Effect of Physical Activity and T-786C Polymorphism in Blood Pressure and Blood Flow in the Elderly

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Abstract

Background: **The T-786C polymorphism of the gene for endothelial nitric oxide synthase (eNOS) and superoxide anion production may reduce production and bioavailability of nitric oxide, affecting the degree of vasodilation. This effect can be reversed by exercise.**

Objective: **To investigate the influence of aerobic training and T-786C polymorphism in the concentrations of nitric oxide metabolites (NOx) in blood flow (BF) and blood pressure (BP).**

Methods: **Thirty-two elderly pre-hypertensive women (59 ± 6 years old) were divided into two groups according to the T-786C polymorphism (TT and TC + CC). We analyzed the concentrations of NOx (plasma) and blood flow by venous occlusion plethysmography at rest, 1, 2 and 3 minutes post-occlusion (BF-0, BF-1 BF-2 BF-3, respectively). Evaluations were performed before and after 6 months of a program of aerobic exercise.**

Results: **In the pre-training evaluations, NOx levels were lower in TC + CC group than in TT group. The TT group showed correlations between NOx and BF-0 (r = 0.6) and diastolic blood pressure (DBP) and BF-0 (r = -0.7), but no correlation was found in TC + CC group. In the post-training evaluations, there were correlations between NOx and BF-0 (r = 0.6) and the changes in NOx and DBP (r = -0.6) in TT group. There were also correlations between DBP and BF-1 (r = -0.8), DBP, and BF-2 (r = -0.6), DBP, and BF-3 (r = -0.6), in the changes between NOx and BF-1 (r = 0.8) and changes in NOx and DBP (r = -0.7) in TC + CC group.**

Conclusion: **It was concluded that 6 months of aerobic exercise can increase the relationship between NO, BP and BF in elderly of allele C carriers. (Arq Bras Cardiol 2010; 95(4): 510-517)**

Key words: **Nitric oxide; superoxide dismutase; exercise; blood pressure; aged; polymorphism, genetic.**

Introduction

Nitric oxide (NO) produced by endothelial nitric oxide synthase (eNOS) has an important role in cardiovascular control, especially for vasodilation[1,2]. When eNOS is activated, the metabolism of L-arginine is increased generating the formation of L-citrulline and NO by endothelial cells[3-5]. Once produced, NO migrates to the vascular smooth muscle cell generating increased activity of guanylate cyclase, resulting in decreased calcium and vascular smooth muscle relaxation[5,6].

In the blood vessel, among the mechanisms involved in maintaining adequate concentrations of NO, it may be noted the activity of eNOS and the bioavailability of NO[7-12].

The activity of eNOS may be genetically determined and some polymorphisms have been identified as potential candidates for the development of arterial hypertension[9]. For example, the C allele of T-786C polymorphism in the promoter region of the eNOS gene has been able to decrease the promoter and transcriptional activity of this gene, reducing the production of NO[7,13].

The bioavailability of NO is closely related to production of superoxide anion (O_2^-) produced by the endothelium, the adventitia layer and vascular smooth muscle cell[1,14-18]. Both physical and humoral factors, such as shear stress, can modulate the activity of NAD(P)H oxidase and induce the production of O_2^-[12,19]. The increase of O_2^- in the cardiovascular system may cause endothelial dysfunction and increased vascular contractility[2,12]. When produced in excess, O_2^- reacts with NO producing peroxynitrite ($ONOO^-$), decreasing the bioavailability of NO[2].

Opposed to the effects of both mechanisms (gene polymorphisms of eNOS and of the production of O_2^-), aerobic exercise has been indicated as a major stimulus to combat these endothelial disorders[2,19,20]. The shear stress induced by the increase in blood flow, especially during physical exercises, may benefit the cardiovascular control mechanism in almost

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two mechanisms. First, the increased expression of eNOS and thus NO production, and second, the production of extracellular superoxide dismutase (ecSOD), which has the ability to react with O_2^-, potentially increasing the availability of NO for vascular smooth muscle cell[11,20].

So, exercise can regulate NO-mediated vasodilation by lowering the blood pressure values in most hypertensive subjects[2]. However, there is still controversy among studies with respect to the beneficial effects of physical exercise in increasing concentrations of NO and the vasodilator response in individuals carrying eNOS gene polymorphism[11].

The objective of this study was to investigate the influence of regular physical activity and T-786C polymorphism of the eNOS gene have in circulating levels of NO metabolites (NOx), the activity of ecSOD, in blood flow (BF) and pressure (BP) in pre-hypertensive elderly women.

Materials and methods

Participants

This study was approved by the Ethics Committee of the University of Maryland College Park/USA and by the Ethics in Research Institute of Biosciences/CEP-IB-UNESP.

On their first visit to the lab, all participants signed an informed consent form. The subjects were 32 apparently healthy women between 50-70 years old, sedentary, pre-hypertensive (without medication), non diabetic and non smokers. Women with overweight and obesity (BMI > 37 kg/m^2) were excluded from the study to avoid the interference of these variables in this study results.

The participants were divided into two groups according to the results of analysis of polymorphism of the eNOS gene, being classified as TT and TC + CC. Women in post-menopausal (83.0% of total participants), who were using hormone replacement therapy (45.0% of participants), maintained medications during the study.

Procedures

All participants performed the Bruce treadmill test with ECG for detection of cardiovascular disease, as described previously[21]. A diet record was performed and participants were instructed to keep the phase 1 diet of the American Heart Association (AHA), with stable weight for at least 3 weeks, as described previously[22]. All basic tests were performed before and after 6 months of a physical training program and the difference between the pre and post evaluations (post - pre = difference) was also used for the purposes of verifying the effectiveness of training in each variable studied.

Basic tests

The evaluation encompassed maximum oxygen consumption test (VO_2max), blood flow (BF), blood pressure (BP) and blood collection.

Maximal oxygen consumption (VO_2max) - was determined by voluntary exhaustion test, through a system of indirect calorimetry (model 2300, Hans Rudolph Inc., Kansas City, Missouri) and gas analyzer (model 1100, Perkin Elmer, Inc Danbury, Conn.) connected to a computer. VO_2max was determined when two of three criteria were met: respiratory exchange > 1.1, heart rate > 220 - age, and an increase greater than 150 ml min^{-1} in VO_2 during the last two minutes of test[23]. During the test, heart rate (HR), BP and ECG were monitored.

Blood flow - All participants were conducted to the laboratory between 7:00 and 9:00 am after fasting for 12 hours. The participants remained at rest in a lying position for 15 minutes, and blood flow was measured at the nondominant forearm using the technique of venous occlusion plethysmography. Briefly, using an automatic inflator, a small cuff was inflated around the wrist and a larger cuff around the upper arm (humerus) to 180 mmHg and 55 mmHg, respectively. To create ischemia of the arm, the cuff was inflated to 50 mmHg above systolic blood pressure at rest and maintained for 5 minutes. After this period of ischemia, the wrist cuff was inflated again and the arm was inflated to 55 mmHg, every minute[24]. It has been shown that the tissue has the ability to recover from ischemia in three minutes and that peak vasodilation occurs in about one minute after the release of the cuff[24,25]. Thus, BF was measured at rest (BF-0) and one, two and three minutes of reactive hyperemia (BF-1 BF-2 and BF-3, respectively), after 5 minutes of ischemia. The HR and BP were measured simultaneously in the other arm. Vascular resistance (VR) of the arm was calculated with the mean BP divided by the BF.

Casual blood pressure - The systolic and diastolic BP (SBP and DBP) were measured after 15 minutes of rest in the sitting position on three separate days according to the JNC 7 guidelines[26]. The mean of three measurements was considered for data analysis.

Blood analysis - Blood samples were collected in heparinized tubes for analysis of ecSOD activity, and in tubes containing EDTA for analysis of concentrations of NO metabolites (NOx), after fasting of 12 hours, and centrifuged at 2000 rpm, at 4ºC for 20 minutes. The plasma supernatant was immediately transferred to Eppendorf tubes and frozen at -80 ºC for later analysis.

Concentration of the metabolites of NO - Plasma was ultrafiltered and nitric oxide metabolites (NOx) were measured by Greiss assay (analysis of concentrations of nitrite after conversion of nitrate to nitrite), as described previously[21,27]. The samples were analyzed in duplicates and the average between them was considered for statistical analysis. The coefficients of variation were within and between 3.3% and 4.9% respectively.

EcSOD activity - The activity of plasma ecSOD levels was measured using commercial kits from Cayman Chemicals (Ann Arbor, MI). The samples were analyzed in duplicates and the average between them was considered for statistical analysis. The coefficients of variation within and between were 22.0% and 7.0% respectively.

Genetic analysis - The DNA of the participants was isolated from leucocytes using PureGene kit (Gentra System, Minneapolis, MN). The T-786C polymorphism of the eNOS gene was detected by polymerase chain reaction (PCR) using the following primers: F: 5'-CACCCAGGCCCACCCCAACT-3'

and R:5'-GCCGCAGGTCGACAGAGAG ACT-3'. The DNA was denatured for 5 minutes to 95°C, followed by 35 cycles of denaturation (30s, 95°C), annealing (15s, 54°C) and extension (30s, 72°C). The PCR product was digested by the enzyme MspI at 37°C, followed by four hours in agarose gel 2.0%. The T allele yields a fragment of 415 bp, and the C allele yields fragments of 370 bp and 45 bp.

C allele carriers were grouped into the same group (TC + CC) and compared with the TT group. The frequency of genotype TT and TC + CC in the population is approximately 32.0% and 68.0%, respectively[28-31]. The C allele is considered capable of generating deleterious effects on the cardiovascular system and both genotypes (TC and CC) showed similar responses, unlike genotype TT[13,29]. Thus, it is appropriate to group both TC and CC genotypes in the same group for analysis in this study.

Aerobic physical training

All participants underwent a supervised program of aerobic exercise on a treadmill for six months, three times a week, with gradual intensity increase over the weeks. With appropriate heating and relaxation, the initial session consisted of 20 minutes of aerobic exercise to 50.0% of VO_2max. The duration of training was increased 5 minutes per week until reaching 40 minutes. Thereafter, the intensity was increased by 5.0% of VO_2max every week until it reaches 70.0%. The intensity control was performed by heart rate monitors.

Only participants who completed 75.0% of the training sessions were included in the final results of this study.

Statistical analysis

A descriptive analysis was performed for both groups (TT and TC + CC) and a one-way ANOVA to detect statistical differences between groups, taking into account the T-786C polymorphism of the eNOS gene as an independent variable and as dependent variable, BMI, BP, VO_2max, the NOx and activity of ecSOD. A two-way ANOVA was performed with genotype as independent variables and physical activity, and as a dependent variable, BF, BP and BMI. The Pearson correlation analysis was also carried out aiming at detecting possible relationships between the variables analyzed in this study.

All results are expressed as mean and standard error of mean. The significance level was $p < 0.05$. Data were analyzed using the statistical package SPSS 13.0.

Results

Thirty-two pre-hypertensive women were divided into two groups according to the T-786C polymorphism of the eNOS gene (TT - 20 participants and TC + CC - 12 participants).

No statistical difference was found between groups in SBP, DBP and BMI, variables before and after the exercise program. VO_2max significantly increased in both groups after the exercise program.

The values of BMI, BP and VO_2max of both groups are summarized in Table 1.

In the pre-training, NOx levels were statistically different ($p < 0.03$) between groups and, as expected, the TC + CC group showed lower values in comparison to the TT group (Figure 1-A). The activity of ecSOD was 30.0% lower in the TC + CC group compared with the TT group, but this difference was not statistically significant (Figure 1-B).

Table 1 - Individual characteristics for both groups before and after 6 months of a regular aerobic exercise program

	TT (n = 20)		TC+CC (n = 12)	
	Pre	Post	Pre	Post
Age (years)	60.2 ± 1.5	-	59.0 ± 2.0	-
BMI (kg/m²)	26.9 ± 0.8	26.0 ± 1.0	29.5 ± 0.9	28.4 ± 0.9
SBP (mmHg)	130.6 ± 2.5	131.1 ± 3.3	133.0 ± 5.5	130.1 ± 4.0
DBP (mmHg)	85.8 ± 1.6	85.9 ± 1.5	88.0 ± 2.9	86.7 ± 2.8
VO_2max (ml/kg/min)	26.1 ± 1.2	28.4 ± 1.7*	25.2 ± 1.5	29.0 ± 1.7*

*BMI - body mass index; SBP - systolic blood pressure; DBP - diastolic blood pressure; VO_2max - maximum volume of oxygen. Values expressed as average ± standard error of average. *Significant difference between pre- and post-training ($p < 0.05$).*

In the post-training evaluation, the TT group maintained the plasma NOx values of the pre-training, while the TC + CC group had an increase in these levels. After the exercise program (Figure 1-A), no significant difference was found between the groups, ie, the difference found in the pre-training did not remain significant after physical training.

Both in pre- and in post-exercise program, no differences were observed between groups in the variable blood flow and vascular resistance. Although this result did not reach statistical differences, the TC + CC group showed higher blood flow values compared to the TT group in the pre-training evaluation, suggesting a lower rate of recovery of blood flow (Figure 2-A). In the post-training evaluation, this small difference was not perceived, with both groups presenting about the same result (Figure 2-B). Basically, vascular resistance had the same pattern of blood flow in relation to both groups and evaluations (Figure 2-C and 2-D).

The Pearson correlation analysis found, in the pre-training evaluation for the TT group, correlations between NOx and BF-0, also found between DBP and the variables of flow and resistance (BF-0, RV-0 and RV-1). In the post-training evaluation, there were correlations between NOx and BF-0 and between the changes in NOx and changes in DBP. For the TC + CC group, no correlation was found in the pre-training evaluation, but in post-training evaluation it was found correlations between DBP and BF-1, BF-2 and BF-3 and between changes in NOx and changes in the BF-1 and DBP. All correlations are summarized in Table 2.

Discussion

This study was conducted to assess the potential effects of T-786C polymorphism of the eNOS gene and six months for a regular program of physical exercise on hemodynamic

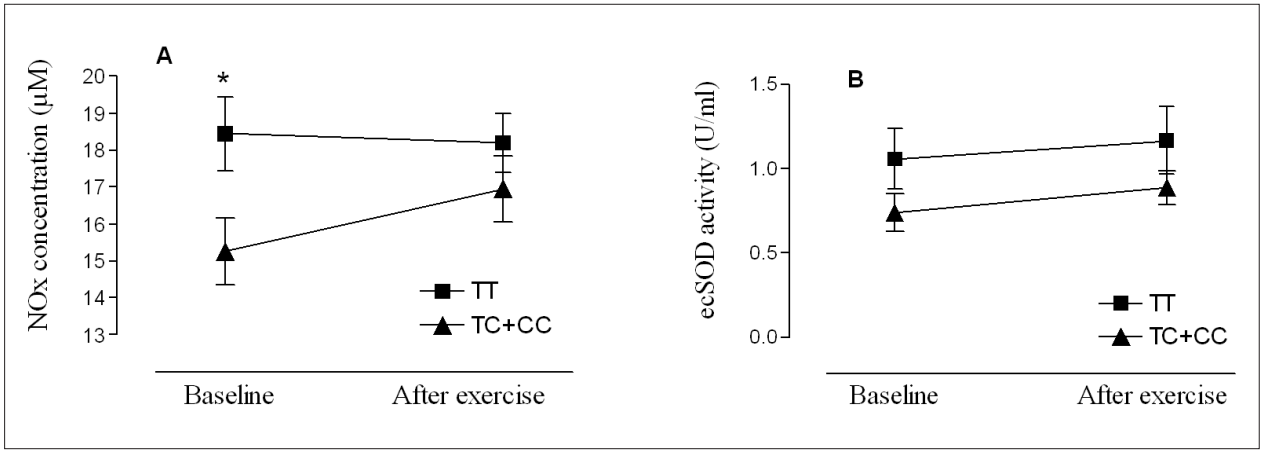

*Figure 1 - Plasma concentrations of NOx (A) and ecSOD (B) activity before and after 6 months of aerobic exercise, among pre-hypertensive elderly women, subdivided as per the T-786C polymorphism of eNOS genes. *Statistical difference between the groups (p < 0.05).*

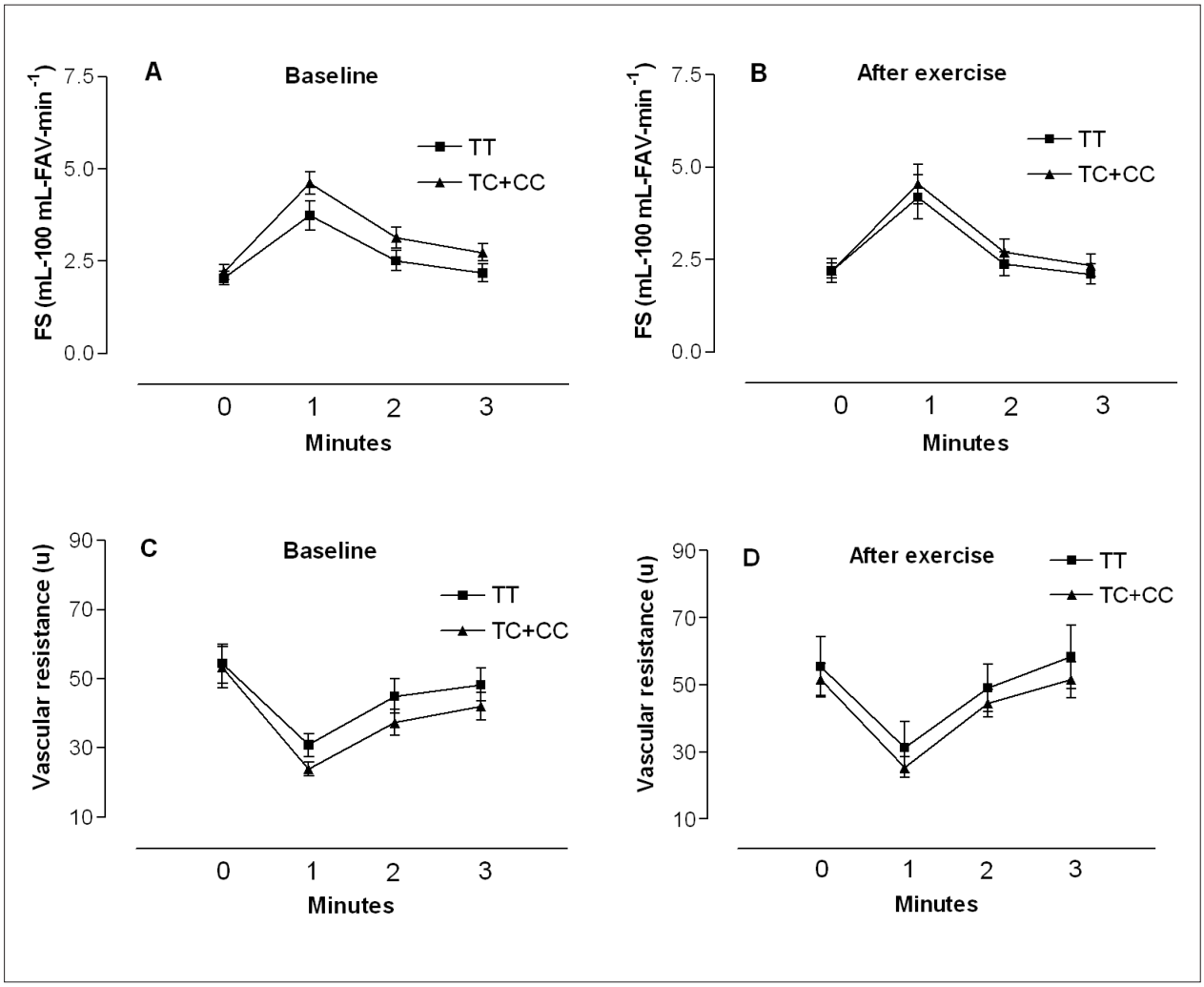

Figure 2 - Blood flow (panel A and B) and vascular resistance (panel C and D) at rest (0) and for three minutes of reactive hyperemia in pre-hypertensive elderly women, subdivided as per the reactive hyperemia minutes in pre-hypertensive elderly women, subdivided as per T-786C polymorphism of eNOS gene, before and after 6 months of an aerobic physical exercise program.

Table 2 - Pearson correlation among NOx, BP and BF variables of pre-hypertensive elderly women, subdivided as per the T-786C polymorphism of eNOS gene, in the pre- and post-physical training moments

	r	p
TT		
Pre-training		
NOx *versus* BF-0	0.6	0.05
DBP *versus* BF-0	-0.7	0.04
DBP *versus* VR-0	0.8	0.01
DBP *versus* VR-1	0.6	0.04
Post-training		
NOx *versus* BF-0	0.6	0.05
Changes with physical training		
NOx *versus* DBP	-0.6	0.05
TC+CC		
Pre-training		
No correlation	-	-
Post-training		
DBP *versus* BF-1	-0.8	0.01
DBP *versus* BF-2	-0.6	0.05
DBP *versus* BF-3	-0.6	0.05
Changes with physical training		
NOx *versus* BF-1	0.8	0.01
NOx *versus* DBP	-0.7	0.03

NOx - nitric oxide metabolites; BF - blood flow (at rest and after one, two or three minutes from reactive hyperemia); DBP - diastolic blood pressure.

responses of elderly women pre-hypertensive.

NO has an important role in vascular control, especially in vasodilation. Both eNOS activity as the mechanisms responsible for the bioavailability of NO contributes to the maintenance of an adequate concentration of nitric oxide in the blood vessel, and both mechanisms may be affected by physical exercise.

As expected, after 6 months of aerobic exercise, the result of VO_2max increased significantly for both groups, demonstrating that the intervention applied possessed sufficient stimulus to improve aerobic capacity of participants.

In blood pressure, both systolic and diastolic, no difference was found between the groups and between pre- and post-training. Several studies have shown a beneficial effect of physical exercise on blood pressure. For example, Hagberg et al[32] found that regular exercise has the ability to reduce BP in approximately 75.0% of hypertensive patients and that training with moderate intensity seems to generate greater benefits than with high intensity, for such reductions. Higashi et al[4] observed a beneficial effect of regular aerobic exercise on blood pressure values for hypertensive patients.

It is noteworthy, however, that these studies refer to the beneficial effects of physical exercise in hypertensive individuals, unlike the present study, which used pre-hypertensive individuals, a fact that could explain the absence of differences in blood pressure values between pre- and post-physical training.

The T-786C polymorphism of the eNOS gene is potentially important for cardiovascular control, as has been reported that the presence of C allele decreases the expression of this gene[33]. In the present study, NO metabolites were significantly lower for the TC + CC group compared with the TT group, in the pre-training. This result is in agreement with previous studies, which also showed that individuals carrying the C allele for the same polymorphism had lower concentrations of NO[13,34,35]. For example, Nakayama et al[13] observed that carriers of C allele have a decrease of 50.0% of promoter activity when compared to individuals carrying the T allele, which is consistent with the concept that the C allele may reduce the concentrations of NO.

In the post-training evaluation, no difference was found for the values of NOx in the TT group. One possible explanation for this finding is that the participants' concentrations of NOx in this group because of the lack of difference between the pre- and post-training, are already at optimal values to maintain BP control. For the TC + CC group, the levels of NOx increased after aerobic training, but this increase was not statistically significant. This result suggests that although physical training has not been enough to increase the concentrations of NOx, an increase of eNOS activity in individuals carrying the C allele, since the difference between the groups in the pre-training evaluation for NOx, did not remain significant in post-training evaluation. This result is in agreement with several other studies that have shown similar results[3,20,36].

There is growing evidence to indicate that chronic exercise increases the antioxidant defense system in human blood vessels by increasing the activity of ecSOD[8,14,37]. Aerobic exercise can increase the activity of ecSOD and potentially increase the bioavailability of NO[11,20]. However, the biological activity of NO is dependent on superoxide anions, which compromise the functionality of NO[38]. In this study, the activity of ecSOD was lower for the TC + CC group compared to the TT group in the pre-training evaluation, but this difference did not reach statistical significance.

After the exercise program, both groups increased the activity of ecSOD proportionally, but this increase remained without statistical differences between evaluatons and groups. Thus, it is suggested that antioxidant activity related to the bioavailability of NO is not impaired in the study participants. This result is in agreement with the study of Di Massimo et al[38] that showed NOx values regardless of the values of ecSOD.

The release of NO has been considered as a strong mediator for reactive hyperemia in peripheral arteries[4]. Thus, low levels of NOx observed in the TC + CC group, associated with the responses of BF among groups in the pre-training evaluation, are in agreement with other studies that have shown similar results[24]. Although no difference was found in variable BF, the TT group had lower responses in the pre-training evaluation. This result indicates that the TT group returned to resting values more rapidly than TC + CC group.

Although the individuals carrying the C allele present lower levels of NOx and lower activity of ecSOD in the pre-

training evaluation, blood flow still remained relatively high. This result suggests that there are several other substances that may be contributing to this cardiovascular response, eg, potassium, oxygen, carbon dioxide and adenosine[39,40]. From the moment that C allele carriers have some vascular dysfunction, it is possible that other vasodilators are working to control responses to blood flow, thereby offsetting the reduced production of NO[17,40].

In TC + CC group, no correlation was found in the pre-training evaluation among variables NOx, BF and BP. This result can be explained by the deleterious effect of C allele in regulating the generation and bioavailability of NO. However, with the correlations found in post-training evaluation among the variables of blood pressure, blood flow and NOx, it can be stated that six months of a regular program of aerobic exercise can increase the relationship between these variables in individuals with C allele.

After the exercise program, apparently, the TT group had a normal response to vascular function, resulting in a reduced effect of the exercise program at rest and after reactive hyperemia in BF. For the TC + CC group, although blood flow values were higher, the subjects had similar responses compared to the responses from the TT group. So it seems that the ability to return to homeostasis more rapidly in the TC + CC group in post-exercise evaluation, may indicate a favorable response to physical training, resulting in improved peripheral vascular function. This result is in agreement with previous studies that showed a beneficial effect of exercise on blood flow in different genotypes[4,24,36].

Although this study has some limitations, such as exclusion of certain population characteristics (obese, diabetic, heart disease patients etc.), the analysis of just a polymorphism and only some substances to the production and to the bioavailability of NO, it is possible to suggest a beneficial effect of physical exercise considering the studies variables.

In summary, this study found a positive effect of physical exercise program with respect to the T-786C polymorphism of the eNOS gene. This suggests that an increased level of regular physical activity can improve the response to cardiovascular control, especially of NO in C allele carriers, contributing to the relationship between blood pressure and blood flow.

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Potential Conflict of Interest

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

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Study Association

This study is not associated with any post-graduation program.

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