Introduction

The angiotensin converting enzyme (ACE) is a zinc membrane-bound metallopeptidase that governs the conversion of angiotensin I to angiotensin II and the degradation of bradykinin at the endothelial surface, thus being important in vasoactive peptide metabolism (26). The control and regulation of ACE gene expression is still largely unknown and might be tissue-specific (5). The human ACE gene is found on Chromosome 17 with a restriction fragment length polymorphism consisting of the presence (insertion, I) or absence (deletion, D) of a 287 base pair Alu repeat sequence in intron 16 (24). The association of the I allele with lower ACE activity in both serum (24) and tissues (9) has ramifications throughout the renin–angiotensin system and the kallikrein–kinin system, and has stimulated much fascinating work with regard to various pathological and physiological states. These three genotypes include ACE D/D and I/I homozygotes and I/D heterozygotes (28). The D allele at the ACE-insertion/deletion (I/D) polymorphism has been shown in association with the renin-angiotensin system and the kallikrein–kinin system, and has stimulated much fascinating work with regard to various pathological and physiological states. These three genotypes include ACE D/D and I/I homozygotes and I/D heterozygotes (28).

Materials and Methods

Subjects

In total 160 DNA samples from individuals belonging to the athlete and sedentary target groups were analyzed to determine the ACE I and D allele frequencies. In sedentary control group, there were eighty healthy volunteers (39 males, 41 females). All samples were taken from the volunteers 20–25 years old both for the sedentary and athletic groups. Healthy people with no routine physical exercise and no connection with any kind of sport activity were included in the study.

Summary: The angiotensin converting enzyme (ACE) gene is located on human chromosome 17 expressing three genotypes within the intron 16 of the related gene structure. These genotypes are classified as I and D alleles which are termed as insertion and deletion, respectively. This study was carried out to identify possible relationships between the insertion/deletion (I/D) polymorphisms and athletic performance in Turkish athletes. To be able to determine these relationships, eighty healthy athletes and eighty healthy sedentary controls were genotyped for the ACE I/D polymorphism at gene level. According to the results obtained, we found significant difference on ACE I/D polymorphism in between athletes and healthy controls ($\chi^2 = 7.32, df = 2, P = 0.026$). This result supports the association in ACE genotype in Turkish athletes, suggesting that this might be a genetic factor influencing the physical performance.
accepted as sedentary control group. For the athletic group (66 males, 14 females), the individuals were selected from volunteer endurance athletes (17 middle-distance running, 10 basketball, 18 handball, 35 football players). Those individuals are graduate students from Pamukkale University Faculty of Education, Department of Sports Education. All the athletes had been competing at the national or international level teams for several years. Individuals from both control and study groups have no special ethnic background, no family relationship and were selected randomly without any specific parameter except that age. They are all Turkish students from different parts of Turkey. The study was approved by the Pamukkale University Ethics Committee, and written informed consent was obtained from each participant.

Genotyping
The human ACE gene is located at chromosome 17q23. The 287–bp I/D polymorphism is located in intron 16 of the ACE gene. Genomic DNA from the subjects was prepared from peripheral blood by standard phenol/chloroform extraction method as published elsewhere (22). Polymerase chain reaction (PCR) was used to detect the presence of the I and D alleles in intron 16 of the ACE gene according to the method described by Rigat et al. (25), using upstream primer

5’-CTGGAGACCACCTCCATCTTTTCT-3’
and the downstream primer

5’-GATGTGGCCATACATTGCAGAT-3’.

Amplification was performed for 35 cycles with denaturation, extension and annealing temperatures of 94°C, 60 °C and 72°C, each of 30 seconds, respectively. The size of amplified fragments was determined by 2% agarose gel electrophoresis, UVI Gel Documentation system.

Statistics
Chi-square test was used for statistical analysis. A value of p<0.05 was considered statistically significant. All analyses were carried out with the computerized SPSS 9.0 program (Statistical Package for Social Sciences, SPSS Inc).

Results
The ACE polymorphisms are determined by the PCR product length on 2% agarose gel electrophoresis. The amplified fragment identification for the I allele is 490 bp and 190 bp for D allele. In Figure 1, the electrophoretic identification of the alleles by their PCR product length is shown. The results obtained from the electrophoretic patterns are summarized in Table 1.

Discussion and Conclusions
ACE is a ubiquitous carboxypeptidase synthesized by the vascular endothelial cells and expressed in their plasma membrane as a class I integral ecto-enzyme (6). I/D polymorphism in intron 16 of the gene coding for the ACE has been used to study the role of this gene in the physical performance. Performance in endurance sports is a multifactorial phenotype, influenced by several factors, such as physique, biomechanical, physiological, metabolic, behavioral, psychological, and social characteristics (23). Investigations have shown that the ACE I allele is associated with improved muscle efficiency (30), a greater anabolic activity (17) in response to physical training and ACE I polymorphism was associated with increased duration of repetitive biceps flexion after a program of general physical training (19). In addition, Montgomery et al. (19) described a significant improvement of duration of exercise

Tab. 1: Distribution of angiotensin converting enzyme I/D polymorphism in athletes and sedentary controls.

| Subjects       | Genotypes | Angiotensin Converting Enzyme | Alleles |
|----------------|-----------|-------------------------------|---------|
|                | DD | ID | II | D | I |
| Controls (n=80)| n  | %  | n  | %  | n  | %  | n  | %  | n  | %  |
| Athletes (n=80)| 56 | 70 | 15 | 18.8 | 9 | 11.2 | 127 | 79.3 | 33 | 20.7 |
|                | 40 | 50 | 29 | 36.3 | 11 | 13.8 | 109 | 68.1 | 51 | 31.9 |

x² = 7.32, df = 2, P = 0.026

Fig. 1: PCR analysis of the angiotensin converting enzyme I/D polymorphism: DD (lanes 1, 2, 3, 5, 9), ID (lanes 4, 7), II (lanes 6, 8), M (Marker, 100 bp ladder).
Among I/I and I/D individuals, in response to a 10-week physical training programme with 11-fold increase in duration of repetitive elbow flexion. Gayagay et al. (11) observed an excess of the I allele (and a concomitant reduction in the D allele) in Australian national rowers. According to the findings of Montgomery et al. (18), individuals with the D/D genotype show a significantly increased left ventricular mass in response to physical training, compared to the I/I genotype (which would be associated with the lowest plasma ACE levels) and the I/D genotype. Thus, the ACE D allele has been associated with a higher ACE activity, compared to the ACE I allele (3, 10). There is also experimental evidence that angiotensinergic neural pathways in the brain probably have an important homeostatic function, especially in regard to osmoregulation and thermoregulation (16). Additionally, the ACE I allele occurred at a significantly higher frequency in 60 Spanish athletes (cyclists, long-distance runners, handball players) compared to controls (3).

On the other hand, some researchers like Taylor et al. (27) and Rankinen et al. (23), do not exclude the possibility that ACE genotype could be related to some attribute relating to a specific type of elite athletic ability or that there may be a difference between genders. Some authors emphasize the importance of carrying out very large studies on athletes involved in various types of sporting activities and on different racial or ethnic groups, because of these previous controversial results (14, 15, 23, 27, 29). In contrast, Nazarov et al. (21) observed no association between the ACE genotypes in Russian athletes.

In normal healthy populations there are many published controversial results (1, 4, 7, 13). For example, Ishir et al. (13) have observed I and D alleles as 50.7% and 49.3%, respectively, in 58 healthy controls. On the other hand, Akar et al. (1) reports the presence of I and D alleles in the control group as 38.7% and 61.2%, respectively, with subject scale of 107. In our observation we found that the presence of I and D alleles as 20.7% and 79.3% in 80 sedentary people. According to our results obtained, we found a significant difference on ACE I/D polymorphism between athletes and healthy controls (X² = 7.32, df = 2, P = 0.026). Our data support with the literature stating the presence of correlation in between ACE I/D polymorphism and athletic performance. Therefore, we suggest the presence of ACE I/D allele could be one of the factors influencing the elite endurance exercise performance. However, these results should be supported with more experimental data on ACE polymorphisms with large elite athletes and sedentary populations in our area. It is this relationship between the I allele, low ACE activity, the increased half-life of bradykinin and reduced production of angiotensin II that might determine the physiological impact of the ACE genotype via enhanced endothelium-dependent vasodilatation (8) and substrate delivery to the working muscles (31).

In summary, our work shows that differences were observed in the genotype frequencies of the ACE I/D polymorphism between Turkish athletes and controls, suggesting that this could be a genetic factor influencing the physical performance. Since there are many controversial results on these observations we suggest the need of the enlargement for sample sizes either on sedentary control and athletic groups. It is also necessary to increase the size of the athletic group under specification of the sport activity type like basketball, handball, and football players, pilots, military personnel having endurance physical activities.

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