The Influence of ACE Genotype on Cardiorespiratory Fitness of Moderately Active Young Men

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Abstract

Background: The angiotensin I-converting enzyme gene (ACE gene) has been broadly studied as for cardiorespiratory fitness phenotypes, but the association of the ACE genotype to middle-distance running has been poorly investigated.

Objective: This study investigated the possible influence of Angiotensin-Converting Enzyme (ACE) genotype (I/D) on cardiovascular fitness and middle-distance running performance of Brazilian young males. The validity of VO_{2max} prediction with regard to the ACE genotype was also analyzed.

Methods: A homogeneous group of moderately active young males were evaluated in a 1,600 m running track test (V1600m; $m.min^{-1}$) and in an incremental treadmill test for VO_{2max} determination. Subsequently, the actual and the predicted [(0.177*V1600m) + 8.101] VO_{2max} were compared to ACE genotypes.

Results: The VO_{2max} and V1600m recorded for DD, ID and II genotypes were 45.6 (1.8); 51.9 (0.8) and 54.4 (1.0) $mL.kg^{-1}.min^{-1}$ and 211.2 (8.3); 249.1 (4.3) and 258.6 (5.4) $m.min^{-1}$ respectively, and were significantly lower for DD carriers ($p < 0.05$). The actual and predicted VO_{2max} did not differ from each other despite ACE genotype, but the agreement between actual and estimated VO_{2max} methods was lower for the DD genotype.

Conclusion: It was concluded that there is a possible association between ACE genotype, cardiovascular fitness and middle-distance running performance of moderately active young males and that the accuracy of VO_{2max} prediction may also depend on the ACE genotype of the participants. (Arq Bras Cardiol 2012;98(4):315-320)

Keywords: Angiotensin-converting enzyme; I/D polymorphism; VO_{2max}, middle-distance running.

Introduction

The angiotensin I-converting enzyme gene (ACE gene) has been broadly studied for cardiorespiratory fitness phenotypes. Concerning the 287bp insertion/deletion (I/D) polymorphism, a body of evidence associates the D allele to a lower aerobic fitness[1,2] with some studies associating it to power-demanding exercises[3,4]. In contrast, the I allele has been related to an improved endothelium-dependent vasodilation[5], higher percentage of the most efficient type I muscle fibers[6], thus suggesting that type II carriers would present higher VO_{2max}. However, few studies have investigated the influence of the ACE genotype on VO_{2max} [7-9], which is considered the gold standard for cardiorespiratory evaluation. Such authors have reported contradictory findings with a higher VO_{2max} observed in DD[9] or II carriers[7], or no relationship reported[10]. Because those studies were conducted on samples with important differences regarding participants' age, gender, and training background; and under different exercise modes, it may be suggested that these differences could be affecting these relationships[1], therefore, it is necessary to use homogeneous samples and protocols to minimize any differences resulting from the phenotypic characteristics of participants.

In addition, the association of the ACE genotype with middle-distance running has been poorly investigated. There are only two studies[11,12] in which a higher middle-distance running performance was reported for young male DD carriers. In both studies, the VO_{2max} was not recorded and its relative influence on running performance could not be evaluated. Consequently, further research ensures the influence of ACE genotype on VO_{2max} as a marker of cardiovascular fitness, as well as on middle distance running performance.

Once the VO_{2max} determination usually requires laboratory facilities, various predictive equations have been proposed for its estimation from practical and inexpensive field tests. An update of the predictive equation of VO_{2max} from a 1.600m running test in a cohort of young men has been recently proposed. These authors have suggested the specificity of predictive equations regarding gender, age and the training background of the participants. The strong difference (12%) detected between the prediction of the previous[14] and the new equation[13] suggests that the geographical origin of participants could be another important factor. It is well known that the allelic frequency of the ACE gene may vary according to the

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location of the sample[15], thus the predictive power of an equation used in a specific population may differ when applied to a population with a different allele frequency.

Thus, this study investigated the possible influence of Angiotensin-Converting Enzyme (ACE) genotype (I/D) on cardiovascular fitness and middle-distance running performance of Brazilian young males. In addition, the validity of VO_{2max} prediction with regard to the ACE genotype was also analyzed.

Methods

Fifty-seven physically active young (practitioners of physical activity at least three times a week for at least 30 min) non-runners were recruited for this study for convenience. We selected this sample for convenience based on previous suggestions about the appropriateness of homogeneous samples for the evaluations of physical performance with regard to genotype[1,15]. All volunteers were informed of the risks and benefits of their participation in the study, so they were instructed to sign a consent form. They were asked to avoid any intense exercise and to abstain from caffeine and alcohol beverages in the 24 hours preceding the tests, which were performed in random order with a minimum of 48h.

The treadmill test (Inbramed Millenium Super ATL, Porto Alegre, Brazil) was performed at 1% inclination, with an initial speed of 6 $km \cdot h^{-1}$ and subsequent increments of 0.75 $km \cdot h^{-1}$ every minute until volitional exhaustion. Expired gases were continuously measured (Cortex Biophysik, Germany) and the VO_{2max} ($mL \cdot kg \cdot min^{-1}$) recorded was the mean of the values reached during the last 20 seconds before exhaustion. Additionally, the following criteria of the American College of Sports Medicine guidelines to determine VO_{2max} were considered: RER > 1.15; VO_{2max} plateau; RPE > 17; and maximal HR within ± 10 $beats \cdot min^{-1}$ of predicted values (HR = 220 – age)[14,16,17].

The middle-distance running test consisted in an all-out 1.600m running track test conducted under thermoneutral conditions (24ºC ±1ºC) and absence of wind[18]. The mean speed ($m \cdot min^{-1}$) from the running performance was calculated (V1600m) and subsequently applied to a previously validated equation: VO_{2max} = (0.177*V1600m) + 8.101.

On a different day, whole venous blood was drawn for DNA extraction (AccuPrep Genomic DNA Extraction Kit – Bioneer HQ) and the ACE I/D polymorphism was identified by polymerase chain reaction using specific primers and subsequent electrophoresis as described elsewhere[19].

The variables are shown as mean (SD in table 1; descriptive statistical and SEM in table 2; inferential statistics). All parameters were normally distributed as confirmed by a Kolmogorov-Smirnov test. ANOVA with Bonferroni as a *post hoc* was conducted to examine possible differences among the groups. The Bland and Altman[20] procedure and the intraclass correlation coefficient (ICC) were used to examine agreement and reliability between measured and predicted VO_{2max} values. Relationships among parameters were determined by Pearson product moment correlation coefficient. Significance level was set at $p < 0.05$.

Results

Table 1 shows the characteristics of participants, though with no evident difference between genotypes. Table 2 presents V1600m and both measured and estimated VO_{2max}

Table 1 - Characteristics of participants according to the ACE genotype (n = 57). Values are expressed as means (± SD)

Genotype	n	Age (years)	Weight (kg)	Height (cm)	BMI $(kg.m^2)^{-1}$
DD	15	22.3 (± 1.2)	71.3 (± 8.4)	177 (± 3)	22.8 (± 2.5)
ID	25	23.7 (± 3.8)	73.2 (± 4.5)	178 (± 4)	23.1 (± 1.3)
II	17	22.5 (± 3.8)	70.5 (± 6.6)	181 (± 4)	21.5 (± 2.2)

BMI – Body max index.

Table 2 - Mean (±SEM) results for 1600 mean velocity (V1600m), VO_{2max} and predicted VO_{2max} according to ACE genotype

Genotype	V1600m ($m \cdot min^{-1}$)	Real VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	Predicted VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)
DD (n = 15)	211.2* (8.28)	45.8* (1.8)	45.2* (1.4)
ID (n = 25)	249.1 (4.28)	52.2 (0.8)	52.6 (0.8)
II (n = 17)	258.6 (5.42)	54.2 (0.9)	53.6 (1.0)

** Statistical difference compared to ID and II genotypes.*

values for the three ACE I/D genotype groups. The recorded VO_{2max} and V1600m for DD, ID and II genotypes were 45.6 (1.8); 51.9 (0.8) and 54.4 (1.0) mL.kg^{-1}.min^{-1} and 211.2 (8.3); 249.1 (4.3) and 258 (5.4) m.min^{-1} respectively and were significantly lower for DD carriers ($P< 0.05$). No differences were observed between the estimated VO_{2max} and that obtained in the cardiopulmonary exercise test. A high correlation was exhibited between VO_{2max} and V1600m for the whole sample ($r=0.94$; $P= 0.0001$).

Strong correlations were detected between predicted and measured VO_{2max} for all genotype groups (DD: 0.89 < ID: 0.99 < II: 0.99; $P<0.05$). The ICCs were also high for all the genotypes but with a lower value for the DD carriers (DD: 0.86 < ID: 0.97 < II: 0.98). Moreover, the Bland and Altman[20] plot showed a lower agreement for the DD carriers (Fig. 1).

Discussion

The main finding of this study was that VO_{2max} values and thus the cardiovascular fitness of physically active young males seemed to be influenced by the I/D polymorphism of the ACE gene. It was observed that, for this homogenous sample studied, the DD carriers presented both a lower VO_{2max} and 1600m running performance compared to II and ID genotypes ($p < 0.05$). Also, V1600m and VO_{2max} were highly correlated in the whole sample ($r = 0.94$; $p = 0.001$) suggesting a great influence of VO_{2max} in participants' middle-distance running ability.

The finding of DD carriers showing a lower mean V1600m and VO_{2max} compared to other genotypes is inconsistent with previous reports[11,12] in which the DD carriers were the best performers of a cohort of young well-trained men in 2,000-2,400m running tests. Although these results may seem opposite, it may be worth noting that the fitness level of the participants of these previous studies[11,12] is higher than those of the current study as for their running times, with a higher mean velocity for longer running distances (~285 and 240 m·min-1 for 2.000 and 2.400 m, respectively). In this regard, Roltsch et al[21] did not find any difference in VO_{2max} in a cycling exercise among ACE genotypes in a group of young women, while the opposite was reported with post-menopausal women with significant lower VO_{2max} values[7]. Furthermore, the study by Zhao et al[9] that evaluated a group of young men in a treadmill graded test, revealed a higher VO_{2max} of DD carriers with a significantly higher VO_{2max} of the whole sample (range ~ 44-76 mL·kg^{-1}·min^{-1}) compared to our participants (range ~ 37-61 mL·kg^{-1}·min^{-1}). Consequently, and despite any possible ethnic or age influence, it may be suggested that the ACE genotype could be influencing middle-distance running and VO_{2max} depending on the fitness level of the sample.

This apparent paradox and the contradictory findings in previous literature can be due to the different protocols employed as only a very few studies have considered field running ability for evaluation of young men[11,12]. This is important as physical demands are quite different depending on the ergometer employed (e.g. treadmill vs. cycle ergometer). Moreover, the intensity and the profile of the running exercises could be also influencing their physiological demands, including both metabolic and neuromuscular factors[22,23]. In this regard, Lucía et al[24] have shown that the DD genotype seemed to be higher in elite cyclists compared with endurance runners, probably because of the higher power demands of cycling. Furthermore, the controversy about the influence of the ACE genotype on the endurance athlete status[25] could be explained by the fact that VO_{2max} is not so important for success as other factors (e.g. running economy) that could be influenced by other genes. Therefore, given the number of associations reported for every allele with different physiological functions before and after training[7,8,10,26], it may be suggested that the specific demands of every testing condition could be interacting with the individuals' fitness level thus modifying the role of the ACE genotype on physical fitness (i.e. VO_{2max}) and subsequent performance (i.e. middle-distance running). Since we recruited a very homogeneous sample of young male physically active non runners, and because the running protocols were selected for the evaluation of individuals' maximum aerobic power, this study demonstrates a more controlled experimental condition for testing our hypotheses properly. In addition, the use of a ramp protocol on a treadmill allows a better assessment of VO_{2max} compared to cycle ergometer (~10-20% higher in the treadmill)[27] because the treadmill provides a common form of physiological stress and the cycle ergometer is limited leading to a peripheral fatigue in many individuals[28].

Besides this, it is well known that both VO_{2max} and distance running are affected from a cardiovascular point of view by both central (e.g. cardiac output) and peripheral factors (e.g. oxygen extraction)[29], with the former being more important. Moreover, it should be pointed out that endurance running is also influenced by neuromuscular factors[23]. In this regard, the II carriers presented: higher maximal arterio-venous O_2 difference[7], higher percentage of type I muscle fibers[6], and larger endothelium-dependent vasodilation in the trained state[5]; whereas the DD carriers have demonstrated: larger skeletal muscle power[30]; and a greater left ventricular hypertrophy in military recruits after a training period[26] and in elite athletes[31]. From these previous studies, it may be suggested that while II carriers may present a greater peripheral function of the cardiovascular system, DD carriers are more benefited from neuromuscular and cardiac central adaptations. Furthermore, DD carriers have demonstrated a larger improvement after training programs in short aerobic efforts[11,12]. This may suggest that lower VO_{2max} and middle-distance running performance of DD carriers of our homogeneous sample could be reversed with respect to the other genotypes after a running training program. Therefore, more studies should be conducted to evaluate the role of the ACE genotype with regard to fitness status (e.g. well-trained vs. moderately trained individuals) and level running intensities (e.g. VT1 vs. VO_{2max}) with attention to physiological changes (i.e. neuromuscular vs. cardiovascular) accounting for such parameters after different training regimes. Nevertheless, our study is the first to report a significant inverse association among VO_{2max} and middle-distance running with the ACE DD genotype in a homogeneous sample of physically active young male non runners exhibiting ~ 50 mL·kg^{-1}·min^{-1}.

On the other hand, in this study, with a greater sample compared to the previous report, we have confirmed the validity of the predictive equation developed for a similar population[13]. Contrary to our hypothesis, the validity of this equation is independent of the ACE genotype. Consequently, this equation may be applied in different geographical locations, thus providing a simple and efficient tool for VO_{2max} prediction in young physically active men from a field running test. Interestingly,

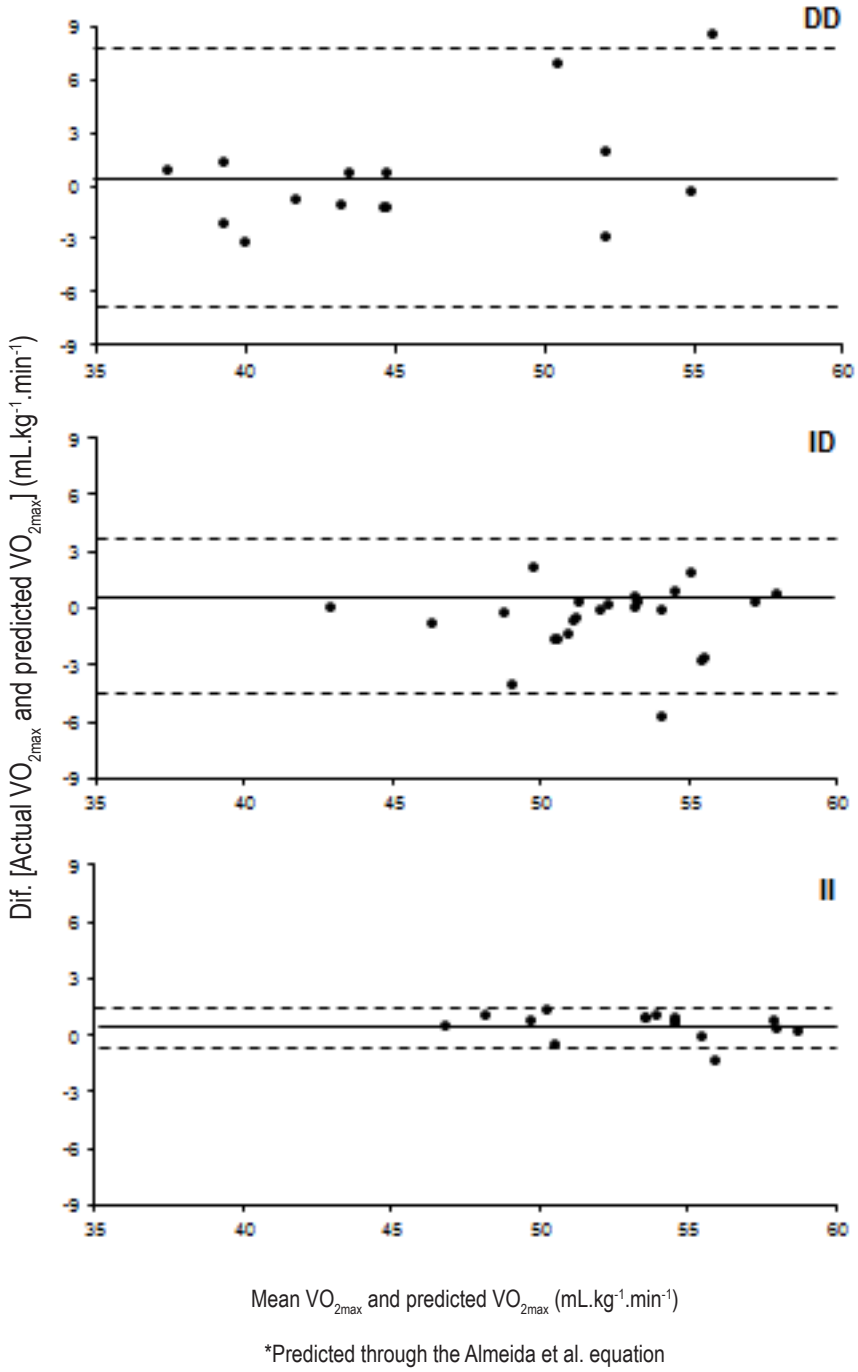

Figure 1 - *Bland-Altman plots showing consistency between methods for every ACE genotype*

although acceptable, consistency between the actual and predicted VO_{2max} was lower for the DD genotype with excellent values detected for the other genotypes (Fig. 1). In this regard, it should also be noted that there are greater SEM values for the DD carriers compared to the other groups (Table 2). We cannot explain these differences among genotypes that could be accounting for another unknown factor. Interestingly, individuals carrying the DD genotype that presented a higher VO_{2max} between measurements showed lower consistency compared to those of smaller VO_{2max} (Fig. 1). Perhaps this may mean that the

carriers of the DD genotype have a lower relationship between performance in middle distance running and VO_{2max} than carriers of other genotypes for ACE gene. Nevertheless, the validity of the equation is ensured in a similar population with such protocols. We suggest taking this aspect into consideration when applying this equation in great samples of those populations in which the D allele could be overrepresented.

Conclusion

Based on the results observed, the classic insertion/deletion polymorphism of the ACE gene has an important association with cardiorespiratory fitness and middle distance running performance in physically active young males with DD genotype carriers exhibiting lower results. The accuracy of VO_{2max} prediction may be slightly lower for DD carriers but with acceptable validity. Additionally, the ACE genotype may be an important factor to be taken into account in the determination/prediction of VO_{2max}. Further studies are required for the assessment of these relationships in similar populations regarding gender, running intensity and fitness status.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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In the original article "Impact of Stenting and Oral Sirolimus on Endothelium-Dependent and Independent Coronary Vasomotion", consider as correct the keywords "Stents, coronary vessels, endothelium sirolimus".

In the original article "The Influence of ACE Genotype on Cardiorespiratory Fitness of Moderately Active Young Men", consider as correct the keywords "Angiotensin-converting enzyme; I/D polymorphism; VO_2max, middle-distance running".