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Original Scientific Paper

Influence of angiotensinogen and angiotensin-converting enzyme polymorphisms on cardiac hypertrophy and improvement on maximal aerobic capacity caused by exercise training

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Background **The allele threonine (T) of the angiotensinogen has been associated with ventricular hypertrophy in hypertensive patients and soccer players. However, the long-term effect of physical exercise in healthy athletes carrying the T allele remains unknown. We investigated the influence of methionine (M) or T allele of the angiotensinogen and D or I allele of the angiotensin-converting enzyme on left-ventricular mass index (LVMI) and maximal aerobic capacity in young healthy individuals after long-term physical exercise training.**

Design **Prospective clinical trial.**

Methods **Eighty-three policemen aged between 20 and 35 years (mean ± SD 26 ± 4.5 years) were genotyped for the M235T gene angiotensinogen polymorphism (TT, $n=25$; MM/MT, $n=58$) and angiotensin-converting enzyme gene insertion/deletion (I/D) polymorphism (II, $n=18$; DD/DI, $n=65$). Left-ventricular morphology was evaluated by echocardiography and maximal aerobic capacity (VO_{2peak}) by cardiopulmonary exercise test before and after 17 weeks of exercise training (50–80% VO_{2peak}).**

Results **Baseline VO_{2peak} and LVMI were similar between TT and MM/MT groups, and II and DD/DI groups. Exercise training increased significantly and similarly VO_{2peak} in homozygous TT and MM/MT individuals, and homozygous II and DD/DI individuals. In addition, exercise training increased significantly LVMI in TT and MM/MT individuals (76.5 ± 3 vs. 86.7 ± 4, $P=0.00001$ and 76.2 ± 2 vs. 81.4 ± 2, $P=0.00001$, respectively), and II and DD/DI individuals (77.7 ± 4 vs. 81.5 ± 4, $P=0.0001$ and 76 ± 2 vs. 83.5 ± 2, $P=0.0001$, respectively). However, LVMI in TT individuals was significantly greater than in MM/MT individuals ($P=0.04$). LVMI was not different between II and DD/DI individuals.**

Conclusion **Left-ventricular hypertrophy caused by exercise training is exacerbated in homozygous TT individuals with angiotensinogen polymorphism.** ***Eur J Cardiovasc Prev Rehabil*** **16:487–492 © 2009 The European Society of Cardiology**

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Introduction

Angiotensin II, the biologically active octapeptide of the renin–angiotensin system, has important functions as vasoconstriction; increases sodium and water reuptake, which in turn increases plasma volume [1] and mediate cell growth and proliferation [2,3].

Recently, a variant of the human angiotensin-converting enzyme (ACE) gene has been identified [4]. It consists of the absence (deletion) of a fragment or D allele, which is associated with higher ACE activity [5] in serum [4,6] and tissue [7]. In contrast, the presence of an extra

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287-bp fragment (insertion) or I allele has been associated with lowered-circulating and tissue ACE activity. This knowledge has led some investigators to suspect that individuals carrying the D allele of the ACE gene are more susceptible to muscle growth. It was interesting that the increase in left-ventricular mass (LVM) after 10 weeks of exercise training (ET) was significantly associated with ACE genotype. More specifically, a positive association of the D allele with left-ventricular hypertrophy was reported in soccer players and endurance athletes [8,9]. In addition, Montgomery *et al.* [10] observed that male military individuals carrying the D allele had a greater increase in LVM than individuals carrying the I allele. In contrast, increased aerobic power has been reported in elite endurance athletes carrying I allele of the ACE [9], and improvement in the duration of repetitive elbow flexion exercise was more pronounced in individuals of II and ID genotypes [11]. In contrast, other investigators failed to find an association between the ID allele of the ACE genotype and cardiorespiratory endurance performance [12]. Sonna *et al.* [13] reported that the ACE genotype did not have any effect on aerobic power. It is important to emphasize that some of these studies were conducted in populations of limited ethnic and geographic diversity [11,14], and the long-term effects of physical exercise were barely studied. These limitations may confound the real influence of the ACE genotype on the gain of LVM and aerobic performance.

Considering the angiotensinogen gene, Jeunemaitre *et al.* [15] showed that a specific variant leading to the substitution of a methionine (M) for a threonine (T) at the codon 235 of the gene was associated with elevated angiotensinogen serum concentrations. The allele T has been associated with ventricular hypertrophy in patients with hypertension and endurance athletes [16,17]. More recently, some investigators reported that the allele D of the ACE and the allele T of the angiotensinogen exert a synergistic effect on LVM in endurance athletes [18]. However, the long-term effect of physical exercise in healthy athlete individuals carrying T and M alleles has not been studied. In addition, the influence of these genotypes on the aerobic capacity remains unknown.

In this study, we investigated the influence of the M or T allele of the angiotensinogen and D or I allele of the ACE on the left-ventricular hypertrophy and maximal aerobic capacity response in young healthy individuals submitted to long-term physical exercise.

Methods

Study population

One hundred and eighty-three preselected healthy male Brazilian policemen recruits were invited to participate in the study. They were genotyped for the M235T gene angiotensinogen polymorphism and for the ACE gene insertion/deletion (I/D) polymorphism. One hundred recruits were excluded for either muscle injury or bad attendance to the ET. Eighty-three recruits completed the study. Twenty-five individuals homozygous for the allele T were compared with 58 individuals heterozygous and homozygous for the allele M. Similarly, 18 individuals homozygous for the allele I were compared with 65 individuals heterozygous and homozygous for the allele D. The individuals had no apparent cardiovascular disease. They were all male from different ethnic backgrounds, healthy, normotensive, and age ranged between 20 and 35 years. Allele and genotype frequencies were in Hardy–Weinberg equilibrium. The Human Subject Protection Committees of the Heart Institute (InCor) and Clinical Hospital, University of São Paulo, Medical School approved the study protocol.

Measurements

Cardiopulmonary exercise test

Peak oxygen consumption (VO_{2peak}) was determined during a progressive exercise test until exhaustion on a treadmill (Quinton Instruments Company, Seattle, Washington, DC, USA). A breath-by-breath gas exchange analyzer (V_{max} 29; Sensor Medics, Buena Vista, California, USA) was used to measure VO_2 and carbon dioxide production. VO_{2peak} was defined as the maximal attained VO_2 at the end of the exercise. Ventilatory thresholds – anaerobic threshold and respiratory compensation point – were determined according to the literature [19,20]. The reproducibility of the VO_{2peak} measured at a different time point in the same individual in our laboratory was $r = 0.95$. Heart rate was continuously monitored by ECG, and blood pressure was measured by sphygmomanometry.

Echocardiography

The morphologic characteristics of the left ventricle were evaluated by echocardiography with ultrasound equipment (Advanced Technology Laboratories, HDI 5000, Philips Medical Systems, Bothell, Washington, DC, USA) and a 2–4 MHz transducer. Left-auricular diameter, left-ventricular end-systolic diameter, left-ventricular end-diastolic diameter, left-ventricular ejection fraction, posterior wall thickness, intraventricular septum thickness (IVST), and LVM were obtained by two-dimensional guided M-mode measurements of the left-ventricle out-flow tract just below the aortic valve obtained from the parasternal long-axis view [21]. Echocardiographic variables are expressed as the average of three consecutive beats. Measurements were made by an experienced observer.

Angiotensin-converting enzyme and angiotensinogen gene polymorphism

The ACE gene I/D polymorphism was determined by means of a 3-primer system and the M235T variant of the ATG gene by a standard polymerase chain reaction detection method [22]. Quality control for these assays

was assessed by randomly selecting 50 samples to be regenotyped by three independent technicians.

Interventions

Exercise training protocol

The ET was performed under supervision and consisted of three sessions per week (60 min), during 4 months. Exercise intensity was graded individually according to the heart rate corresponding to ventilatory thresholds. At the first 2 months, the running intensity was moderate with heart rate between the ventilatory thresholds, and in the last 2 months the heart rate was kept slightly above the respiratory compensation point.

Statistical analysis

All data are presented as mean ± SE. Differences in age between groups were compared by unpaired *t*-test. The echocardiographic parameters and VO_{2peak} within-groups and between-groups before and after the ET were compared by two-way analysis of variance for repeated measurements. LVM, left-ventricular mass index (LVMI), and VO_{2peak} were adjusted for covariants, such age, height, weight, and body mass index. When significance was found, the Scheffè's post-hoc comparison test was applied. Significant differences were assumed to be at a P value of less than 0.05.

Results

Angiotensinogen and angiotensin-converting enzyme gene polymorphisms

There was no significant difference in age between MM/MT and TT groups (MM/TT = 26 ± 1 vs. TT = 27 ± 1 years, $P = 0.3$) or between DD/DI and II groups (DD/DI = 26 ± 1 vs. II = 26 ± 1 years, $P = 0.8$). Baseline physical, hemodynamic, and echocardiographic characteristics in individuals of MM/MT or TT group of the angiotensinogen, and individuals of DD/DI or II group of the ACE are shown in Table 1. There were no significant differences in all these parameters between individuals of the MM/MT and TT or for DD/DI and II group even after adjusted for age, height, weight, and body mass index.

The ET did not change the body mass index and mean arterial pressure in individuals homozygous for the T allele and MM/MT individuals or for the individuals of the II and DD/DI groups (Table 1). However, the ET increased significantly VO_{2peak} in individuals of MM/MT (10%) and TT (8.5%) groups (Fig. 1a), and also for individuals carrying the DD/DI allele (6%) and the II allele (8%) (Fig. 1a). In fact, resting heart rate was significantly reduced after the ET in individuals of TT and MM/MT groups and in individuals of II and DD/DI groups (Table 1). The comparisons between groups showed that the effects of the ET on VO_{2peak} and resting heart rate were similar in individuals homozygous for the T allele and MM/MT group and also for individuals carrying II and DD/DI allele.

Table 1 Physical, hemodynamic, and echocardiographic characteristics preexercise training and postexercise training in individuals homozygous for the M allele, T allele of the angiotensinogen and D allele, I allele of the angiotensin-converting enzyme

	Pre training	Post training		Pre training	Post training
Physical characteristics					
BMI (kg/m^2)					
MM/MT	24 ± 0.5	24 ± 0.4	DD/DI	24 ± 0.5	24 ± 0.5
TT	24 ± 1	24 ± 1	II	23 ± 1	23 ± 1
VO_{2peak} (ml/kg per min)					
MM/MT	50 ± 1	55 ± 1*	DD/DI	49 ± 1	52 ± 1*
TT	47 ± 2	51 ± 2*	II	51 ± 2	55 ± 2*
Hemodynamic characteristics					
MAP (mmHg)					
MM/MT	91 ± 1	91 ± 1	DD/DI	91 ± 1	91 ± 1
TT	92 ± 1	91 ± 1.5	II	91 ± 1	90 ± 2
HR (bpm)					
MM/MT	76 ± 2	69 ± 2*	DD/DI	79 ± 2	70 ± 2*
TT	79 ± 2	69 ± 2*	II	74 ± 2	67 ± 2*
Echocardiographic characteristics					
LVESD (mm)					
MM/MT	32 ± 0.4	32 ± 0.5	DD/DI	31.4 ± 0.4	31.2 ± 0.5
TT	29.7 ± 1.3	30.3 ± 0.8	II	31.5 ± 0.8	31.3 ± 0.8
LVEDD (mm)					
MM/MT	51 ± 0.5	51.5 ± 0.5	DD/DI	51 ± 0.4	51.3 ± 0.4
TT	50 ± 1	50.5 ± 1	II	50.2 ± 0.8	50.6 ± 0.8
PWT (mm)					
MM/MT	7.6 ± 0.1	8 ± 0.1*	DD/DI	7.6 ± 0.1	8.1 ± 0.1*
TT	7.8 ± 0.2	8.3 ± 0.2*	II	7.7 ± 0.2	8 ± 0.2*
IVST (mm)					
MM/MT	8.3 ± 0.1	8.6 ± 0.1*	DD/DI	8.3 ± 0.1	8.8 ± 0.1*
TT	8.4 ± 0.2	9.2 ± 0.3*,**	II	8.3 ± 0.2	8.5 ± 0.2*
LVM (g)					
MM/MT	145 ± 4	155 ± 4*	DD/DI	145 ± 3	159.3 ± 4*
TT	144 ± 7	163 ± 8.6*	II	143 ± 8	149.5 ± 7.5*
LVMI (g/m^2)					
MM/MT	76 ± 2	81 ± 2*	DD/DI	76 ± 2	83.5 ± 2*
TT	76.5 ± 3.3	86.7 ± 4.2*,**	II	77.7 ± 4.4	81.5 ± 4.3*

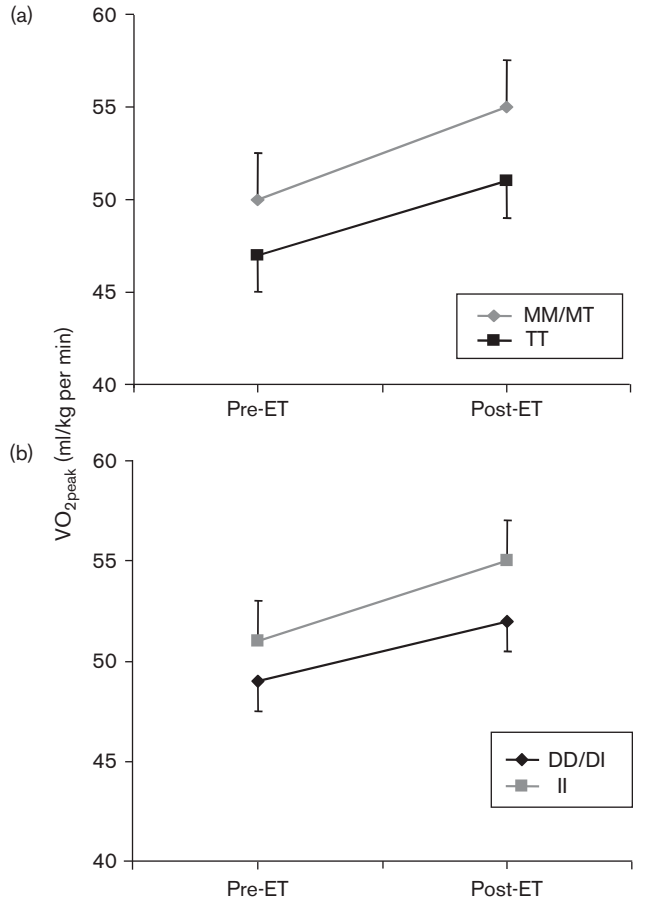
BMI, body mass index; HR, heart rate; IVST, intraventricular septum thickness; LVEDD, left-ventricular end-diastolic diameter; LVESD, left-ventricular end-systolic diameter; LVM, left-ventricular mass; LVMI, left-ventricular mass index; M, methionine; MAP, mean arterial pressure; PWT, posterior wall thickness; T, threonine; VO_{2peak}, peak oxygen consumption. *Within-group difference, $P<0.05$. **Between-group difference, $P<0.05$.

The ET caused no significant changes in left-ventricular end-systolic diameter, left-ventricular end-diastolic diameter (Table 1), auricular diameter, and left-ventricular ejection fraction (not shown) in individuals homozygous for the T allele and MM/MT or for individuals of II and DD/DI groups. The ET significantly increased the posterior wall thickness, IVST, LVM, and LVMI in individuals homozygous for the TT and MM/MT groups (Fig. 2a) and for individuals of the II and DD/DI groups (Fig. 2b). The comparisons between groups showed that the ET caused a more pronounced increase in IVST and LVMI in individuals homozygous for the T allele than in individuals of the MM/MT group (Fig. 2a) but, in contrast, these changes were similar in individuals of II and DD/DI groups.

Discussion

The main findings of this study are: (i) left-ventricular hypertrophy caused by the ET is greater in individuals with the TT genotype than in individuals with the MM/MT genotypes for the position 235 of the angiotensinogen gene; (ii) the improvement in VO_{2peak} after the ET is

Fig. 1



Absolute change in peak oxygen consumption (VO_{2peak}) provoked by exercise training (ET) (pre-ET and post-ET) in individuals with MM/MT and TT polymorphism (a) and in individuals with DD/DI and II polymorphism (b). D, deletion; I, insertion; M, methionine; T, threonine.

similar in individuals carriers of the TT and MM/MT genotypes of the angiotensinogen gene; (iii) the increase in LVM and the improvement in VO_{2peak} after the ET occurs in the II and DD/ID genotypes of the ACE gene.

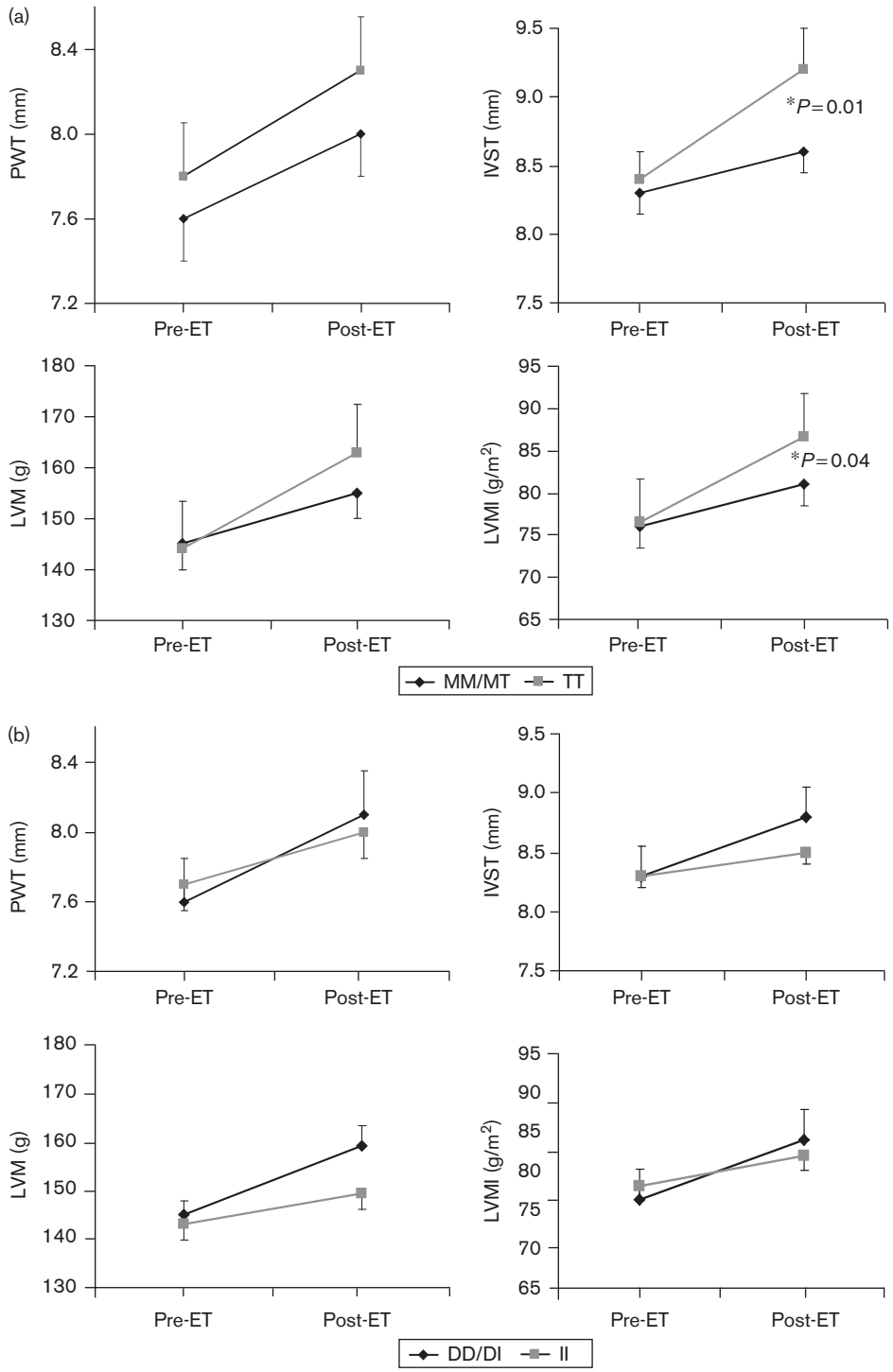
Earlier studies showed that individuals carrying the allele T of the angiotensinogen express more angiotensin II in the final cascade of the renin-angiotensin system [16]. As angiotensin II is associated with myocyte hypertrophy, we could expect that individuals carrying the TT polymorphism would have greater cardiac mass than the one carrying the MM polymorphism. This hypothesis was not confirmed in the Evangelista and Krieger's study with mice [23] and in this study. We found no differences in LVM between individuals who were homozygous for the T allele of the angiotensinogen when compared with individuals who were homozygous for the M allele and heterozygous. However, when individuals carrying TT allele form were exposed to the ET, this phenotype was intensively expressed, but with a low-statistic power. Thus, our study extends the concept that individuals carrying TT form of the angiotensinogen have augmented susceptibility to cardiac hypertrophy when submitted to moderate/vigorous ET. In an associative study, Karjalainen *et al*. [17] showed that athletes carrying TT form of the angiotensinogen had greater myocardial hypertrophy than athletes carrying MM form of the angiotensinogen.

Our study shows that the increase in VO_{2peak} was similar in individuals homozygous for the T allele and individuals homozygous for the M allele and heterozygous. This observation shows that the naturally occurring polymorphism of the angiotensinogen caused by the mutation encoding for amino acid M235T does not influence the aerobic power.

This study does not confirm earlier observations [24,25] that LVM is augmented in individuals carrying the D allele of ACE gene. In fact, LVM was similar between individuals carrying II and DD/DI form of ACE gene. Moreover, our study shows that the left-ventricular hypertrophy caused by long-term exercise is not different in individuals carrying DD/DI and II genotypes. These findings contrast with earlier reports that showed greater LVM in athletes carrying DD form [8,9,26]. It is very likely that this controversy is because of the sampling and methodology involved across studies. Most of those studies were limited to the association of ACE genotype and cardiac morphology in highly selected athletes. In addition, not all athletes practiced the same sport and it has been well documented that the training paradigm plays a crucial role in the gain of cardiac mass [27,28]. The ET based on prolonged moderate/vigorous exercise provokes concentric and eccentric cardiac hypertrophy [29,30], whereas the ET based on strengthening exercises provokes primarily concentric cardiac hypertrophy [23,31]. Montgomery *et al.* [10] reported that 10 weeks of aerobic training caused greater cardiac hypertrophy in Caucasian military recruits carrying the DD form of ACE gene than in those carrying the II form of the ACE gene. The ethnic and geographic diversity was limited in this study, which might have confounded the actual effect of the ACE genotype on the gain of LVM. We studied healthy males from different ethnic backgrounds aged between 20 and 35 years. The effectiveness of the ET in Montgomery's study was uncertain, as there was no change in resting heart rate after the ET. In this study, we found that VO_{2peak} was increased and resting heart rate was reduced, which prove the effectiveness of our exercise strategy.

The association of the I allele of the ACE and aerobic power has been claimed by some investigators [12,32,33]. In this study, as well as in other [25], no association between this genotype and VO_{2peak} was observed. Notably, the effect of ACE genotype on aerobic capacity

Fig. 2



Absolute change in left-ventricular parameters provoked by exercise training (ET) (pre-ET and post-ET) in individuals with MM/MT and TT polymorphism (a) and in individuals with DD/DI and II polymorphism (b). D, deletion; I, insertion; IVST, intraventricular wall thickness; LVM, left-ventricular mass; LVMI, left ventricular mass index; M, methionine; PWT, posterior wall thickness; T, threonine. *Between-group difference, $P<0.05$.

has been found in homogeneous populations, which may not be representative of the whole population. In addition, the genetic distribution could have been influenced by different ethnic features. This bias may result in the overestimation of the association between ACE genotype and aerobic capacity.

Limitations

We recognized some limitations in our study. Considering the fact that we have studied individuals of different ethnic background, our findings may be restricted to our populations. However, there is growing evidence that the expression of a phenotype does not depend on a specific genotype, but on the combination of two or more genotypes. Thus, the combined effects of ACE and angiotensinogen polymorphisms would likely improve the extension of our observational study. As our study sample included only men, sex may limit the generalization of our findings. Our ET consisted mostly of jogging (40–60 min). This study has low-statistic power to identify light effects of polymorphisms in phenotypes. In conclusion, the present findings improve our understanding with regard to the angiotensinogen and ACE genotypes regulation in the ventricular hypertrophy and aerobic power in humans. They show an influence of angiotensinogen genotype in the cardiac hypertrophy caused by long-term physical exercise in humans.

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