Alpha2A-adrenergic receptor and eNOS genetic polymorphisms are associated with exercise muscle vasodilatation in apparently healthy individuals

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Abstract

Purpose: Muscle vasodilatation during exercise has been associated with cardiovascular health and may be influenced by genetic variability. The purpose of this study was to evaluate functional genetic polymorphisms of physiologic pathways related to the regulation of the cardiovascular function (alpha-adrenergic receptors, endothelial nitric oxide synthase and bradykinin B2 receptor) and exercise muscle vasodilatation in apparently healthy men and women.

Methods: We enrolled 689 individuals without established cardiovascular disease that had attended a check-up program. The vasodilatation was studied with venous occlusion plethysmography and determined by the increase of vascular conductance during handgrip exercise. Genotypes for ADRA1A Arg347Cys (rs1048101), ADRA2A 1780 C > T (rs553668), ADRA2B Del 301–303 (rs28365031), eNOS 786 T > C (rs2070744), eNOS Glu298Asp (rs1799983) and BDKRB2 (rs5810761) polymorphisms were assessed by polymerase chain reaction followed by high resolution melting analysis.

Results: The eNOS rs2070744 polymorphism was significantly associated with forearm vascular conductance during exercise in women. Women with CC genotype showed higher vasodilatation than carriers of TC and TT genotypes (p = 0.043). The ADRA2A rs553668 polymorphism was significantly associated with forearm vascular conductance during exercise in men. Men with TT genotype had higher vasodilatation than carriers of CT and CC genotypes (p = 0.025).

Conclusions: eNOS rs207074 polymorphism in women and ADRA2A rs553668 polymorphism in men were associated with the increase of forearm vascular conductance during handgrip exercise. These findings suggest that eNOS and ADRA2A genetic polymorphisms may be potential markers of exercise muscle vasodilatation.

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1. Introduction

Peripheral vasodilator capacity has been proven to be an important marker of cardiovascular health in different populations, including individuals with or without overt heart disease [1–3]. The regulation of the muscle blood flow during the exercise involves a complex interaction between muscle contraction, local vasoactive substances, nitric oxide produced by the endothelium and the balance between sympathetic and parasympathetic tonus [4]. Muscle vasodilatation is influenced by factors such as age [5,6], sex [7], lipid profile and body mass index [8], and also by genetic variability [9].

Studies with small samples, ranging from 33 to 72 participants, have suggested that muscle vasodilatation during exercise may be modulated by functional polymorphisms in genes of physiologic pathways linked to cardiovascular regulation such as beta-adrenergic receptors, bradykinin B2 receptor (BDKRB2) and endothelial nitric oxide synthase (eNOS) [10–13]. Additionally, experimental studies with healthy individuals have demonstrated that alpha-adrenergic receptors mediate an important role on the modulation of the vascular tonus at rest [14] and during exercise [15]. Despite that, the contribution of functional polymorphisms in alpha1 and alpha2-adrenergic receptors genes (ADRA1 and ADRA2) to the control of exercise muscle vasodilatation remains unknown.

The purpose of this study was to evaluate possible associations between functional genetic polymorphisms of physiologic pathways related to the regulation of the cardiovascular function (alpha-adrenergic receptors, endothelial nitric oxide synthase and bradykinin B2 receptor) and exercise muscle vasodilatation estimated by the increase of forearm vascular conductance during forearm exercise in a large sample of men and women without overt heart disease.

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2. Methods

2.1. Study sample

We studied 689 unrelated asymptomatic participants of a cohort launched to follow-up volunteers interested in cardiological medical evaluation (check-up) at an academic medical center devoted to the prevention, diagnosis and treatment of heart diseases. Women and men aged older than 18 years without a past medical history of cardiovascular disease were informed and asked to participate in this study. A physical exam, laboratory work-up, and interview were collected. Individuals were excluded for the following: pre-existing hypertension, diabetes mellitus, chronic obstructive pulmonary disease or thyroid diseases, were excluded.

Weight and height were measured, and body mass index (BMI) was calculated. Ethnicity was classified for the Brazilian population according to a set of phenotypic characteristics (such as skin color, hair texture, shape of the nose, and aspect of the lips). Ethnic groups were classified as white, pardo, black, or Asian descendant. Smoking status was classified as current smokers or non-smokers.

2.2. Forearm blood flow and vascular conductance

Venous occlusion plethysmography was used to measure forearm blood flow (FBF) and vascular conductance as previously described. The non-dominant arm was elevated above heart level to achieve the venous drainage. A mercury-filled silastic tube attached to a low pressure transducer was placed around the forearm and connected to a plethysmograph (model EC6, Hokanson®, Bellevue, WA). Sphygmonanometer cuffs were placed around the wrist and upper arm. At 15-second intervals, the upper cuff was inflated above venous pressure for 7 to 8 s. Forearm blood flow was estimated on the basis of 4 separate readings. Forearm vascular conductance was described as the ratio of forearm blood flow and mean arterial pressure.

Mean blood pressure was monitored noninvasively and intermittently from an automatic and oscillometric cuff (DX 2710, Dixtal) placed on the left ankle with cuff width adjusted to ankle circumference. Heart rate was monitored continuously using lead II of electrocardiography. The electrocardiography signal was recorded at a frequency of 500 Hz and then analyzed in an AT/CODAS program.

Handgrip exercise at 30% of maximal voluntary contraction was performed in the contralateral arm by using a handgrip dynamometer. This maneuver was associated with significant forearm vasodilatation in previous studies. Forearm blood flow, mean blood pressure, and heart rate were recorded every minute during the protocol. Vasodilatation was estimated by the increase of the forearm vascular conductance during the 3 min-exercise.

2.3. Genotyping

Genomic DNA from subjects was extracted from a peripheral blood sample using standard salting-out procedure. Genotypes for the polymorphisms ADRA1A rs1048101 (Arg347Cys), ADRA2A rs553668 (1780T>C), ADRA2B rs28365031 (Del 301–303), eNOS rs2070744 (786T>C), eNOS rs1799983 (Glu298Asp), and BDKRB2 rs5810761 were determined using polymerase chain reaction (PCR) followed by high-resolution melting (HRM) analysis with the Rotor Gene 6000® instrument (Qiagen®, Courtabeuf, France). Details about the genotyping process are published elsewhere.

2.4. Statistical analysis

Continuous data are expressed as mean ± standard deviation. Categorical data are expressed as number and percentage. Differences between proportions and Hardy–Weinberg proportions for each studied polymorphism were determined using chi-squared test. Student’s t-test or Mann–Whitney U test (when appropriate) were performed for comparison of the means between two groups.

Mixed linear models were performed to study associations between the forearm vascular conductance during exercise and the genetic polymorphisms in men and women. Demographic and laboratory covariates included in the models were age, ethnicity, body mass index, smoking status, rest blood pressure, HDL-cholesterol, total cholesterol and fasting glucose. p-Values < 0.05 were considered significant. Analysis of residuals was performed to test whether the requirements of the models were properly met.

Due to significant differences between men and women relative to the forearm blood flow and vascular conductance responses during forearm exercise published elsewhere, the statistic models were conducted separately for men and women.

2.5. Ethics

The study protocol was approved by the Committee of Ethics on Human Research of the Hospital and all participants were instructed about the study and signed an informed consent.

3. Results

3.1. Clinical characteristics and genetic distribution of the study population

The demographic, clinical and laboratory characteristics of the study population are shown in Table 1.

| Variable          | Men (n = 317) | Women (n = 372) | p-Value |
|-------------------|---------------|-----------------|---------|
| Age (years)       | 42.4 ± 13     | 43.6 ± 13       | NS      |
| Body mass index (kg/m²) | 26.4 ± 3.9    | 26.3 ± 4.7      | NS      |
| Systolic blood pressure (mm Hg) | 124.8 ± 12.6  | 122.3 ± 13.4    | 0.015   |
| Diastolic blood pressure (mm Hg) | 81.7 ± 9.2    | 79.4 ± 8.6      | <0.001  |
| Smoking           | 55 (17.3%)    | 76 (20.4%)      | NS      |
| Fasting glucose (mg/dL) | 94.4 ± 8.5    | 90.7 ± 8.4      | <0.001  |
| Total cholesterol (mg/dL) | 192.1 ± 37.9  | 194.7 ± 38.2    | NS      |
| HDL-cholesterol (mg/dL) | 142.5 ± 12.6  | 122.5 ± 13.5    | <0.001  |
| LDL-cholesterol (mg/dL) | 120.2 ± 31.4  | 122.5 ± 33.7    | NS      |
| Triglyceride (mg/dL) | 137.9 ± 94.5  | 100.80 ± 54.5   | <0.001  |
| Creatinine (mg/dL)  | 0.97 ± 0.14   | 0.74 ± 0.12     | <0.001  |
| Hemoglobin (g/dL)  | 15.4 ± 0.9    | 13.6 ± 1        | <0.001  |
| LVEF (%)          | 64.8 ± 5.1    | 67.1 ± 4.6      | <0.001  |

Table 1

Clinical characteristics of the study sample among men (n = 317) and women (n = 372). Continuous variables are described as mean ± standard deviation. Categorical variables are described as number (percentage).

NS, not significant; LVEF, left ventricular ejection fraction.
* Number (%) of smokers among men.
** Number (%) of smoker among women.
4. Discussion

Exercise muscle vasodilatation during exercise. Carriers of CC genotype showed greater increase in forearm vascular conductance during exercise than carriers of allele T. In a study with 72 healthy and physically active individuals, carriers of eNOS 786TT genotype showed impaired exercise forearm vasodilation than carriers of eNOS 786TC and 786CC genotypes, which allowed the hypothesis that T allele may increase susceptibility for endothelial dysfunction [12]. Interestingly, in this study, carriers of eNOS 786TT genotype, despite worse baseline exercise vasodilation, had better improvement in muscle vasodilation after 18-week-exercise training than carriers of eNOS 786C allele. Other study that included patients with hypertension found different results, with higher endothelium-dependent vasodilation in carriers of eNOS 786TT genotype when compared with carriers of eNOS 786TC and 786CC genotypes [20]. However, the same group of researchers found in an analysis of GENICA (Genetic and Environmental Factors in Coronary Atherosclerosis) study that in patients with coronary artery disease, the eNOS 786TT genotype was associated with lower survival and impaired markers of oxidative stress, with higher levels of myeloperoxidase and lower levels of nitrotyrosine [21]. While eNOS 786C allele has been related to worse expression of eNOS enzyme and NO production [22,23], our study demonstrated better exercise muscle vasodilation in homozygote eNOS 786CC women. A possible explanation for this find is that depressed activity of eNOS enzyme in carriers of 786CC genotype may be counterbalanced by increased activation of other vasodilator pathways such as prostaglandins and endothelium-derived hyperpolarizing factor.

In men, the ADRA2A rs553668 (1780 C > T) polymorphism was associated with exercise forearm vascular conductance (Table 5). Relative to CT genotype, carriers of CC genotype had a greater increase in vascular conductance during exercise (p = 0.043), while carriers of TT genotype showed no significant difference.

In women, we found association between eNOS rs2070744 (786 T > C) and muscle vasodilation during exercise. Carriers of CC genotype showed greater increase in forearm vascular conductance during exercise than carriers of allele T. In a study with 72 healthy and physically active individuals, carriers of eNOS 786TT genotype showed impaired exercise forearm vasodilation than carriers of eNOS 786TC and 786CC genotypes, which allowed the hypothesis that T allele may increase susceptibility for endothelial dysfunction [12]. Interestingly, in this study, carriers of eNOS 786TT genotype, despite worse baseline exercise vasodilation, had better improvement in muscle vasodilation after 18-week-exercise training than carriers of eNOS 786C allele. Other study that included patients with hypertension found different results, with higher endothelium-dependent vasodilation in carriers of eNOS 786TT genotype when compared with carriers of eNOS 786TC and 786CC genotypes [20]. However, the same group of researchers found in an analysis of GENICA (Genetic and Environmental Factors in Coronary Atherosclerosis) study that in patients with coronary artery disease, the eNOS 786TT genotype was associated with lower survival and impaired markers of oxidative stress, with higher levels of myeloperoxidase and lower levels of nitrotyrosine [21]. While eNOS 786C allele has been related to worse expression of eNOS enzyme and NO production [22,23], our study demonstrated better exercise muscle vasodilation in homozygote eNOS 786CC women. A possible explanation for this find is that depressed activity of eNOS enzyme in carriers of 786CC genotype may be counterbalanced by increased activation of other vasodilator pathways such as prostaglandins and endothelium-derived hyperpolarizing factor.

In men, the ADRA2A rs553668 (1780 C > T) was associated with muscle vasodilation during exercise. Carriers of 1780T genotype presented higher muscle vasodilation than carriers of 1780C allele. In a study with 10 healthy men, it was demonstrated that forearm vascular tonus was more affected after brachial artery administration of the alpha2-adrenoceptor antagonist yohimbine than the administration of alpha1-adrenoceptor antagonist prazosin, which suggests that alpha2-adrenergic receptor may participate of the regulation of the vascular reactivity during exercise [14]. In a study with 73 healthy individuals, homozygous carriers of ADRA2A rs553668 had higher vasodilation and blood pressure reduction in response to alpha-agonist dexmedetomidine, which demonstrated a link

3.2. Exercise muscle vasodilator response

During the handgrip exercise maneuver, there were significant progressive increases in mean blood pressure, heart-rate, forearm blood flow and vascular conductance (Table 3). We observed differences between women and men with regard to vasodilator responses during exercise (Table 4). The forearm blood flow baseline values and increase during exercise were higher in men than in women. The increase in vascular conductance was higher in men at the first minute of handgrip exercise, but not at the second and third minutes of forearm exercise.

3.3. Exercise muscle vasodilatation and genetic polymorphisms

In women, the eNOS rs1799983 polymorphism, we observed higher prevalence of GC genotype in pardos (p < 0.01) when compared with other ethnic groups. In eNOS rs1799983 polymorphism, we observed higher prevalence of T allele in Asians descendants than in other groups (p = 0.019).

Table 2

| Allelic distribution of genetic polymorphisms in the study population. |
|-----------------------------|-----------------------------|
| Genetic polymorphism       | Genotype N (%)              | Allele N (%) |
|                            | CC                         | CT           | TT           | C            | T            |
| ADRA1A Arg347Cys (rs1048101) | 182 (30.4)                 | 291 (48.6)   | 126 (21)    | 655 (54.7)  | 543 (45.3)   |
| ADRA2A 1780 C > T (rs553668) | 371 (61.8)                 | 199 (33.2)   | 30 (5)      | 941 (78.4)  | 259 (21.6)   |
| eNOS 786 T > C (rs2070744)  | 66 (11.1)                  | 242 (40.8)   | 285 (48.1)  | 374 (31.5)  | 812 (68.5)   |
|                            | II                         | ID           | DD          | I            | D            |
| ADRA2B DEL301-303 (rs28365031) | 368 (62.9)                 | 142 (24.3)   | 75 (12.8)   | 878 (75)    | 292 (25)     |
| BDKRB2 (rs5810761)         | 173 (28.7)                 | 308 (51.1)   | 122 (20.2)  | 654 (54.2)  | 652 (45.8)   |
|                            | GG                         | GT           | TT          | G            | T            |
| eNOS Glu298Asp (rs1799983) | 297 (49.7)                 | 250 (41.8)   | 51 (8.5)    | 844 (70.6)  | 352 (9.4)    |

* Single Nucleotide Polymorphism with a change between Cytosine (C) and Thymine (T).
† Single Nucleotide Polymorphism with a change between Guanine (G) and Thymine (T).
‡ Insertion (I) and Deletion (D) Polymorphism.

Table 3

| Variable                  | Baseline (SD) | Δ exercise 1st minute (SD) | Δ exercise 2nd minute (SD) | Δ exercise 3rd minute (SD) |
|---------------------------|---------------|----------------------------|----------------------------|----------------------------|
| Mean blood pressure (mm Hg) | 94.6 (11.3)   | 5.7 (9.8)                  | 11.9 (10.8)                | 15.7 (13.4)                |
| Heart-rate (b.p.m)        | 65.1 (9.3)    | 4.7 (5.6)                  | 7.2 (8.6)                  | 8.8 (7.6)                  |
| FBF (ml/min 1.100 g−1)    | 1.91 (0.63)   | 0.41 (0.46)                | 0.64 (0.58)                | 0.87 (0.66)                |
| PVC (units)               | 2.04 (0.09)   | 0.29 (0.48)                | 0.37 (0.53)                | 0.52 (0.66)                |

SD, standard deviation; FBF, forearm blood flow; PVC, forearm vascular conductance.
between this genetic variant in the ADRA2A gene and the vasodilator response to a vasoactive substance [24]. In animal models, the ADRA2A rs553668 was related to overexpression of Alpha2A-adrenergic receptors [25], which may be a possible mechanism for the blood flow variation and different vasodilator responses during exercise among carriers of ADRA rs553668 genotypes. A previous study demonstrated that ADRA rs553668 polymorphism were associated with blood pressure responses during treadmill stress testing, suggesting this variant could also play a role in cardiovascular regulation during aerobic exercise [26].

In a study with 33 apparently healthy individuals, the muscle vasodilatation during handgrip exercise, estimated by variation in forearm vascular conductance, was significantly different in carriers of eNOS rs1799983 (Glu298Asp) polymorphism [11]. Different from these findings, we did not observe associations between eNOS rs1799983 and muscle vasodilatation. However, in our study we evaluated eNOS rs1799983 variant together with other functional genetic polymorphisms and covariates that may have overshadowed its impact on genetic modulation of exercise-induced vasodilatation.

We observed different associations between the exercise-induced vasodilatation and the study genetic polymorphisms in men and women. These differences in genetic modulation of exercise vasodilatation between the genders may be multifactorial. Nitric oxide bio-synthesis is greater in women than in men, which may suggest that endothelium-dependent vasodilatation may be more pronounced in women than in men [27]. Regarding the adrenergic system, differences have been also described between the genders. Markers of sympathetic activity such as muscle sympathetic activity (MSNA) and baroreflex sensitivity have been shown to be influenced by gender [28–30]. In addition, vasoconstrictor sensitivity to α1-adrenergic stimulation is reduced and vasodilator sensitivity to α2-receptor stimulation is higher in women when compared to men, which may imply in differences between men and women regarding autonomic regulation of vascular function [31–33].

Our study has several limitations. It is a cross-sectional data, which limits our capacity to make conclusions about the relation between the genetic polymorphisms and the study phenotypes. The stimulus for forearm vasodilatation was performed non-invasively in the contra-lateral arm, which differs from others studies that evaluated endothelial function and vasodilatation with a direct stimulus in the vascular bed such as intra-arterial infusion of pharmacologic substances or reactive hyperemia after ischemic occlusion of the limb [20,34]. However, it has been demonstrated in previous studies that the handgrip maneuver used in our study cause substantial muscle vasodilatation and is associated with cardiovascular variables [17–19,35]. We did not have enough data regarding the use of oral contraceptives, hormone replacement therapy and information about the menstrual cycle in women, which may have influenced the vasodilator responses in the female group. Nevertheless, a previous study with healthy premenopausal women demonstrated that forearm blood flow responses to vasodilator stimulus were not different between women using or not using oral contraceptives [36].

5. Conclusions

In a cohort of individuals apparently healthy, the eNOS rs2070744 polymorphism in women and the ADRA2A rs553668 polymorphism in men were associated with the response of forearm vascular conductance during handgrip exercise. These findings suggest that eNOS and ADRA2A genetic polymorphisms may be potential markers of exercise muscle vasodilatation.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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