

Lack of Association Between ACE Indel Polymorphism and Cardiorespiratory Fitness in Physically Active and Sedentary Young Women

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Background: Polymorphisms at the angiotensin-converting enzyme gene (ACE), such as the indel [rs1799752] variant in intron 16, have been shown to be associated with aerobic performance of athletes and non-athletes. However, the relationship between ACE indel polymorphism and cardiorespiratory fitness has not been always demonstrated.

Objectives: The relationship between ACE indel polymorphism and cardiorespiratory fitness was investigated in a sample of young Caucasian Brazilian women.

Patients and Methods: This study investigated 117 healthy women (aged 18 to 30 years) who were grouped as physically active (n = 59) or sedentary (n = 58). All subjects performed an incremental exercise test (ramp protocol) on a cycle-ergometer with 20-25 W/min increments. Blood samples were obtained for DNA extraction and to analyze metabolic and hormonal profiles. ACE indel polymorphism was determined by polymerase chain reaction (PCR) and fragment size analysis.

Results: The physically active group had higher values of peak oxygen uptake (VO₂ peak), carbon dioxide output (VCO₂), ventilation (VE) and power output than the sedentary group (P < 0.05) at the peak of the exercise test. However, heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) did not differ between groups. There was no relationship between ACE indel polymorphism and cardiorespiratory variables during the test in both the physically active and sedentary groups, even when the dominant (DD vs. DI + 2) and recessive (2 vs. DI + DD) models of inheritance were tested.

Conclusions: These results do not support the concept that the genetic variation at the ACE locus contributes to the cardiorespiratory responses at the peak of exercise test in physically active or sedentary healthy women. This indicates that other factors might mediate these responses, including the physical training level of the women.

Keywords: Exercise Test; Angiotensin-Converting Enzyme; Polymorphism, Genetic; Motor Activity

1. Background

Physical exercise requires the interaction of various physiological mechanisms that enable the cardio-respiratory system to support the increased energy demands of contracting muscles (1). Oxygen uptake (VO₂) is influenced by the interaction of several variables including environmental, morphophysiological, and genetic factors (2, 3). The most accurate measure of VO₂ involves a direct measurement of expired gases during a maximal cardiopulmonary exercise test (CPET). This parameter has been widely used for the measurement of cardiorespiratory performance, as a basis for prescribing training intensities, and as a prognostic tool for health status (4, 5). Angiotensin-converting enzyme (ACE) is a key enzyme within the renin-angiotensin system (RAS), where the conversion of angiotensin I (ANG I) produces angiotensin 2 (ANG 2), a vasoconstrictor. ACE is also associ-

ated with the degradation of other vasodilators, such as bradykinin. The RAS influences circulatory and local systems, including skeletal muscle. These roles of ACE may contribute to the improvement of metabolic efficiency and endurance performance (6, 7).

Polymorphisms at the ACE gene, such as the insertion/deletion (indel) variant of 287 bp in intron 16 (rs1799752), have been shown to be associated with ACE activity variations and with phenotypes of strength/power or endurance performance (8, 9). In this context, ACE genotype II is predominant in elite athletes that require high cardiorespiratory capacity, such as rowers (10, 11), long distance runners (12, 13), long distance swimmers (13), ironman triathletes (14) and climbers (15, 16). Individuals carrying genotype II are thus prone to having better cardiorespiratory parameters and consequently better endurance

performance. The peak or maximum oxygen uptake (VO_2 peak or max), a standard measure of cardiorespiratory system and aerobic metabolism; shows great variability in response to physical training and it was estimated that at least 50% are influenced by genetic factors (17). However, the influence of the ACE indel polymorphism on VO_2 peak or max values has not been consistently demonstrated. Hagberg et al. reported that, in postmenopausal women, ACE II genotype carriers presented higher VO_2 max values compared with those carrying the DD genotype (18, 19). On the other hand, other studies have failed to establish a positive relationship between the ACE indel variant and maximal aerobic power in young sedentary women (20), sedentary Caucasian and black subjects (21), army recruits (22), and sedentary and endurance-trained women (23).

2. Objectives

This study investigated the relationship between ACE indel polymorphisms and cardiorespiratory fitness in a sample of both physically active and sedentary Caucasian women from Brazil.

3. Patients and Methods

3.1. Subjects and Study Design

The sample size was calculated based on the frequency of the ACE II genotype described in a sample of the European population (18). Assuming a margin of error of 10% for the estimate with 0.80 of power in the test, and a significance level of 5%, a minimum number of 118 independent individuals were obtained. All participants completed a health questionnaire and signed an informed consent document after being informed about the research. The present study was approved by the Research Ethics Committee of the Methodist University of Piracicaba (Protocol n° 43/06) and is in accordance with the legal requirements of the Declaration of Helsinki.

A group of 117 healthy Caucasian women who were 18 to 30 years of age in the Brazilian cohort were selected and grouped as sedentary ($n = 58$) and physically active ($n = 59$). The physically active women performed continuous physical activity at least 3 days per week (for at least 6 months) at an intensity of 65-90% of maximum heart rate with durations of 30-60 minutes in accordance with the American College of Sports Medicine (24). The inclusion criteria for all subjects were: body mass index (BMI) between 19.0 and 24.9 kg/m^2 ; ovulation confirmed by serum progesterone above 4.0 mg/mL on the 21st day of the menstrual cycle.

The exclusion criteria included cardiovascular dysfunction (arrhythmia, myocardial ischemia), blood pressure (BP) alterations at rest, depression, gynecological, musculoskeletal or neurological diseases, hyperandrogenism, pregnancy, smoking, and the use of any type of medication that could interfere in the cardiorespiratory system.

3.2. Physical and Biochemical Assessment

All subjects underwent a clinical examination and an assessment was performed between the 7th and 10th day of the menstrual cycle, in consideration of the lower hormonal fluctuations during this phase (follicular phase). The exam included the use of a standard 12-lead electrocardiogram (ECG) at rest. Cardiac auscultation, heart rate (HR), and BP were evaluated after 5 minutes of rest in the supine and sitting positions. The BP was measured three times at rest by auscultation of Korotkoff sounds using a mercury-column sphygmomanometer (WanMed, Sao Paulo, SP, Brazil) and stethoscope (Littman, St. Paul, MN, USA).

For biochemical measurements, venous blood samples were drawn after 12 hours of an overnight fast. Serum concentrations of total cholesterol, triglycerides, urea, and creatinine were determined by enzymatic colorimetric assays (BioSystems, Barcelona, Spain). The low-density lipoprotein (LDL) was estimated by Friedewald's formula (25) (since triglycerides values were less than 400 mg/dL). All the volunteers were in good health and had biochemical parameters within normal ranges.

3.3. Evaluation of Cardiorespiratory Fitness

Experiments were always carried out between 2:00 to 4:00 p.m. to avoid different responses caused by circadian changes. Room temperature was kept at 23°C and relative air humidity between 40-60%. Subjects were acquainted with the experimental protocol and instructed to abstain from stimulants and alcoholic beverages (coffee, tea, soft drinks), avoid any exhausting physical activity during the 24 hours preceding the exam, and to ingest a light meal at least 2 hours before the measurements.

CPET was performed on a cycle ergometer with electromagnetic braking (Quinton Corival 400, Seattle, WA, USA) whose seat was adjusted to allow approximately 5 to 10 degrees of knee flexion. The protocol consisted of 1 minute pre-testing while seated in a resting position on the cycle ergometer followed by a 4 minute warm-up period at 4 W. The workload was then increased continuously by 20 to 25 W/minute until physical exhaustion, i.e. the moment at which the subject could no longer maintain 60 rpm or the occurrence of a limiting symptom or respiratory fatigue. Workload increments were determined for each subject according to the formula proposed by Wasserman et al. ($\text{Workload increase (W)} = [(\text{height-age}) \times 20] - [150 + (6 \times \text{body mass})]/100$ (1).

During the CPET, ventilatory and metabolic variables, such as the relative oxygen uptake (VO_2 $\text{mL}/\text{kg}/\text{minute}$), carbon dioxide output (VCO_2), and ventilation (VE) were obtained on a breath-by-breath basis using a specific metabolic analyzer (CPX/D MedGraphics Breeze, St. Paul, MN, USA). These variables were subsequently processed and calculated as moving means after every eight respiratory cycles for a better kinetic observation during the exercise testing. The highest value of oxygen consumption achieved during the CPET was considered VO_2 peak.

The standard criteria of exhaustion were verified by at least three conditions including: A) plateau in oxygen consumption (change < 2 mL/kg/minute); B) a respiratory exchange ratio (RER) ≥ 1.1 ; C) ≤ 10 beats/min of age predicted maximum (220-years of age) and d) rating of perceived exertion (RPE) ≥ 18 (26).

We also determined HR (Polar, Vantage NV, Finland), systolic blood pressure (SBP), and diastolic blood pressure (DBP), which were determined at the highest values of these variables achieved during the maximal CPET.

3.4. ACE Indel Polymorphism Analysis

DNA was isolated from white blood cells EDTA-treated, anti-coagulated blood using a standard protocol as described by Salazar et al. (27) The ACE indel (rs1799752) polymorphism was analyzed by polymerase chain reaction (PCR) and fragment analysis as previously described (28). Sequences of PCR primers were: sense 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and anti-sense 5'-GAT GTG GCC ATC ACA TTC AGA T-3'. The PCR assays were carried out in a thermocycler (T-Gradient, Whatman Biometra, Goettingen, Germany) under the following conditions: one cycle at 95°C for 5 min; 35 cycles at 95°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute and one cycle at 72°C for 10 minutes. ACE indel fragments were detected by a 1.5% agarose gel electrophoresis. To avoid a misclassification of ID heterozygotes as DD homozygotes, a second PCR assay was performed in all of the samples initially classified as DD with the insertion-specific primers: sense 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and anti-sense 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3' (29). The PCR conditions were similar as previously stated except for the annealing temperature (64°C). Only I allele generates a 335 bp amplicon

that is also detected by a 1.5% agarose gel electrophoresis.

Genotyping quality control was performed with all genotypes determined by two independent technicians with the results entered into the database in duplicate. Additionally, 10% of the samples were randomly reanalyzed.

3.5. Statistical Methods

The allele and genotype frequencies were estimated by counting. The agreement of genotype frequencies with the Hardy-Weinberg equilibrium expectations was tested using the chi-square test. The SPSS statistical program version 13 was used to compare categorical variables among groups and adjusted residuals. Comparisons of mean values of quantitative variables were carried out by independent t-tests and ANOVA by ranks with Tukey's post hoc test correction for multiple comparisons. Significance was considered at $P < 0.05$.

4. Results

Physical and anthropometric variables of the subjects are presented in Table 1. Age, weight, height, and BMI mean values did not differ significantly between the sedentary and physically active groups ($P > 0.05$). Supine and sitting HR was higher in the sedentary group than in the physically active group ($P = 0.01$), while SBP, and DBP did not differ between both groups ($P > 0.05$). Analysis of biochemical variables showed that total cholesterol, LDL cholesterol, and triglyceride levels in serum were higher in the sedentary group than in the physically active group ($P < 0.05$). Other variables, such as fasting glucose, urea, creatinine, HDL cholesterol, progesterone, and estradiol were not statistically different between the sedentary and physically active groups ($P > 0.05$).

Table 1. Anthropometric, Clinical Data and Biochemical Variables in Sedentary and Physically Active Women ^a

Variables	Sedentary (n = 58)	Physically Active (n = 59)	P Value ^b
Age, y	23.7 ± 4.5	22.1 ± 4.4	0.07
Weight, kg	59.5 ± 7.0	58.7 ± 7.2	0.52
Height, cm	164.7 ± 7.0	165.5 ± 6.7	0.51
Body mass index, kg/m ²	21.9 ± 2.0	21.5 ± 1.8	0.24
Supine heart rate, bpm	68.3 ± 8.8	64.3 ± 9.9	0.02
Sitting heart rate, bpm	74.7 ± 9.6	69.6 ± 10.4	0.01
Systolic blood pressure, mmHg	110.6 ± 9.4	110.4 ± 7.8	0.88
Diastolic blood pressure, mmHg	71.6 ± 7.9	74 ± 6.8	0.09
Fasting glucose, mg/dL	72 ± 7.0	70 ± 5.0	0.88
Urea, mg/dL	22 ± 5.0	24 ± 4.0	0.75
Creatinine, mg/dL	0.6 ± 0.3	0.7 ± 0.3	0.65
Total cholesterol, mg/dL	161 ± 29.0	175 ± 26.0	0.03
Low-density lipoprotein cholesterol, mg/dL	109 ± 24.0	75 ± 23.0	0.01
High-density lipoprotein cholesterol, mg/dL	41 ± 8.0	53 ± 16.0	0.09
Triglycerides, mg/dL	92 ± 30.0	77 ± 15.0	0.04
Progesterone, ng/mL	1.8 ± 6.0	1.6 ± 5.0	0.33
Estradiol, pg/mL	102 ± 57.0	92 ± 25.0	0.21

^a Data are presented as mean ± SD.

^b Compared by independent t-test.

Genotype distribution of ACE indel polymorphism was within the expectations of the Hardy-Weinberg equilibrium for both the physically active group ($II = 0.413$, $P = 0.5$) and the sedentary state group ($II = 0.004$, $P = 0.9$). The ACE indel genotype and allele frequencies did not differ between the physically active and the sedentary state groups ($P > 0.05$; Table 2).

4.1. Aerobic Function Capacity During the Cardiopulmonary Exercise Test

Table 3 summarizes aerobic function data including the ventilatory response at the peak of the CPET, according to the genotypes of ACE indel polymorphism using co-dominant, dominant and recessive models of inheri-

tance. VO_2 peak, VCO_2 , VE, power output, HR, SBP, and DBP mean values were not different among the ACE genotypes in the sedentary and active groups ($P > 0.05$). There was also no relationship between cardiorespiratory variables during the test ($P > 0.05$) using dominant (DD vs. DI + II) or recessive (II vs. DD + DI) models of inheritance. These results therefore revealed that, independent of the ACE genotype carried, physically active women had higher VO_2 peak, VCO_2 , VE, and power output mean values than the sedentary group had at the peak of the exercise test ($P < 0.05$). Moreover, HR, SBP and DBP did not show significant differences ($P > 0.05$) at the peak of the exercise test. The ACE indel polymorphism had no effect ($P > 0.05$) on the variables VO_2 peak, VCO_2 , VE, power output, HR, SBP, and DBP in the data from total sample (data not shown).

Table 2. Frequencies of ACE Indel Polymorphism in Sedentary and Physically Active Women ^a

	Active (n = 59)	Sedentary (n = 58)	P Value
Genotypes			0.2
DD	17 (28.8)	21 (36.2)	
DI	27 (45.8)	28 (48.3)	
II	15 (25.4)	9 (15.5)	
Alleles			0.2
D	30 (50.9)	35 (60.3)	
I	29 (49.1)	23 (39.7)	

^a Data are presented as No. (%).

Table 3. Dependent Variables for Cardiorespiratory Responses in Physically Active and Sedentary (DD, DI, II Genotypes) Groups During the Cardiopulmonary Test ^{a, b, c}

	VO_2 , mL/kg/ min	VO_2 , mL/kg/ min	Ventilation, L/min	Power, Watts	Heart Rate, bpm	SBP, mmHg	DBP, mmHg
Physically active							
DD	31.0 ± 3.9 ^d	2.1 ± 0.2 ^d	60.6 ± 9.8 ^d	168.5 ± 26.4 ^d	180.1 ± 9.2	177.1 ± 2.8	79.3 ± 5.5
DI	32.6 ± 4.0 ^e	2.1 ± 0.3 ^e	60.9 ± 12.3 ^e	168.6 ± 24.9 ^e	177.5 ± 12.4	179.5 ± 2.4	78.1 ± 4.8
II	33.7 ± 1.9 ^f	2.3 ± 0.3 ^f	66.4 ± 10.1 ^f	173.2 ± 25.7 ^f	180.0 ± 11.5	178.0 ± 1.5	77.8 ± 3.2
P Value							
C	0.2	0.4	0.9	0.1	0.1	0.9	0.8
D	0.9	0.1	0.7	0.3	0.3	0.2	0.8
R	0.5	0.7	0.6	0.7	0.6	1	0.4
Sedentary							
DD	24.6 ± 2.4	1.7 ± 0.2	51.1 ± 10.3	132.9 ± 18.1	179.4 ± 12.3	179.5 ± 1.4	80.1 ± 3.4
DI	23.9 ± 3.1	1.6 ± 0.2	50.3 ± 12.3	130.5 ± 17.7	175.4 ± 9.8	179.4 ± 2.5	82.6 ± 2.7
II	29.2 ± 5.9	1.8 ± 0.4	51.4 ± 15.7	134.6 ± 18.8	177.8 ± 20.2	177.1 ± 3.5	79.0 ± 3.3
P Value ^d							
C	0.07	0.4	0.2	0.1	0.2	0.7	0.7
D	0.08	0.7	0.6	0.2	0.7	0.5	0.5
R	0.09	0.7	0.6	0.3	1	0.7	0.6

^a Abbreviations: C, co-dominant model (DD vs. DI vs. II); D, dominant model (DD vs. DI + II); DBP, diastolic blood pressure; R, recessive model (II vs. DD + DI); SBP, systolic blood pressure; VO_2 , oxygen uptake; VCO_2 , carbon dioxide output.

^b Data are presented as mean ± SD.

^c Values were compared by ANOVA (co-dominant model) and independent t-test (dominant and recessive models).

^d Significantly different from genotype DD sedentary group ($P < 0.05$).

^e Significantly different from genotype DI sedentary group ($P < 0.05$).

^f Significantly different from genotype II sedentary group ($P < 0.05$).

5. Discussion

In the present study, the sedentary group was classified as having low cardiorespiratory fitness according to the American Heart Association (30), and the physically active group was classified as having regular cardiorespiratory fitness. Additionally, total cholesterol, LDL cholesterol, and plasma triglycerides concentrations were significantly lower in the physically active group. These data reinforce the importance of physical activity for the maintenance of cardiorespiratory fitness and metabolic status (24). No significant difference was found in anthropometric variables, blood pressure, hormonal profiles, and renal function (creatinine, urea), which show the homogeneity of the sample. The main finding of this study was no association of ACE variant with VO_2 peak, VCO_2 , VE and power output at the maximal CPET in the sample of healthy women, independent of the physical activity status. According to the population studied, our data show that the level of aerobic physical activity is associated with improvements in cardiorespiratory fitness. However, the improvements were not enough to show a positive interaction with the polymorphism, and were independent of the physical activity status in the population studied. The frequencies of the ACE D allele in the physically active group (51.7%) and sedentary group (60.3%) were similar to those previously reported in sample populations in North American (18), European (20), Australian (31), and Brazilian (32). The similarity of D allele frequencies between physically active and sedentary women is suggestive that this variant is not associated with the physical activity status in young women. Aerobic physical activity is responsible for several physiological adaptations in the cardiorespiratory system and skeletal muscle that lead to increased maximal aerobic power and endurance performance (33, 34). Likewise, Rankinen et al. evaluated a cohort of sedentary Caucasian families ($n = 476$) and sedentary Africans ($n = 248$) before and after 20 weeks of an aerobic training program performed on a cycle ergometer (21). The results demonstrated no effects of ACE indel polymorphism on VO_2 max values before and after the physical training program. Day et al. also did not detect a significant association between the ACE indel polymorphism and VO_2 max and the mechanical efficiency of muscle contractions during a cycle ergometry exercise test in sedentary women (20). Plasma ACE activity was also not related to the maximal aerobic power or mechanical efficiency (20). In accordance with Rankinen et al. (21) and Day et al. (20), our data demonstrated no sufficient influence of a single polymorphism (rs1799752) on the phenotype of cardiorespiratory performance of physically active and sedentary young women. This leads to the discussion concerning the analysis of interaction between two or more gene polymorphisms on human performance (35) or other regulatory mechanisms such as epigenetics (36). Alternatively, our data disagree with Hagberg et

al. who evaluated the cardiorespiratory fitness in postmenopausal women (sedentary, physically active, and athlete groups) and observed that women carrying the II genotype had higher VO_2 max values and estimated maximal arteriovenous O_2 difference (a-v Do_2) (18, 19). This suggests an increase in regulation of peripheral vascular tone with increased capillary perfusion and red cell transit time when compared with DI and DD genotypes. However, there were no differences in the variables of maximal stroke volume and maximal cardiac output index.

The physiological mechanism by which ACE indel polymorphism may modulate cardiorespiratory fitness would be that carriers of the II genotype have low plasma ACE activity (28), reducing the conversion rate of angiotensin I to angiotensin 2 (a vasoconstrictor), and decreasing the degradation of vasodilators such as bradykinin (8). Against the evidence that the II genotype is associated with better cardiorespiratory fitness, Zhao et al. investigated the relationship of the ACE indel polymorphism in 67 young Chinese men (37). In this study he found that the VO_2 max values were significantly higher for the DD genotype when compared to other genotypes (I/D and 2), contradicting previous studies (18, 19). These contradictory results must be explained due to the heterogeneity of genetic variants of the population involved in the studies and the aerobic training status. Thus, most studies that have found positive effects of ACE indel polymorphism on maximal aerobic power have typically been conducted in homogeneous ethnic populations and training status. This could overestimate the effect of a single polymorphism in physical performance, as observed in studies conducted with elite endurance athletes (12, 13, 16).

In physically active women, the level of trainability was not large enough to promote significant cardiovascular and muscular adaptations, as observed by the VO_2 peak values, when compared with studies that evaluated athletes (38, 39). Therefore, our data indicate that in sedentary or physically active women with low and moderate levels of trainability, a single ACE polymorphism does not significantly affect cardiorespiratory performance, since the central and peripheral factors are limiting the VO_2 peak values in the maximal cardiopulmonary test (2).

Our study has some limitations that need to be addressed. Firstly, the small sample size studied (both physically active and sedentary Brazilian women) could never adequately represent the global population. Secondly, we did not evaluate systemic plasma ACE activity, a factor that could elucidate the relationship between ACE indel genotypes and cardiorespiratory response during a physical test. These results do not support the concept that the genetic variation at the ACE locus contributes to the cardiorespiratory responses at the peak of exercise test in physically active or sedentary healthy women. This indicates that other factors might mediate these responses, including the physical training level of the women.

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Authors' Contributions

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