Circ J 2005; **69:** 1105 – 1110

Improved Endothelium Dependent Vasodilation in Endurance Athletes and Its Relation With ACE I/D Polymorphism

Halil Tanriverdi, MD; Harun Evrengul, MD; Seyhan Tanriverdi, MD*;
Sebahat Turgut, VDPhD**,†, Beyza Akdağ, PhD††;
H. Asuman Kaftan, MD; Ender Semiz, MD

Background Aerobic exercise enhances endothelium-dependent vasodilation in healthy individuals. It is thought that exercise increases nitric oxide (NO) production and decreases NO inactivation, leading to an increase in NO bioavailability. Angiotensin II and NO have important roles in maintaining vascular tone. There are polymorphisms of the angiotensin converting enzyme (ACE) gene and the presence of the deletion (D) allele has been associated with higher concentrations of circulating and tissue ACE. In this study, the relationship between endothelial function and ACE gene polymorphisms was investigated in athletes and sedentary subjects.
Methods and Results The study group comprised 56 endurance athletes and 46 sedentary subjects who underwent brachial artery ultrasonographic examination. ACE insertion (I) and D allele frequencies were analyzed in all patients. Baseline brachial artery diameter and resting blood flow were similar in athletes and controls ($p>0.05$). The flow-mediated dilation (FMD) was 8.48±3.65% in athletes and 5.16±2.5% in controls ($p=0.0001$). FMD was significantly different between ACE genotypes in the athletes ($p<0.0001$): it was higher in ACE II (10.5±1.6%) subjects than in the DI (8.4±2.3%) or DD (7±1.2%) subgroups.
Conclusion Regular isotonic exercise can improve endothelium-dependent vasodilation especially in those with the ACE II genotype. (*Circ J* 2005; **69:** 1105 – 1110)

Key Words: ACE I/D polymorphism; Athlete; Endothelial function; Flow mediated dilation

The vascular endothelium plays an important role in the regulation of vascular tone and the maintenance of cardiovascular homeostasis[1]. Importantly, endothelial dysfunction, particularly impaired endothelium-dependent vasodilation, has been linked to the pathogenesis of atherosclerotic vascular disease and acute cardiovascular events[2]. Indeed, reduced endothelial vasodilatory function occurs early in atherogenesis before histological and angiographic evidence[3,4].

Epidemiologic studies have shown that high levels of physical activity and cardiorespiratory fitness reduce cardiovascular morbidity and mortality in the general population, including healthy subjects[5,6]. It is clinically important to select the appropriate kind of exercise. Regular aerobic exercise is associated with beneficial changes in blood pressure, lipid metabolism, glucose metabolism, neurohormonal factors, body weight, and shear stress[7,8]. Although the mechanism of improvement in endothelial function during exercise has not been fully clarified, it is thought that nitric oxide (NO) production is increased by up-regulation of endothelial NO synthase gene expression and vascular endothelial growth factor-induced angiogenesis, as well as decreased NO inactivation with augmented antioxidants, such as superoxide dismutase and glutathione peroxidase, and attenuation of nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (NADH/NADPH) oxidase activity, all leading to an increase in NO bioavailability[9].

Polymorphisms of the angiotensin converting enzyme (ACE) gene, located on chromosome 17, have been found and the polymorphism is characterized by the presence (insertion (I)) or absence (deletion (D)) of a 287-base-pair alu repeat within intron 16. The presence of the D allele has been associated with higher concentrations of circulating and tissue ACE. Increased ACE activity might lead to high Angiotensin II (Ang II) concentrations[10]. Of the several candidate genes for endothelial dysfunction, the ACE gene appears to be a likely one because (1) it is anchored via its carboxyl terminus to the endoluminal side of the endothelial cell plasma membrane, from where it can be released in the bloodstream[11,12] and (2) the increased plasma ACE activity found in subjects with the D allele could decrease bradykinin bioactivity with ensuing blunting of receptor-mediated release of NO[13]. Furthermore, even though the literature is variable on whether Ang II effects are increased in subjects with the D allele[14,15], enhanced Ang II production can increase the concentrations of superoxide through increased activity of NADH/NADPH oxidase activity[16] and thus lower the bioactivity of NO[17]. The balance of vasodilators and vasoconstrictors also plays an important role in the physiologic regulation of vascular tone;[18] However, it is not clear whether the ACE genotype can modify the endothelial response to exercise in athletes.

The aim of this study was to investigate the relationship between the ACE genotype and endothelial function in athletes and sedentary subjects by measuring flow-mediated

(Received March 23, 2005; revised manuscript received June 2, 2005; accepted June 8, 2005)
*Departments of Cardiology, *Radiology, **Physiology, Pamukkale University School of Medicine, †Pamukkale University Research Center for Genetic Engineering and Biotechnology (PAMGEN) and ††Department of Bioistatistic, Pamukkale University School of Medicine, Denizli, Turkey*
Mailing address: Halil Tanriverdi, MD, Pamukkale Üniversitesi Tip Fakültesi, Kardiyoloji AD, Kinikli, Denizli,Turkey. E-mail: htanriverdi @posta.pamukkale.edu.tr

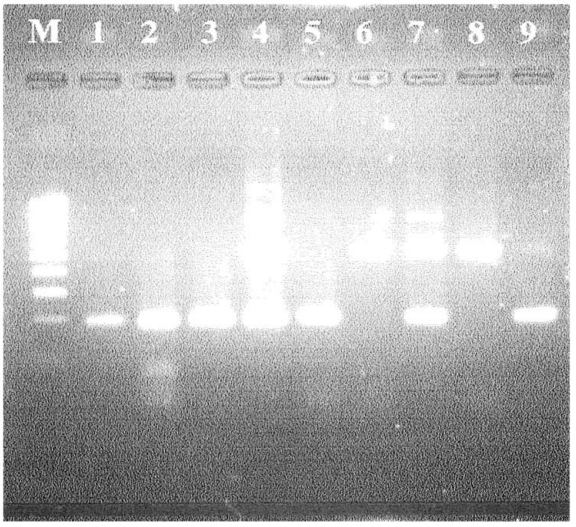

Fig 1. Polymerase chain reaction analysis of the I/D polymorphism of the angiotensin-converting enzyme. DD (lanes 1–3, 5, 9), ID (lanes 4, 7), II (lanes 6, 8), M (Marker, 100 bp ladder).

dilation (FMD) in the brachial artery ultrasonographically.

Methods

Subjects

The study group comprised 56 athletes (43 men, 13 women, mean age 23±1.7 years) and 46 sedentary subjects (36 men, 10 women, mean age 22.5±1.5 years) who underwent vascular ultrasonographic assessment and analysis of ACE I and D allele frequencies. The athletic individuals were selected from volunteer endurance athletes (35 middle-distance running, 21 football players). All the athletes had been competing at the national or international level for several years. None of the subjects had a history of cigarette smoking, hypertension, coronary artery disease, diabetes mellitus, renal or hepatic dysfunction, or a positive family history of hypertrophic cardiomyopathy. Likewise, no medication was taken by any of the subjects for any reason, including anabolic steroids. The study was approved by the Local Ethic's Committee, and written informed consent was obtained from each participant.

Lipid Parameters and ACE Genotyping

Fasting serum samples were analyzed for total cholesterol and high-density lipoprotein cholesterol (HDL-C) by standard clinical laboratory techniques.

A total of 102 DNA samples from the athletes and controls were analyzed to determine the ACE I and D allele frequencies, and they were separated into 3 subgroups: ACE DD (n=46), DI (n=31) and II (n=25) genotypes.

The human ACE gene is located at chromosome 17q23 and the 287-bp ID polymorphism is located in intron 16 of the gene. Genomic DNA from the subjects was prepared from peripheral blood by standard phenol/chloroform extraction method as detailed elsewhere![19] Polymerase chain reaction was used to detect the I and D alleles in intron 16 of the ACE gene according to the method described by Rigat et al;[20] using upstream primer 5'-CTGGAGACC-ACTCCCATCCTTTCT-3' and the downstream primer 5'-GATGTGGCCATCACATTCGTCAGAT-3'. Amplification was performed for 35 cycles with denaturation, extension and annealing temperatures of 94°C, 60°C and 72°C, respectively. The size of the amplified fragments was determined by 2% agarose gel electrophoresis, UVI gel documentation system. A representative sample from the athletes is shown in Fig 1.

Vascular Reactivity Study

The technique for assessing endothelium-dependent and endothelium-independent vasodilation by non-invasive ultrasound has been described in detail by others?[21] Briefly, the diameter of the brachial artery is measured in triplicate at rest, during reactive hyperemia after administration of sublingual glyceryl trinitrate (GTN), using a high-resolution ultrasound machine with a 12.0 MHz linear array transducer (VST-Masters, Diasonics, Santa Clara, CA, USA). Longitudinal images of the brachial artery are obtained proximal to the antecubital fossa. Transmit focus zones are set to the approximate depths of the anterior and the posterior vessel walls. Images are magnified, and the depth and gain settings are used to optimize the image of the vessel wall, in particular, the media–adventitia interface. Other investigators have demonstrated that conduit artery dilation in response to flow increase is endothelium-dependent;[22] whereas the dilator response to GTN is endothelium-independent?[23]

Brachial artery ultrasound was performed in a quiet room after each patient had rested for at least 10 min. After a baseline scan, increased flow was then induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 300 mmHg for 4–5 min. A second scan was obtained 45–60 s after cuff deflation. After a recovery phase of 15 min, sublingual GTN (0.4 mg) was administered and 3–4 min later the last scan was obtained. Vessel diameters (VD) after reactive hyperemia and GTN administration were compared to the resting diameters and expressed as a percentage of the average lumen diameter at rest, which was considered 100% ($FMD\% = [(VD_{\text{reactive hyperemia}} - VD_{\text{rest}}) \times 100]/VD_{\text{rest}}$; $GTN\% = [(VD_{\text{after GTN}} - VD_{\text{rest}}) \times 100]/VD_{\text{rest}}$). Arterial blood flow was measured as Doppler flow velocity multiplied by the cross–sectional area ($\pi \times r^2$).

Statistical Analysis

All analyses were performed with the SPSS 11.5 program (Chicago, IL, USA). Results are given as mean±standard deviation. Independent samples t-test was used for comparison of the controls and athletes. The Kruskal-Wallis test was used to test for the difference between ACE genotypes and then the Mann-Whitney U test with Bonferroni correction was used to compare the difference between each group. A p-value of <0.05 was considered statistically significant.

Results

Basic Characteristics of the Subjects

The characteristics of the subjects are summarized in Table 1. There was no difference statistically between the athletes and controls ($p>0.05$), according to age, gender, waist circumference, body mass index, systolic and diastolic blood pressures. As expected the heart rates of the athletes were lower than the controls ($p<0.001$).

Lipid Parameters

Concentrations of total cholesterol and high-density lipoprotein were not different statistically between athletes

Table 1 Comparison of Group Characteristics

	Athletes (n=56)	Controls (n=46)
Age (years)	23±1.7	22.5±1.5
Gender (%) (M/F)	76.8/23.2	78.3/21.7
BMI (kg/m^2)	22.1±2.4	23.6±2.4
Waist circumference (cm)	71.1±8.1	73.5±9.5
SBP (mmHg)	116.8±6.5	115.4±7.4
DBP (mmHg)	73.5±5.4	72.5±4.6
Heart rate (beats/min)	58.8±4.3	71.3±9.1*

*BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure. *For comparison of athletes, p=0.0001.*

Table 2 Lipid Profile and Endothelial Parameters in All Study Subjects

	Athletes (n=56)	Controls (n=46)
Total cholesterol (mg/dl)	153.1±38.9	158.2±35.9
HDL-C (mg/dl)	45.3±11.2	42.4±12.4
Brachial artery diameter (mm)	4.31±0.68	4.36±0.65
Baseline blood flow (ml/min)	46.4±16.5	43.7±19.3
RH (% increase in flow)	438.2±87.3	424.4±99.1
Flow-mediated dilation (%)	8.48±3.65	5.16±2.5**
GTN-mediated dilation (%)	14.48±3.8	14.22±4.11

*HDL-C, high-density lipoprotein cholesterol; RH, reactive hyperemia; GTN, glyceryl trinitrate. **For comparison of athletes, p=0.0001.*

Table 3 Lipid and Endothelial Parameters in Athletes and Controls According to ACE Polymorphism

	ACE genotypes							
	Athletes (n=56)				Controls (n=46)			
	DD (n=22)	DI (n=18)	II (n=16)	p value	DD (n=24)	DI (n=13)	II (n=9)	p value
Cholesterol (mg/dl)	161.4±24.6	170.7±38.4	139.7±31.4	NS	144.7±32.2	174.7±35.8	170.3±34.7	0.03
HDL (mg/dl)	46.2±8.2	41.5±7.3	48.3±12	NS	42.4±9.4	39.5±8.8	44.7±12.6	NS
FMD (%)	7±1.2	8.4±2.3	10.5±1.6	0.0001	4.9±1.3	5.5±1.7	5.5±1.9	NS
GTN-MD (%)	14.6±3.4	14.2±2.8	14.5±3.2	NS	14.2±3.9	14.6±4.3	14.2±4.1	NS

ACE, angiotensin converting enzyme; D, deletion; I, insertion; HDL, high-density lipoprotein; FMD, flow-mediated dilation; GTN-MD, glyceryl trinitrate-mediated dilation.

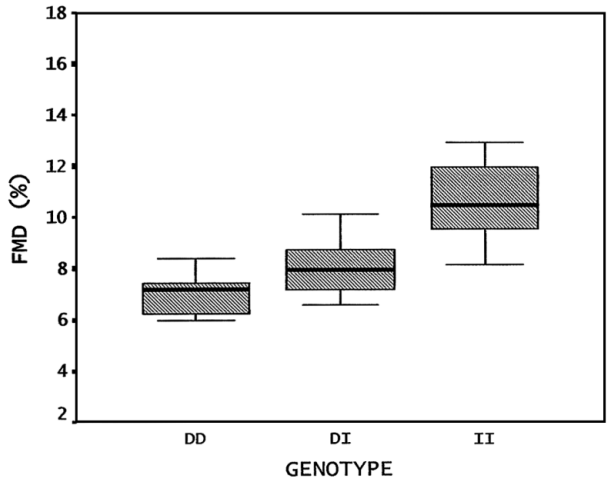

Fig 2. Box plots of flow-mediated dilation (FMD) in athletes according to the polymorphisms of the angiotensin-converting enzyme.

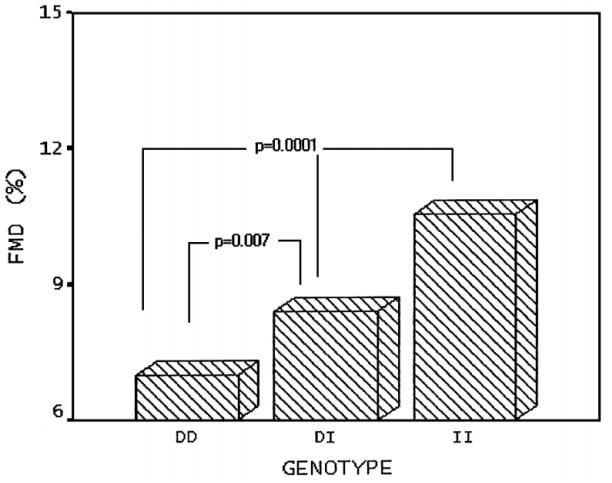

Fig 3. Comparison of flow-mediated dilatation (FMD) in athletes according to the polymorphisms of the angiotensin-converting enzyme.

and controls (Table 2), and those parameters were similar in athletes and controls according to ACE genotypes (Tables 3, p>0.05).

Vascular Studies

Baseline brachial artery diameter, resting blood flow, hyperemia response, FMD and GTN-mediated dilation for athletes and controls are shown in Table 2. Baseline brachial artery diameter (4.31±0.68 vs 4.36±0.65 mm), resting blood flow (46.4±16.5 vs 43.7±19.3 ml/min) and the degree of reactive hyperemia by cuff inflation and release (438.2±87.3% vs 424.4±99.1%) were similar in athletes and controls (p>0.05). FMD was 8.48±3.65% in athletes and 5.16±2.5% in controls (p=0.0001). Dilatation in response to GTN was similar in athletes and controls (14.48±3.8% vs 14.22±4.11%, p>0.05). FMD and GTN-mediated dilation in the athletes according to ACE genotype are shown in Table 3. FMD differed significantly between ACE genotypes (p<0.0001): it was higher in the ACE II (10.5±1.6%) subgroup than in either the DI (8.4±2.3%) or DD (7±1.2%) subgroup (Figs 2,3). Dilatation in response to GTN was similar in athletes according to ACE genotypes (p>0.05). According to ACE genotype FMD and GTN-mediated dilation were not different statistically in controls (Table 3, p>0.05).

Discussion

In the present study, we evaluated the endothelial function in athletes and sedentary subjects in relation to ACE I/D polymorphism.

Healthy conduit arteries are capable of accommodating changes in blood flow by increasing their internal diameter, a phenomenon termed flow-mediated vasodilation. In vitro

studies have shown that this physiological vessel response is endothelium-dependent[24] and that the crucial mediator is NO[25] Therefore, the FMD of the brachial artery is used as an in vivo index of endothelial function[26,27] because it can be induced by hyperemia and then measured non-invasively, accurately and reproducibly by high-resolution ultrasound.

Exercise and Endothelial Function

Previous animal studies suggest that exercise training improves NO-dependent vascular function and upregulates constitutive endothelial NO-synthase expression[28] Exercise programs also improve endothelium-dependent function in adults with cardiac failure,[29,30] coronary disease,[31,32] and diabetes.[33] Shear stress-mediated upregulation of NO-synthase expression, resulting from increased blood flow across the endothelium, is the likely physiologic mechanism. Acute changes in flow and shear stress stimulate the release of NO during exercise in animals, and increases in blood flow through conduit arteries are associated with flow-mediated stress on the vessel wall, which, in turn, liberates NO from the endothelium.[28] In humans, FMD is attenuated by coinfusion of NG-monomethyl-l-arginine, suggesting that conduit vessel dilation during exercise may, at least in part, be NO-dependent.[34] Improvement in vascular function in human training studies is not restricted to the vessels of the exercising musculature[29,33] and occurs in the absence of changes in lipid fractions, blood pressure, or glycemic control.[35] Epidemiologic studies have demonstrated that daily physical aerobic exercise prevents cardiovascular mortality and morbidity.[8] Physical inactivity (sedentary state) is a risk factor for cardiovascular diseases. Recent experimental studies demonstrated continued exercise-augmented vasodilation evoked by the endothelium-dependent vasodilator acetylcholine (ACh) in dogs and rats[36] Other studies have shown that even in normal control animals[37] and healthy subjects[38] exercise training augments endothelial function.

There are also many human studies showing positive effects of exercise on endothelial function. Clarkson et al showed improvement in the endothelial function compared with the baseline values at the end of a 10-week exercise program in young healthy adults.[39] Higashi et al found that exercise improved endothelial function in both normotensive and hypertensive subjects.[9] In both these studies, isotonic exercise was shown to have positive effects on endothelial function. In a study done by Ebenbicher et al body-builders taking anabolic steroids and doing isometric exercise had impaired endothelial function.[40] We found that endothelial function was improved with long-term isotonic exercise training in athletes compared with sedentary subjects, which was in accordance with the results of Clarkson et al and Higashi et al. The beneficial effects were independent of the known influences of exercise on total cholesterol, HDL-C, or resting blood pressure.

ACE Gene Polymorphism and Endothelial Function

I/D polymorphism of the ACE gene has recently been identified as a possible risk factor for several cardiovascular disorders. Because the polymorphism is located in an intron, it is believed to be a neutral marker in strong linkage disequilibrium with one or more unknown functional variants located in or close to the ACE gene.[41] The 3 genotypes include ACE DD and II homozygotes and ID heterozygotes.[42] Compelling evidence indicates that the D allele of the ACE gene is related to increased plasma concentrations of ACE.[10,20,41,43]

Angiotensin-converting enzyme, a key component of the circulating and vascular renin–angiotensin systems promotes the synthesis of Ang II, which is an important regulator of vascular function.[44] By stimulating the AT1 receptor, Ang II promotes vasoconstriction, both directly and through endothelin release or augmentation of sympathetic tone. Moreover, increased free radical generation by Ang II may also contribute to endothelial dysfunction.[45,46] In addition, ACE also metabolizes locally synthesized bradykinin, which would promote vasoconstriction by reducing bradykinin-dependent NO release. This vasoconstriction is partly inhibited by Ang II-mediated release of NO from the endothelium.[47,48] Because individuals with the ACE D allele have higher plasma and tissue ACE concentrations, we hypothesized that the D allele of the ACE gene, by increasing local Ang II generation and decreasing bradykinin activity, may modulate vascular tone.[10,49]

In the present study, we found no difference in endothelial function in the sedentary subjects according to ACE genotypes, as has been found in other studies.[50,51] According to Perticone et al, who studied a smaller population of never-treated primary hypertensive patients, the DD genotype was associated with significant blunting of endothelium-dependent vasodilation, but endothelium dependent vasodilation was normal in the healthy subjects.[52] They claimed that the mechanism of forearm vasodilation in response to ACh in hypertensives was probably reduction of endothelium-dependent vasodilation in response to ACh infusion, or the increased breakdown of endothelium derived relaxing factor (EDRF) by the scavenger for oxygen radicals in DD homozygous subjects. It is also possible that the breakdown of bradykinin, a potent releaser of EDRF, may be involved in this effect. More recently, Butler et al reported. A blunted endothelium-dependent vasodilation in healthy young normotensive university students carrying the D allele compared with II homozygous subjects.[43] They found that the ACE gene D allele in homozygosity was associated with reduced ACh-induced vasodilation in healthy people. However, in this latter study, a blunted endothelium-independent vasodilation was also found in DD homozygous subjects, thereby suggesting that the blunted endothelium-dependent vasodilation could be in part accounted for by either a dysfunctional cGMP pathway or enhanced arteriolar structural changes, or both. Thus, their observed defect in vasomotion may not have been necessarily caused by endothelial dysfunction.

Consistent with our results, Rossi et al found no effect of the I/D genotype on the endothelium-dependent and -independent vasodilation in mild-to-moderate hypertensive and normotensive subjects.[53] Similarly, Celermajer et al also found no differences between D/I genotypes in the in vivo brachial artery responses of 184 normotensive nondiabetic lifelong nonsmokers using FMD.[51] Finally, van Dijk et al found no differences in bradykinin-induced vasodilation in 8 II and 8 DD normotensive males.[54] The possible explanation for these differences could reside in the different criteria used for subject enrollment. We did our best to exclude previously heavy cigarette smokers because it has been contended that the ACE genotype and smoking produce additive detrimental effects on endothelial function, although the blunting of endothelial function by smoking was not dose-dependently related to the D allele.

Long-term evaluation of the athletes revealed that endothelium-dependent vasodilation was better in the ACE II

genotype subjects than in those with the DI or DD genotype. To date, there has not been a study showing the relationship between ACE I/D polymorphism and the positive effect of the exercise on endothelial function. Sanada et al researched the effect of hormone replacement therapy on endothelial function and the relationship between ACE gene polymorphism in postmenapausal women.[55] They found that hormone replacement therapy showed more significant improvement in the subjects with II and DI genotype than in the DD genotype subjects. The effect of ACE inhibition on endothelial function in relation to the ACE genotype was investigated in hypertensive patients by Prasad et al,[56] who found that the improving effects of captopril therapy on endothelial function were most prominent in the D allele group, which has the highest serum concentration of the ACE enzyme. Because ACE is identical to kininase II and is present on the vascular endothelium, bradykinin is inactivated by ACE. Thus, it is assumed that ACE inhibitors may act on kininase II and increase the tissue concentration of bradykinin, which, in turn, augments the vasodilation induced by bradykinin, leading to an increase in NO synthesis.[57] They claimed that plasma ACE enzyme concentrations decreased significantly with ACE inhibitory treatment in subjects with the DD genotype, and as a result of this therapy the improvement in endothelial function was more prominent than in the other genotypes.[56]

It is well known that a balance between Ang II and NO is important in the regulation of vascular tone.[18,58] Ang II increases vascular superoxide production through activation of membrane-associated nicotinamide adenine dinucleotide diaphorase/nicotinamide adenine dinucleotide phosphate diaphorase oxidase, resulting in NO inactivation and toxic peroxynitrite production. Therefore, exercise may increase NO by inhibiting Ang II production. Furthermore, under physiologic conditions, endogenous bradykinin is limited by ACE. Bradykinin binds to B_2 receptors on the endothelial cell surface, causing the release of NO.[59] Recently, Higashi and Goto have shown that plasma Ang II concentrations do not alter during aerobic exercise of mild, moderate, or high intensity in healthy young men or during exercise of moderate intensity in hypertensive patients.[60,61] Therefore, it is unlikely that Ang II plays a critical role in augmentation of endothelial function during exercise training in healthy subjects. It is thought that having the D allele, which is associated with high plasma ACE and Ang II concentrations, contributes negatively to endothelial function, which is positively affected by the release of NO by exercise.

Study Limitations

We could not measure the serum and tissue ACE-Ang II concentrations in relation to ACE gene polymorphism, but Rigat et al[10] and Butler et al[43] showed that serum and tissue ACE-Ang II concentrations were increased in the presence of D allele.

Conclusion

To our knowledge, this study is the first to investigate the effects of ACE I/D genotypes on endothelial function in athletes. The results of our study indicate that endothelium-dependent vasodilation is increased by prolonged training in endurance athletes, particularly those with the ACE II genotype. However, ACE polymorphism had no effect on endothelial function in sedentary subjects.

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