 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 admixture 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 six 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 were undergone an identical training program with double training session per day and six days a week for six months. The program consists of flexibility exercises, circuit trainings, 2,400 m and/or 3,000 m runs, 1,000 to 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 two weeks, there were approximately 30 min whole body flexibility exercises and circuit trainings every weekday, 30 to 45 min anaerobic threshold runs and 45 to 60 min aerobic threshold runs alternately except Sundays. From third week onwards, one hurdling course training, and one or two of the 1,000 m to 3,000 m run with military equipment and/or the 2,400 m 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 sec 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 $MgCl_2$, 1x 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'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'-ATGTGGCCATCACATTCGTCAGAT-3' (Rigat et al. 1992). DNA is amplified for 35 cycles, each cycle comprising denaturation at 94^{o} C for 30 s, annealing at 50^{o} C for 30 s, extension at 72^{o} C for 1 min with final extension time of 7 min. The initial denaturizing stage was carried out at 95^{o} 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 and 2). Around 2,400 m performance enhancement ratios (variation %) 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 suggest that their exertion is at least equal or even higher than VO_{2max}. Running performances corresponding to VO_{2max} resulted in 8-12mM blood lactate concentrations (Noakes 1998).Ohkuwa et al. (1984) had shown that mean peak blood lactate levels were 12 mM after an exhaustive 3,000m running in truck and field athletes. Thus, it may be postulated a high anaerobic energy contribution exists in 2,400 m maksimal running performance.

ACE D allele is seems related with a higher VO_2max (Rankinen et al. 2000a; Zhao et al. 2003) and superior performance 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 (Çolakoğlu et al. 2005; Folland et al. 2000; Hopkinson et al. 2004) and a higher anaerobic capacity (Woods et al. 2004). 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 percantage (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 elite athletic performance in achort, 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 short-duration 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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Table 1- Differences amongst *ACE* genotype groups in 2,400 m performance

	***ACE* II**	***ACE* ID**	***ACE* DD**	**ANOVA**	
	(n=31)	(n=86)	(n=69)	χ^2	P
Baseline	599.1 ± 33.0	591.9 ± 33.9	601.0 ± 40.3	1.305	0.274
Post-training	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*

*: p<005; **: p<0.01; [a]: {[last value/previous value]-1}x 100

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

	***ACE* II**	***ACE* ID**	***ACE* DD**
	(n=31)	(n=86)	(n=69)
***ACE* II**	—	1.000	0.013*
***ACE* ID**	1.000	—	0.004**

*: p<0.05; **: p<0.01(Bonferroni test)