IJC Heart & Vasculature 13 (2016) 14–18

Contents lists available at ScienceDirect

IJC Heart & Vasculature

journal homepage: http://www.journals.elsevier.com/ijc-heart-and-vasculature

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

Rafael Amorim Belo Nunes *, Lucia Pereira Barroso, Alexandre da Costa Pereira, Maria Urbana Pinto Brandão Rondon, Carlos Eduardo Negrão, José Eduardo Krieger, Alfredo José Mansur

Unidade Clínica de Ambulatório Geral, Instituto do Coração (InCor) do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Av. Dr. Enéas de Carvalho Aguiar, 44, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 2 July 2016
Received in revised form 22 September 2016
Accepted 7 October 2016
Available online 13 October 2016

Keywords:
Muscle vasodilatation
Forearm vascular conductance
Exercise
Genetic polymorphism
Alpha-adrenergic receptor
Endothelial nitric oxide synthase
Bradykinin B2 receptor
Cardiovascular genetics

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 B_2 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 $BDKRB_2$ (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.

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 B_2 receptor ($BDKRB_2$) 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 vasodilation 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.

* Corresponding author.
E-mail address: rafael.nunes@incor.usp.br (R. Amorim Belo Nunes).

http://dx.doi.org/10.1016/j.ijcha.2016.10.001

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 after informed consent underwent clinical examination (clinical history and physical examination), 12-lead electrocardiogram, chest X-ray, echocardiogram, and laboratory work-up. The laboratory work-up included complete blood count, lipid profile, fasting blood glucose, serum creatinine and high-sensitivity C-reactive protein. Individuals with evidence of heart disease or other significant systemic diseases, such as 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 [16]. The non-dominant arm was elevated above heart level to adequate 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). Sphygmomanometer 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 [17–19]. 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 following standard salting-out procedure. Genotypes for the polymorphisms ADRA1A rs1048101 (Arg347Cys), ADRA2A rs553668 (1780 C > T), ADRA2B rs28365031 (Del 301–303), eNOS rs2070744 (786 T > C), eNOS rs1799983 (Glu298Asp), and $BDKRB_2$ rs5810761 were detected by polymerase chain reaction (PCR) followed by high-resolution melting (HRM) analysis with the Rotor Gene 6000® instrument (Qiagen®, Courtaboeuf, France). Details about the genotyping process are published elsewhere [26].

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 [8], 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 laboratorial characteristics of the study population are shown in Table 1.

The distribution of the genetic polymorphism in the study sample is shown in Table 2. The genotyping success rate for each polymorphism was: ADRA1A rs1048101 99%, ADRA2A rs553668 99.2%, ADRA2B rs28365031 97%, eNOS rs2070744 98.3%, eNOS rs1799983 98.9%, and $BDKRB_2$ rs5810761 99.7%. The distributions of the genotypes were compatible with Hardy–Weinberg equilibrium, except for ADRA2B rs28365031 polymorphism.

The ethnicity distributions were the following: 530 (76.9%) white, 39 (5.6%) black, 110 (16%) pardo, 10 (1.5%) Asian descendant. Relative to the different ethnicities, we observed no differences in genotype distribution for ADRA2A rs553668, ADRA2B rs28365031 and eNOS rs2070744 polymorphism. We found in ADRA1A rs1048101 polymorphism, higher prevalence of TT genotype in whites ($p < 0.01$) and CC

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).

Variables	Men	Women	p-Value
Age (years)	42.4 ± 13	43.6 ± 13.5	NS
Body mass index (kg/m^2)	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)	45.2 ± 12.6	52.5 ± 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

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

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)
$BDKRB_2$ (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.

genotype in pardos ($p < 0.01$) when compared with other ethnic groups. In eNOS rs1799983 polymorphism, we observed higher prevalence of GG genotype in pardos ($p = 0.012$) and TT genotype in whites ($p = 0.01$). In $BDKRB_2$ rs5810761, the deletion/deletion genotype was more prevalent in Asians descendants than in other groups ($p = 0.019$).

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 rs2070744 (786 T > C) polymorphism was associated with exercise forearm vascular conductance (Table 5). Relative to TC 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 men, ADRA2A rs553668 (1780 C > T) polymorphism was associated with exercise forearm vascular conductance (Table 6). Relative to CT genotype, carriers of CC genotype showed a lower increase in vascular conductance during exercise ($p = 0.025$), while carriers of TT genotype showed no significant difference.

4. Discussion

The main findings of this study were associations between genetic polymorphisms of eNOS and ADRA2A genes and exercise muscle vasodilatation in individuals without heart disease, suggesting that these pathways may play a role in regulation of vascular function during exercise.

In women, we found association between eNOS rs2070744 (786 T > C) and 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 vasodilatation 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 vasodilatation, had better improvement in muscle vasodilatation 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 vasodilatation 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 vasodilatation 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 (1780C > T) was associated with muscle vasodilatation during exercise. Carriers of 1780TT genotype presented higher muscle vasodilatation 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 vasodilatation and blood pressure reduction in response to alpha-agonist dexmedetomidine, which demonstrated a link

Table 3
Blood pressure, heart-rate and forearm blood flow responses during handgrip isometric exercise.

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)
FVC (units)	2.04 (0.69)	0.29 (0.48)	0.37 (0.53)	0.52 (0.66)

SD, standard deviation; FBF, forearm blood flow; FVC, forearm vascular conductance.

Table 4
Forearm Blood flow (FBF) and forearm vascular conductance (FVC) during handgrip isometric exercise in men and women.

Variable	Men		Women		p
	Mean	SD	Mean	SD	
Baseline FBF	2.02	0.59	1.82	0.65	<0.001
Δ exercise - 1st minute	0.51	0.54	0.32	0.34	<0.001
Δ exercise - 2nd minute	0.77	0.71	0.53	0.4	<0.001
Δ exercise - 3rd minute	1	0.77	0.76	0.52	<0.001
Baseline FVC	2.1	0.67	2	0.71	0.107
Δ exercise - 1st minute	0.34	0.51	0.25	0.44	0.035
Δ exercise - 2nd minute	0.4	0.63	0.34	0.43	0.205
Δ exercise - 3rd minute	0.51	0.73	0.53	0.59	0.735

SD, standard deviation.

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 rs5536668 genotypes. A previous study demonstrated that ADRA rs5536668 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 biosynthesis 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 α_1-adrenergic stimulation is reduced and vasodilator sensitivity to α_2-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].

Table 5
Association between eNOS rs2070744 (786 T>C) polymorphism and vascular conductance during handgrip exercise in women.

Forearm vascular conductance	Estimate	p-Value
Genotype TT	0.04	0.552*
Genotype CC	0.22	0.043†
Allele T	−0.23	0.026‡

C, nucleotide Cytosine; T, nucleotide Thymine.
* p-Value relative to TT versus TC genotype.
† p-Value relative to CC versus TC genotype.
‡ p-Value relative to T versus C allele.

Table 6
Association between ADRA2A rs553668 (1780 C > T) polymorphism and vascular conductance response during handgrip exercise in men.

Forearm vascular conductance	Estimate	p-Value
Genotype CC	−0.18	0.028*
Genotype TT	0.47	0.025†
Allele C	−0.42	0.036‡

C, nucleotide Cytosine; T, nucleotide Thymine.
* p-Value relative to CC versus CT genotype.
† p-Value relative to TT versus CT genotype.
‡ p-value relative to C versus T allele.

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.

Funding sources

This study was supported in part by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grant number 2009/52992-1.

Study association

This article is part of the doctoral thesis submitted by Rafael Amorim Belo Nunes, of Faculdade de Medicina da Universidade de São Paulo.

References

[1] S.H. Kubo, T.S. Rector, A.J. Bank, R.E. Williams, S.M. Heifetz, Endothelium-dependent vasodilation is attenuated in patients with heart failure, Circulation 84 (1991) 1589–1596.
[2] T. Heitzer, T. Schlinzig, K. Krohn, T. Meinertz, T. Münzel, Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease, Circulation 104 (2001) 2673–2678.
[3] Y. Matsuzawa, S. Sugiyama, H. Sumida, et al., Peripheral endothelial function and cardiovascular events in high-risk patients, J. Am. Heart Assoc. 2 (2013) e000426.
[4] B. Saltin, G. Rådegran, M.D. Koskolou, R.C. Roach, Skeletal muscle blood flow in humans and its regulation during exercise, Acta Physiol. Scand. 162 (1998) 421–436.
[5] D.N. Proctor, D.W. Koch, S.C. Newcomer, K.U. Le, S.L. Smithmyer, U.A. Leuenberger, Leg blood flow and VO2 during peak cycle exercise in younger and older women, Med. Sci. Sports Exerc. 36 (2004) 623–631.
[6] Z. Barrett-O'Keefe, S.J. Ives, J.D. Trinity, et al., Endothelin-A-mediated vasoconstriction during exercise with advancing age, J. Gerontol. A Biol. Sci. Med. Sci. 70 (2015) 554–565.

[7] D.P. Casey, J.R. Shepherd, M.J. Joyner, Sex and vasodilator responses to hypoxia at rest and during exercise, J. Appl. Physiol. 116 (2014) 927–936.
[8] T.E. Sartori, R.A. Nunes, G.T. da Silva, et al., Influence of demographic and metabolic variables on forearm blood flow and vascular conductance in individuals without overt heart disease, Vasc. Health Risk Manag. 6 (2010) 431–437.
[9] E. Ingelsson, A.C. Syvänen, L. Lind, Endothelium-dependent vasodilation in conduit and resistance vessels in relation to the endothelial nitric oxide synthase gene, J. Hum. Hypertens. 22 (2008) 569–578.
[10] I.C. Trombetta, L.T. Batalha, M.U. Rondon, M.C. Laterza, E. Frazzatto, M.J. Alves, A.C. Santos, P.C. Brum, A.C. Barretto, A. Halpern, S.M. Villares, C.E. Negrão, Gly16 + Glu27 ß2-adrenoceptor polymorphism cause increased forearm blood flow responses to mental stress and handgrip in human, J. Appl. Physiol. 98 (2005) 787–794.
[11] R.G. Dias, M.N. Alves, A.C. Pereira, et al., Glu298Asp eNOS gene polymorphism causes attenuation in nonexercising muscle vasodilatation, Physiol. Genomics 37 (2009) 99–107.
[12] M.V. Negrão, C.R. Alves, G.B. Alves, et al., Exercise training improves muscle vasodilatation in individuals with T786C polymorphism of endothelial nitric oxide synthase gene, Physiol. Genomics 42A (2010) 71–77.
[13] C.R. Alves, G.B. Alves, A.C. Pereira, et al., Vascular reactivity and ACE activity response to exercise training are modulated by the +9/−9 bradykinin B_2 receptor gene functional polymorphism, Physiol. Genomics 45 (2013) 487–492.
[14] F.A. Dinenno, J.H. Eisenach, N.M. Dietz, M.J. Joyner, Post-junctional alpha-adrenoceptors and basal limb vascular tone in healthy men, J. Physiol. 540 (2002) 1103–1110.
[15] J.C. Richards, G.J. Luckasen, D.G. Larson, F.A. Dinenno, Role of α-adrenergic vasoconstriction in regulating skeletal muscle blood flow and vascular conductance during forearm exercise in ageing humans, J. Physiol. 592 (2014) 4775–4788.
[16] C.E. Negrão, E. Hamilton, G. Foronow, A. Hage, J.D. Moriguchi, H.R. Middlekauff, Impaired endothelium-mediated vasodilation is not the principal cause of blood vasoconstriction in heart failure, Am. J. Physiol. Heart Circ. Physiol. 278 (2000) H168–H174.
[17] I.C. Trombetta, L.T. Batalha, M.U. Rondon, et al., Weight loss improves neurovascular and muscle metaboreflex control in obesity, Am. J. Physiol. Heart Circ. Physiol. 285 (2003) H974–H982.
[18] M.M. Ribeiro, A.G. Silva, N.S. Santos, et al., Diet and exercise training restore blood pressure and vasodilatory responses during physiological maneuvers in obese children, Circulation 111 (2005) 1915–1923.
[19] A.G. da Silva, M.M. Ribeiro, I.C. Trombetta, et al., Augmented muscle vasodilatory responses in obese children with Glu27 beta-2-adrenoceptor polymorphism, Pediatr. Exerc. Sci. 20 (2008) 157–168.
[20] G.P. Rossi, S. Taddei, A. Virdis, et al., The T-786C and Glu298Asp polymorphisms of the endothelial nitric oxide gene affect the forearm blood flow responses of Caucasian hypertensive patients, J. Am. Coll. Cardiol. 41 (2003) 938–945.
[21] G.P. Rossi, G. Maiolino, M. Zanchetta, et al., The T(−786)C endothelial nitric oxide synthase genotype predicts cardiovascular mortality in high-risk patients, J. Am. Coll. Cardiol. 48 (2006) 1166–1174.
[22] M. Nakayama, H. Yasue, M. Yoshimura, et al., T-786–>C mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm, Circulation 99 (1999) 2864–2870.
[23] V.E. Dosenko, V.Y. Zagoriy, N.V. Haytovich, O.A. Gordok, A.A. Moibenko, Allelic polymorphism of endothelial NO-synthase gene and its functional manifestations, Acta Biochim. Pol. 53 (2006) 299–302.
[24] D. Kurnik, M. Muszkat, C. Li, et al., Genetic variations in the α(2A)-adrenoreceptor are associated with blood pressure response to the agonist dexmedetomidine, Circ Cardiovasc Genet 4 (2011) 179–187.
[25] A.H. Rosengren, R. Jokubka, D. Tojjar, et al., Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes, Science 327 (2010) 217–220.
[26] R.A. Nunes, L.P. Barroso, A.C. Pereira, J.E. Krieger, A.J. Mansur, Gender-related associations of genetic polymorphisms of α-adrenergic receptors, endothelial nitric oxide synthase and bradykinin B2 receptor with treadmill exercise test responses, Open Heart 1 (2014) e000132.
[27] B.J. Kneale, P.J. Chowienczyk, J.R. Cockcroft, D.J. Coltart, J.M. Ritter, Vasoconstrictor sensitivity to noradrenaline and NG-monomethyl-l-arginine in men and women, Clin. Sci. 93 (1997) 513–518.
[28] B.J. Kneale, P.J. Chowienczyk, S.E. Brett, et al., Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature, J. Am. Coll. Cardiol. 36 (2000) 1233–1238.
[29] C.T. Minson, J.R. Halliwill, T.M. Young, et al., Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women, Circulation 101 (2000) 862–868.
[30] N. Charkoudian, Influences of female reproductive hormones on sympathetic control of the circulation in humans, Clin. Auton. Res. 11 (2001) 295–301.
[31] A.B. Luzier, J.J. Nawarskas, J. Añonuevo, et al., The effects of gender on adrenergic receptor responsiveness, J. Clin. Pharmacol. 38 (1998) 618–624.
[32] J.A. Schmitt, M.J. Joyner, N. Charkoudian, et al., Sex differences in alpha-adrenergic support of blood pressure, Clin. Auton. Res. 20 (2010) 271–275.
[33] B.J. Kneale, P.J. Chowienczyk, J.R. Cockcroft, et al., Vasoconstrictor sensitivity to noradrenaline and NG-monomethyl-l-arginine in men and women, Clin. Sci. (Lond.) 93 (1997) 513–518.
[34] K. Suzuki, S.H. Juo, T. Rundek, et al., Genetic contribution to brachial artery flow-mediated dilation: the Northern Manhattan Family Study, Atherosclerosis 197 (2008) 212–216.
[35] R.A. Nunes, V. Giampaoli, H.F. de Freitas, et al., Exercise-induced muscle vasodilatation and treadmill exercise test responses in individuals without overt heart disease, Cardiology 127 (2014) 38–44.
[36] S. John, J. Jacobi, M.P. Schlaich, C. Delles, R.E. Schmieder, Effects of oral contraceptives on vascular endothelium in premenopausal women, Am. J. Obstet. Gynecol. 183 (2000) 28–33.