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Influence of the I/D Polymorphism of the Angiotensin Converting Enzyme Gene and Acute Aerobic Exercise in the Ambulatory Arterial Stiffness Index of Elderly Women

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ABSTRACT

Coelho JM, Sales MM, Moraes JFVN, Asano RY, Neto WB, Santana HAP, Silva CB, Moreira SR, Simões HG, Lima RM, Campbell CSG. Influence of the I/D Polymorphism of the Angiotensin Converting Enzyme Gene and Acute Aerobic Exercise in the Ambulatory Arterial Stiffness Index of Elderly Women. **JEPonline** *2*011;14(5):1-9. Acute bouts of exercise can reduce arterial stiffness in elderly, but the effect of exercise seems to be influenced by genetic factors. The aim of this study was to verify the influence of the angiotensin converting enzyme (ACE) gene's insertion/deletion polymorphism and acute aerobic exercise in the ambulatory arterial stiffness index (AASI) of elderly women. Twenty-five elderly women (70.9 ± 6.1 yrs; 25.2 ± 2.7 kg/m^2), previously genotyped for the ACE gene's I/D polymorphism, participated in this study. The volunteers were submitted to an incremental test to identify anaerobic threshold (AT). Afterwards, they underwent two sessions: a 90% AT Session and a Control Session. The AASI was measured during the 24 hrs after the sessions. In conclusion, exercise performed at 90% AT reduced arterial stiffness of the elderly subjects', especially carriers of the D/D ACE gene genotype.

Key Words: Exercise, Polymorphism, Hypotension, Elderly

INTRODUCTION

An increase in blood pressure (BP) associated with aging predisposes large arteries to increased arterial stiffness, which results in changes in the extracellular matrix of the arterial wall and also in its pathological hypertrophy (7). The increase in the mechanical stress promoted by high BP is one of the main determinants of the stiffness of the arterial wall (10,17,26). The increase in arterial stiffness leads to systemic arterial hypertension (SAH) that hastens the vascular aging process (8).

On the other hand, regular exercise helps to prevent arterial stiffness and, therefore, as a non-pharmacological treatment, helps to slow the vascular aging process. In fact, studies show that short periods of training or even acute bouts of exercise can reduce BP (17) and arterial stiffness (25,26). However, the hemodynamic response to exercise (29) varies from one person to the next. This suggests that the effects of exercise may be mediated partially by genetic variations. The study of "candidate genes" to certain phenotypic responses is one of the strategies used to verify possible associations among genetic variants and the effect of exercise (1,18).

The above mentioned studies have investigated the genes from the angiotensin-renin-aldosterone system (ARAS), which seem to play an important role in BP regulation. One of the intermediaries of the ARAS is the angiotensin converting enzyme (ACE). This enzyme presents an important physiological influence in BP homeostasis. The gene that expresses ACE, located at chromosome 16 of the human genome is considered a polymorphism. The scientific literature has distinguished it by the insertion (I) or deletion (D) of 287 base pairs in the DNA sequence, thus categorizing the individuals as homozygous for deletion (D/D genotype), homozygous for insertion (I/I genotype), and heterozygous (I/D genotype) (22).

The conformation of the different genotypes results in distinct serum levels of ACE, with the D/D genotype having almost twice the concentration of ACE when compared to the I/I genotype, and the I/D genotype (9,11,22). Although some controversy exists, most of the literature demonstrates an association between the D/D genotype and the cardiovascular risk factors (2,13). Even though individuals respond differently to exercise, which may be explained by genetic variants, studies indicate that elderly people of both sexes who exercise on a regular basis present lower levels of arterial stiffness or even absence of stiffness associated to aging when compared to sedentary pairs. In other words, aerobic fitness is inversely associated with arterial stiffness (24,26).

In addition, longitudinal studies have confirmed the beneficial effects of regular exercise on arterial stiffness, where relatively short periods of aerobic training (e.g., 2 to 6 months) have demonstrated an improvement in arterial compliance in individuals of different ages (5,12,27). Therefore, we hypothesized that the ACE gene polymorphism and the acute effects of aerobic exercise may influence the arterial stiffness in elderly individuals. Furthermore, no study has investigated the influence of the ACE gene polymorphism and acute aerobic exercise on arterial stiffness in elderly women. The aim of this study was to determine the influence of the ACE gene's insertion/deletion polymorphism and acute aerobic exercise in elderly women, using the ambulatory arterial stiffness index (AASI).

METHODS

Subjects

The study was conducted at the Physical Training and Evaluation Laboratory of the Catholic University of Brasília. It was approved by the local ethics committee (nº63/2008). Twenty-five elderly women (70.9 ± 6.1 yrs; 25.2 ± 2.7 kg/m^2), who were previously genotyped for the ACE gene's I/D

polymorphism, answered a health questionnaire and, then, signed an informed consent in which they agreed to participate in the study.

Procedures
The subjects submitted to an incremental test to identify the anaerobic threshold (AT). They also underwent two sessions: a 90% AT Session and a Control Session. Both sessions were carried out at the same time of day and in randomized order.

Incremental Test (IT)
The IT was performed on a cycle ergometer (Lode, model Excalibur, Netherlands) with 15 watts of initial workload followed by increments of 15 watts at each 3-min stage until volitional exhaustion. All subjects maintained 60 revolutions per minute.

During the last 20 sec of each incremental stage, a 25 µL sample of capillarized blood was collected from the ear lobe through heparinized and calibrated glass capillaries, being deposited in microtubes (Eppendorf) containing 50 µL of sodium fluoride (NaF) 1% for later analysis of the blood lactate concentrations through an electroenzimatic method (Yellow Springs 2.700 STAT, OH, USA). Furthermore, gas analysis was used to determine ventilatory parameters (Cortex Biophysik model Metalyzer 3B, Germany). Rate of perceived exertion (RPE) (Borg scale ranged from 6 to 20) and heart rate (HR) (Polar Electro Oy, model S-810, Finland) were also measured until the volitional exhaustion of the subjects.

Anaerobic Threshold Determination (AT)
Expired gases were collected breath by breath, and the data from the last 20 sec were used for analysis. Ventilatory threshold was determined through the analysis of the ventilatory equivalents of O_2 (V_E/VO_2) and CO_2 (V_E/VCO_2). Threshold was considered the intensity that corresponded to the moment V_E/VO_2 presented an over proportional increase in relation to V_E/VCO_2 (25).

The blood lactate kinetics during the IT stages identified the lactate threshold considered as an exercise intensity above which an over proportional increase in blood lactate was observed when compared to the increase in workload (29). The AT was calculated by the mean of intensity found in both the ventilatory and lactate thresholds.

90% Anaerobic Threshold Session (90%AT)
The 90%AT session was performed on a cycle ergometer (Lode, model Excalibur, Netherlands) during 20 min at an intensity that corresponded to 90%AT, which was previously determined in the IT.

Control Session (CONT)
The CONT session was carried out according to the same procedures used in the 90%AT session, but without performing exercise. The volunteers remained seated for 20 min in a comfortable position.

Ambulatory Arterial Stiffness Index (AASI)
The AASI was calculated from the difference between 1 and the regression slope of DBP on SPB during the 24 hrs monitoring of blood pressure (28) (Figure 1). Desirable values for AASI are <0.50 for youths and <0.70 for the elderly (28).

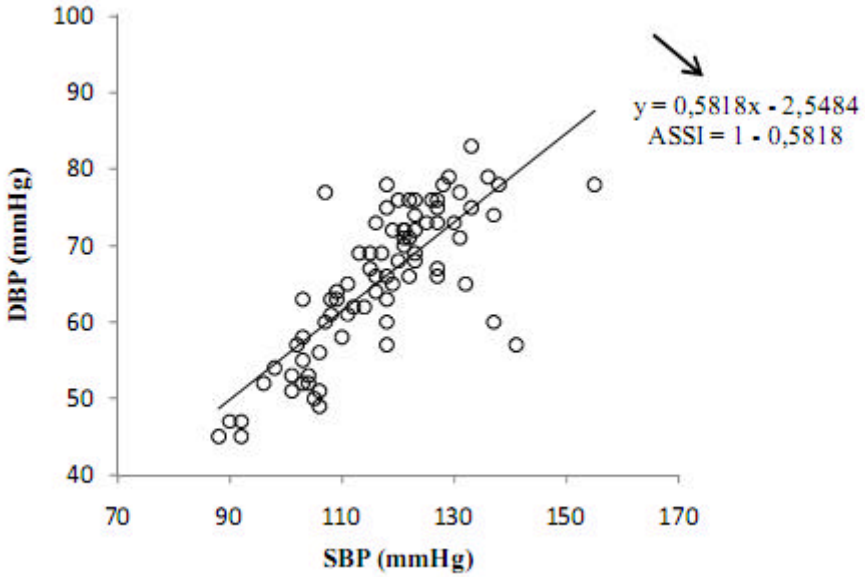

Figure 1. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) values of one of the volunteers during the 24 hrs of blood pressure monitoring after the 90%AT session. The arrow (→) points to the regression slope value.

DNA Extraction and Genotyping of the I/D polymorphism of the ACE gene

Total DNA was isolated from the peripheral blood according to standard procedures (16). The insertion(I) / deletion(D) polymorphism in the human ACE gene (rs4646994) was determined by inspection of the electrophoretic profile of polymerase chain-reaction (PCR) products, which was performed as described by Marre et al. (15) with modifications.

Either the 490 bp (I allele) or the 190 bp (D allele) products were amplified using primers: 5'-CTGCAGACCACTCCCATCCTTTCT-3' and 5'-GATGTGGCCATCACATTCGTCAGAT-3', which flank the polymorphic site. Reaction tubes contained 100 ng of DNA, 10 mmol/L Tris–HCl pH8.3, 75 mmol/L KCl, 3.5 mmol/L MgCl2, 0.2 mmol/L dNTP, 20 pmol of each primer, 0.5 µg of purified chicken albumin and 1U of Taq DNA polymerase (Phoneutria®, Minas Gerais, Brazil) in a final volume of 25 µL. After 1 min of heating at 80°C and an initial denaturation for 2 min at 94°C, the amplifications were performed by 30 cycles of 40 sec at 94°C, 45 sec at 64°C and 50 sec at 72°C followed by a final 5 min extension at 72°C. Inspection of DD subjects was carried out using specific oligonucleotides (5'-TGGGACCACAGCGCCCGCCACTAC-3' and 5'-TCGCCAGCCCTCCCATGCCCATAA-3') to amplify a 335 bp fragment of the insertion sequence. Afterwards, DNA was amplified for 30 cycles with denaturation at 92°C for 40 sec, annealing at 63°C for 40 sec, and extension at 72°C for 40 sec.

All PCR products were separated by electrophoresis on 2% agarose gels containing ethidium bromide at 50 µg/mL, visualized by using CCD camera (Vilber Lourmat®, Eberhardzell, Germany), examined by using the gel analysis software enclosed (Photo Capt 1D), and confirmed by visual inspection.

Statistical Analyses

Results are shown using descriptive statistics (mean ± standard deviation). Student's t-test for independent samples was used to compare resting values between the groups. After evaluating for normality though skewness and kurtosis, Analysis of Variance Split-Plot (SPANOVA) with Bonferroni test as *post hoc* was performed to compare AASI between and within the groups. Gpower 3.0.10 software was used to determine that the sample size of 25 subjects was the minimum necessary to

provide a statistical power of 80%, with an alpha level of 5% (P<0.05) for the analysis. The level of significance adopted was 5% (P<0.05). The software used was the SPSS v15.0 (SPSS Inc. USA).

RESULTS

Table 1 shows the general characteristics of the subjects divided by groups (D/D and I/I+I/D).

Table 1. D/D group (n = 10), I/I+I/D group (n = 15). Values shown in mean ± standard deviation.

	D/D	I/I+I/D
Age (yrs)	70.6 ± 5.8	71.1 ± 6.5
BM (kg)	60.0 ± 9.1	59.1 ± 6.7
Height (cm)	153.7 ± 4.7	153.5 ± 6.9
BMI (kg/m^2)	25.4 ± 3.2	25.1 ± 2.4
SBP_{rest} (mmHg)	118.4 ± 7.0	118.6 ± 16.0
DBP_{rest} (mmHg)	73.0 ± 6.7	72.4 ± 8.4
VO_2 90%AT ($ml \cdot kg^{-1} \cdot min^{-1}$)	15.8 ± 3.3	14.9 ± 2.7
VO_2 max (%)	78.6 ± 11.3	73.0 ± 8.1
P 90%AT (watts)	35.6 ± 13.3	33.8 ± 14.7
P max (%)	61.4 ± 7.3*	53.8 ± 10
[Lac] 90%AT ($mmol \cdot L^{-1}$)	3.3 ± 1.2	2.8 ± 1.2
RPE 90%AT (Borg)	13.8 ± 1.1	13.3 ± 1.7

BM = body mass; BMI = Body mass index; SBP_{rest} = systolic blood pressure at rest; DBP_{rest} = diastolic blood pressure at rest; VO_2 90%AT = oxygen uptake at 90% of anaerobic threshold; VO_2 max (%) = maximal oxygen uptake percentage in exercise performed at 90%AT; P 90%AT = workload performed at 90% of anaerobic threshold; P max (%) = maximal workload percentage in exercise performed at 90%AT; [Lac] 90%AT = lactate concentrations at 90% of anaerobic threshold; RPE 90%AT = rate of perceived exertion at 90% of anaerobic threshold. *P<0.05 to the I/I+I/D group.

The AASI values in the CONT and 90%AT sessions between the I/I+I/D and D/D groups are presented in Figure 2. Significantly lower values (P<0.05) were found in the 90%AT session when compared to CONT only in the D/D group.

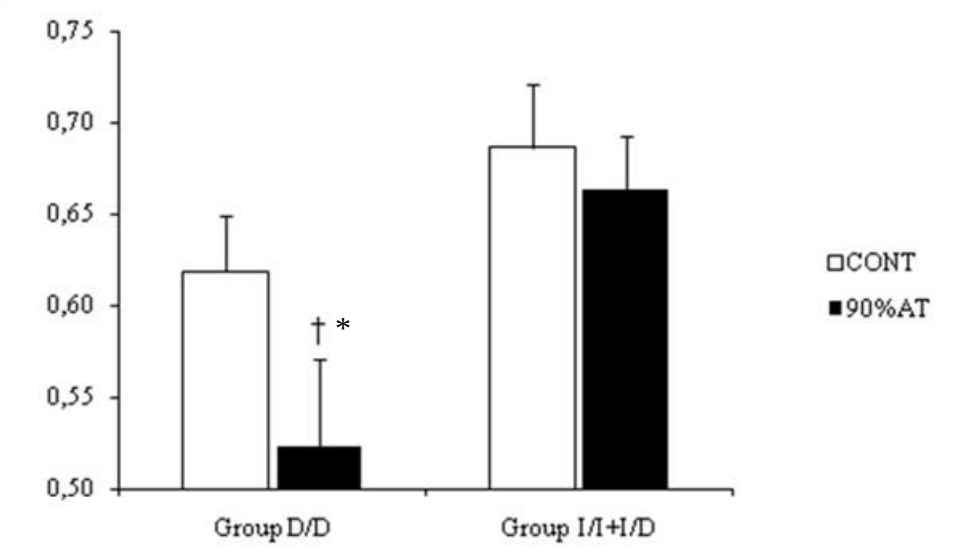

Figure 2. The Mean Ambulatory Arterial Stiffness Index (AASI) values for the D/D group (n = 10) and the I/I+I/D (n = 15) after 24 hrs of blood pressure monitoring subsequent to the aerobic exercise (90%AT) and the control (CONT) sessions. †P<0.05 to CONT in the same group; *P<0.05 to aerobic exercise (90%AT) Group I/I+I/D.

DISCUSSION

When compared to the CONT session, the D/D group presented lower AASI values ($P<0.05$) (Figure 2) after performing the exercise bout at 90% of anaerobic threshold (90%AT). Lima and colleagues (14) compared two exercise sessions at different intensities (90% and 110% of AT) in individuals with type 2 diabetes. They found that the exercise session performed at 110% of AT promoted a greater decrease in systolic and diastolic blood pressures; a fact that may reflect the stiffness of the arteries. Thus, we can infer that the D/D group showed lower values for AASI due to greater shear stress in the walls of arteries and, therefore, resulting in a higher release of substances that induced vasodilation after exercise (e.g., bradykinin and nitric oxide) (18,19,22).

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In accordance with our results, Tanaka and colleagues (24) showed that low to moderate aerobic physical exercise (60% to 75% of HR max) reduced in 20% to 30% the stiffness of large arteries in elderly trained individuals when compared to sedentary subjects of the same age. According to the authors, exercise has a direct influence on the arterial compliance, since the study found no other alterations in maximal aerobic capacity, body mass and serum cholesterol levels. In addition, regular exercise reduces the sympatho-adrenergic response by a direct mechanism or through the sympathetic-inhibitory effect of nitric oxide (20).

Bonithon-Kopp et al. (4) and Castellano et al. (6) observed an association between the thickness of the wall of the carotid artery with higher levels of ACE in the blood plasma and the presence of the D allele, suggesting an important influence of this allele in arterial stiffness. However, in the present study, although not reaching statistical significance, the results showed higher AASI values in the CONT session for the I/I+I/D group when compared to the D/D group. Since the AASI represents an estimate of the index of arterial stiffness, this means that in a normal day situation, without physical activity, the I allele carriers, defined in the present study by the I/I+I/D group, seem to have a higher arterial stiffness when compared to the D allele carriers (Figure 2). These findings are also in agreement with Benetos and colleagues (3), which demonstrated an association with arterial stiffness and the presence of the I allele.

Likewise, the present study also showed a better benefit derived from exercise in the D/D group. When comparing the 90%AT with the CONT session (in the D/D group), there was a significant decrease in AASI ($P<0.05$) (Figure 2). With the purpose of explaining the D/D genotype carriers' significant decrease in arterial stiffness, it is possible to suggest that exercise at an intensity of 90%AT was more intense for this group when compared to the I/I+I/D group. Although the relative intensity was the same, the maximal workload percentage (Pmax) in which the D/D group exercised was higher than when compared to the I allele carriers (D/D: 61.4 ± 7.3 vs. I/I+I/D: 53.8 ± 10.0; $P<0.05$). This could have generated a higher mechanical stress in the walls of the arteries in the D/D subjects; consequently, a higher release of substances that induce vasodilation (18-20) after exercise may have induced a lower arterial stiffness (Table 1).

CONCLUSION

Exercise performed at 90% of AT was sufficient to reduce arterial stiffness in elderly women subjects, especially carriers of the D/D ACE gene genotype.

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