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Influence of eNOS gene polymorphism on cardiometabolic parameters in response to physical training in postmenopausal women

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Abstract

The health-promoting effects of exercise training (ET) are related to nitric oxide (NO) production and/or its bioavailability. The objective of this study was to determine whether single nucleotide polymorphism of the endothelial NO synthase (eNOS) gene at positions -786T>C, G894T (Glu298Asp) and at the variable number of tandem repeat (VNTR) Intron 4b/a would interfere with the cardiometabolic responses of postmenopausal women submitted to physical training. Forty-nine postmenopausal women were trained in sessions of 30-40 min, 3 days a week for 8 weeks. Genotypes, oxidative stress status and cardiometabolic parameters were then evaluated in a double-blind design. Both systolic and diastolic blood pressure values were significantly reduced after ET, which was genotype-independent. However, women without eNOS gene polymorphism at position -786T>C (TT genotype) and Intron 4b/a (bb genotype) presented a better reduction of total cholesterol levels (-786T>C: before = 213 ± 12.1, after = 159.8 ± 14.4, Δ = -24.9% and Intron 4b/a: before = 211.8 ± 7.4, after = 180.12 ± 6.4 mg/dL, Δ = -15%), and LDL cholesterol (-786T>C: before = 146.1 ± 13.3, after = 82.8 ± 9.2, Δ = -43.3% and Intron 4b/a: before = 143.2 ± 8, after = 102.7 ± 5.8 mg/dL, Δ = -28.3%) in response to ET compared to those who carried the mutant allele. Superoxide dismutase activity was significantly increased in trained women whereas no changes were observed in malondialdehyde levels. Women without eNOS gene polymorphism at position -786T>C and Intron 4b/a showed a greater reduction of plasma cholesterol levels in response to ET. Furthermore, no genotype influence was observed on arterial blood pressure or oxidative stress status in this population.

Key words: Exercise training; eNOS polymorphism; Oxidative stress; Nitric oxide; Women

Introduction

Epidemiological studies have shown that the incidence of cardiovascular disease (CVD) increases in women after menopause, and dyslipidemia, arterial hypertension, diabetes mellitus, physical inactivity, and weight gain are considered as the main risk factors for CVD in this population (1). Conversely, exercise training has been considered to be an important tool in preventing as well as managing cardiovascular and endocrine-metabolic diseases (2). Accordingly, the health-beneficial effect of physical exercise is related to an increase of nitric oxide (NO) production (by up-regulation of endothelial NO synthase, eNOS) and/or decrease of NO inactivation (mainly by up-regulation of superoxide dismutase, SOD) (3,4). Nevertheless, the mechanisms by which shear stress induced by physical exercise promotes alterations in gene expression, especially in eNOS and SOD activities, are not fully understood (5).

Since NO plays a key role in the cardiovascular system by regulating vascular tone, platelet aggregation, and vascular smooth muscle growth (6), a number of studies have examined the relationship between genetic variations

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in the eNOS gene and cardiovascular diseases (7-9). Indeed, the presence of single nucleotide polymorphisms (SNPs) at position -786T>C and in exon 7 G894T (Glu298Asp) as well as at the variable number of tandem repeat (VNTR) Intron 4b/a of the eNOS gene has been associated with a reduction in plasma NO levels, decreased eNOS expression or reduced enzyme activity, which in turn were positively associated with coronary spasm and arterial hypertension (10,11). On the other hand, some studies have failed to find a positive relationship between polymorphisms and cardiovascular disease. In fact, no measurable differences were found in nitrite/nitrate levels (NO_x^-) in subjects with eNOS gene polymorphism carrying a mutant allele under basal conditions (12,13). Recently, we showed that plasma NO_x^- levels were similar under basal conditions in postmenopausal women with or without eNOS gene polymorphism at position -786T>C (14).

It has also been reported that genetic variations in the eNOS gene are implicated in the genesis of endocrine-metabolic diseases such as insulin resistance, type 2 diabetes mellitus, higher plasma-oxidized low-density lipoprotein (LDL), and metabolic syndrome (15-18). Since exercise training exerts beneficial effects on both the cardiovascular and endocrine-metabolic systems and that these health-promoting effects are strongly related to NO production and/or its bioavailability to the tissues, the objective of the present study was to determine whether SNP of the eNOS gene at positions -786T>C and G894T (Glu298Asp) as well as at VNTR Intron 4b/a would interfere with the cardiovascular and metabolic parameters in response to an exercise training program in postmenopausal women. Therefore, in the present study we tested the hypothesis that postmenopausal women without eNOS gene polymorphism at position -786T>C, G894T (Glu298Asp) and Intron 4b/a would be more responsive to the beneficial effect of exercise training than those who carry the mutant alleles.

Material and Methods

Participant screening

The study was approved by the Institutional Review Board of Universidade Estadual Paulista (UNESP). Volunteers from the community were recruited by advertisements in the Campus of UNESP and surrounding area. To be included in the study, women had to be sedentary, non-smoking, non-diabetic (fasting glucose level <100 mg/dL), never treated with hormone replacement therapy, in the postmenopausal age, and having no medical condition that would exclude exercise training. The exclusion criteria were subjects with cardiovascular disease (angina, valvular disease, stroke), arthritis, alcohol consumption >3 drinks per day, orthopedic conditions, or any serious medical condition that prevented the participants from adhering to the protocol or from exercising safely. To detect cardiovascular, pulmonary or other disease the volunteers were examined by a physician. A questionnaire was applied to the participants before the inclusion in this study reporting medical, exercise training and dietary data. Eligible subjects were informed of the procedures and risks of the study and signed a written informed consent in accordance with the policies and with the Ethics Committee of the Institute of Bioscience, UNESP (Rio Claro).

Anthropometric and cardiovascular parameters

Height was measured with a clinical stadiometer with the subject barefoot and body weight was measured with a calibrated digital precision scale (Thinner MS-7400, USA). Body mass index (BMI) was determined as weight (kg) divided by height in meters squared. Waist circumference was also determined. Women were instructed not to exercise outside the laboratory before blood pressure measurement. After 15 min of seated quiet rest, blood pressure was measured by auscultation with aneroid sphygmomanometer (Tycos, USA) in three sessions.

Cardiorespiratory capacity and exercise training

In the first week, the participants performed 30 min of physical exercise at 50% of maximum heart rate (HR), so that they could become familiar with the cycle ergometer. Individual exercise prescription was determined by a prior aerobic capacity evaluation as previously described (19). Briefly, each subject was asked to describe her habitual physical activity and her fitness level was determined based on this description. Initial work loads of 50/75 W were used and if the HR values for the 5th and 6th min did not differ by more than 5 bpm and if their mean values were between 130 and 170 bpm, the test was stopped. If mean HR was less than 130 bpm, the work load was increased and the exercise test was continued. After that, the predicted VO_2 max was determined from the nomogram and multiplied by correction factors (body weight and age). The maximal HR was calculated according to the following equation: 220 - age. The reserve HR (rHR) was calculated according to the following equation (20): rHR = [(maximal HR - rest HR) x % of intensity] + rest HR.

The exercise training program started with appropriate warm-up and cool-down activities. All exercise sessions were performed under the supervision of a physical trainer. Subjects were submitted to an aerobic exercise program on a cycle ergometer (Moviment, Brazil) in a quiet room with temperature at 25°C. Exercise training was performed for 3 days a week, each session consisting of 30-40 min over an 8-week period. In the initial 4 weeks, the intensity of training was that which produced 50-60% of rHR. An adjustment of the intensity of the exercise training program was done for 4 additional weeks (60-70% of rHR). The protocol design is schematically illustrated in Figure 1.

Lipid profile and fasting glucose

Venous blood samples (6 mL) were collected after 12 h of overnight fasting and serum was separated by centrifugation. LDL cholesterol (LDL-C; Wiener, Argentina), high-density lipoprotein cholesterol (HDL-C), total cholesterol, triglycerides, and glucose levels were measured enzymatically with commercial

colorimetric kits (LaborLab, Brazil). The atherogenic index was calculated by dividing total cholesterol by HDL-C levels. Values were considered to be elevated when LDL-C (≥130 mg/dL), HDL-C (<50 mg/dL for women), total cholesterol (≥200 mg/dL), triglycerides (≥150 mg/dL), and glucose (≥100 mg/dL).

Determination of plasma nitrite/nitrate (NO_x^-) levels

In order to evaluate NO production, the plasma levels of nitrite (NO_2^-) and nitrate (NO_3^-) were measured. Briefly, immediately after arterial blood collection, the samples were centrifuged at 8000 *g* for 10 min, and the resulting plasma supernatant was stored at -80°C. Plasma samples were ultra-filtered through microfilter cups (Microcon Centrifugal Filter Units, 10 kDa; Millipore, USA). The NO_x^- concentration of the resulting filtrate solution was determined by ELISA using a commercially available kit (Cayman Chemical Co., USA) according to manufacturer instructions. This assay determines the total NO based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. After conversion, the spectrophotometric measurement of nitrite is performed using the Griess reaction. The resulting deep purple azo compound absorbs light at 540 nm.

Plasma SOD and malondialdehyde (MDA) levels

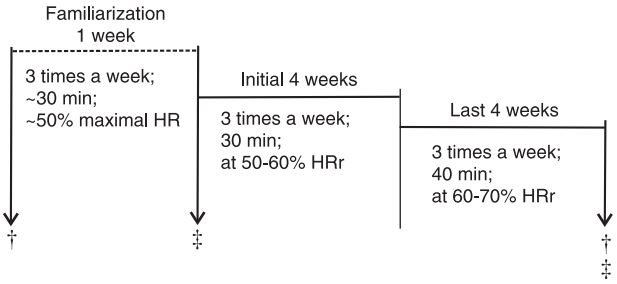
Blood samples were centrifuged at 8000 *g* for 10 min and the plasma supernatant was ultrafiltered through microfilter cups (Microcon Centrifugal Filter Units, 10 kDa; Millipore). The white buffy layer was removed and discarded. The supernatant was collected and kept in ice for assaying. Samples, standards and radical detector were prepared and processed as described in commercial kits (SOD assay kit and plasma MDA kit, Cayman Chemical Co.). The assays were performed in duplicate using different sample dilutions.

Genotype determination

Genomic DNA was extracted from leukocytes of blood cells by the chloroform-phenol method and the eNOS -786T>C, G894T (Glu298Asp) and Intron 4b/a sites were determined as described previously. The polymerase chain reaction (PCR) cycling conditions were 1 cycle at 96°C for 2 min followed by 35 cycles of denaturation at 96°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min; ending with extension at 72°C for 5 min for all positions.

-786T>C

Genotyping for the eNOS -786 region was performed using PCR amplification with flanking primers: sense: 5'-CACCCAGGCCCACCCCAACT-3'; antisense: 5'-GCCGCAGGTCGACAGAGAGACT-3' (21). The amplicon was digested for 5 min at 37°C using 2 U Fast *Msp*I enzyme followed by electrophoresis for 3 h on 2.5% agarose gel. The T allele yields a fragment of 394 bp and the C allele yields fragments of 352 and 42 bp.



† - Anthropometric, cardiovascular and biochemical parameters

‡ - Cardiorespiratory capacity

Figure 1. Schematic presentation of the protocol design employed for a training program applied to 49 postmenopausal women. HR = heart rate; HRr = reserve HR.

G894T (Glu298Asp)

The 298 eNOS gene region was amplified by the PCR using the following flanking primers: sense: 5'-AAGGCAGGAGACAGTGGATGGA-3'; antisense: 5'-CCCAGTCAATCCCTTTGGTGCTCA-3' (22). The amplicon was digested overnight at 37°C using 2 U *Mbo*I enzyme followed by electrophoresis for 3 h on 2.5% agarose gel. The G allele yields a fragment of 248 bp and the T allele yields fragments of 190 and 58 bp.

Intron 4b/a

Subjects were genotyped for the eNOS 27-bp repeat region in Intron 4b/a by PCR amplification with the following flanking primers: sense: 5'-FAM-AGGCCCTATGGTAGTGCCTTG-3'; antisense: 5'-TCTCTTAGTGCTGTGGTCACAG-3', according to Ref. 22, slightly modified. Genotyping was performed with a MegaBACE™ (USA), sequencer and the results were analyzed using the Fragment Profiler (USA) software version 1.2. The 4a allele generated a fragment of 393 bp and the 4b allele a fragment of 420 bp.

Genotype characterization was performed after 2 months of exercise training in a double-blind fashion for both volunteers and researchers.

Statistical analysis

Descriptive statistics for continuous variables consisted of the determination of central tendency and dispersion indicators, which are reported as means ± SEM. The distribution of genotypes for each polymorphism (Hardy-Weinberg equilibrium) and pharmacologic therapy were assessed using the chi-square (χ^2) test. The paired and unpaired Student *t*-tests were used to analyze the effect of exercise training within each group (baseline and after exercise training) and the difference between groups (non-polymorphism and polymorphic group), respectively. A P value <0.05 was accepted as significant.

Results

The distribution of genotypes for the three polymor-

phisms showed no deviation from Hardy-Weinberg equilibrium with allele frequencies of 0.73, 0.55, and 0.67 for T, G, and 4b at position -786T>C, G894T (Glu298Asp), and Intron 4b/a, respectively. Correspondingly, the allele frequency for C, T, and 4a were 0.16, 0.02, and 0.02, respectively. Forty-nine postmenopausal women with a mean age of 50.3 ± 0.92 years were studied in a double-blind design. Exercise training for 8 weeks did not modify BMI (25.50 ± 0.39 kg/m^2) as compared to the baseline (25.46 ± 0.39 kg/m^2), whereas waist circumference values were reduced in trained postmenopausal women (84.43 ± 1.07 cm) as compared to baseline values (85.96 ± 1.11 cm), approximately 2%. Similarly, the cardiorespiratory capacity was markedly increased after aerobic exercise training (32.99 ± 1.13 mL·kg^{-1}·min^{-1}) as compared to baseline values (27.07 ± 0.99 mL·kg^{-1}·min^{-1}) by approximately 22%, showing the effectiveness of the exercise training employed in this study. Table 1 shows the values of systolic and diastolic blood pressure, glycemia, lipid profile, plasma NO_x^- levels, SOD activity, and MDA levels before and after 8 weeks of exercise training in the overall samples. Exercise training was effective in promoting a significant reduction in arterial blood pressure, total cholesterol, LDL-C, as well as an increase in HDL-C and SOD activity. No changes were found in glycemia, triglycerides, NO_x^-, or MDA levels when we analyzed the population as a whole before and after exercise training.

Table 1. Effect of aerobic exercise training on cardiometabolic parameters of post-menopausal women.

	Before	After	Δ%
SBP (mmHg)	113.07 ± 1.59	107.13 ± 1.50*	-5.2
DBP (mmHg)	74.57 ± 1.16	69.20 ± 1.09*	-7.2
Glycemia (mg/dL)	85.33 ± 2.03	82.71 ± 1.66	-3.07
TG (mg/dL)	110.26 ± 9.01	105.18 ± 6.30	-4.6
TC (mg/dL)	208.27 ± 5.85	183.47 ± 5.18*	-11.9
HDL-C (mg/dL)	65.06 ± 1.78	73.51 ± 1.97*	-12.98
LDL-C (mg/dL)	141.10 ± 6.06	107.80 ± 4.96*	-23.6
NO_x^- (µM)	19.48 ± 2.32	23.14 ± 2.51	18.8
SOD (U/mL)	4.86 ± 0.23	5.68 ± 0.24*	16.9
MDA (µM)	5.81 ± 0.33	5.65 ± 0.24	-2.75

Data are reported as means ± SEM before and after 8 weeks of aerobic exercise training for 49 women. SBP = systolic blood pressure; DBP = diastolic blood pressure; TG = triglycerides; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; NO_x^- = plasma nitrite/nitrate levels; SOD = superoxide dismutase activity; MDA = malondialdehyde. *P < 0.05, compared with before intervention (paired Student *t*-test).

After eNOS genotype analysis, postmenopausal women were divided into three groups according to the presence of SNPs at positions -786T>C, G894T (Glu298Asp) and the VNTR Intron 4b/a. Subsequently, the volunteers were subdivided into non-polymorphic groups [-786: TT (N = 13), Glu298Asp: GG (N = 27), Intron 4: bb (N = 31)], and polymorphic groups -786: TC+CC (N = 36), Glu298Asp: GT+TT (N = 22), Intron 4: ba+aa (N = 18)]. The genotype distribution for the polymorphic groups was 73.4% for -786 TC+CC, 44.8% for Glu298Asp GT+TT and 36.7% for Intron 4 ba+aa. The incidence of chronic diseases and pharmacologic therapy was genotype independent for dyslipidemia [-786T>C (TT: 6.1 and TC+CC: 4.1%); G894T (GG: 6.1 and GT+TT: 4.1%); Intron 4 (bb: 4.1 and ba+aa: 6.1%)].

Table 2. Effect of 8 weeks aerobic training on blood pressure and plasma nitrite/nitrate levels of 49 postmenopausal women with or without polymorphism for the eNOS gene at positions -786T>C, Glu298Asp and Intron 4b/a.

	Before	After	Δ%	Before	After	Δ%
	-786T>C					
	TT (N = 13)			TC+CC (N = 36)		
SBP	113.78 ± 3.59	105.36 ± 2.63*	-7.4	112.81 ± 1.77	107.77 ± 1.82*	-4.5
DBP	75.14 ± 2.38	68.78 ± 2.16*	-8.5	74.36 ± 1.34	69.35 ± 1.28*	-6.7
NO_x^-	21.99 ± 5.13	21.07 ± 4.38	-4.2	18.55 ± 2.59	23.91 ± 3.06	28.9
	Glu298Asp					
	GG (N = 27)			GT+TT (N = 22)		
SBP	112.56 ± 1.92	106.83 ± 2.00*	-5.1	113.70 ± 2.70	107.49 ± 2.31*	-5.5
DBP	73.98 ± 1.57	68.81 ± 1.40*	-7.0	75.29 ± 1.75	69.67 ± 1.75*	-7.5
NO_x^-	21.82 ± 3.54	25.43 ± 3.73	16.5	16.72 ± 2.84	20.43 ± 3.26	22.2
	Intron 4b/a					
	bb (N = 31)			ba+aa (N = 18)		
SBP	112.73 ± 2.16	107.64 ± 1.82*	-4.5	113.66 ± 2.27	106.24 ± 2.68*	-6.5
DBP	74.06 ± 1.36	69.49 ± 1.30*	-6.2	75.44 ± 2.16	68.70 ± 1.99*	-8.9
NO_x^-	19.88 ± 3.10	23.25 ± 3.47	17.0	18.81 ± 3.50	22.95 ± 3.51	22.0

Data are reported as means ± SEM. TT, GG, and bb = non-polymorphic; TC+CC, GT+TT, and ba+aa = with polymorphism for the eNOS gene; SBP, DBP = systolic and diastolic blood pressure, respectively (mmHg); NO_x^- = plasma nitrite/nitrate levels (µM). *P < 0.05, compared with before intervention (paired Student *t*-test).

Cardiovascular parameters, physical training and eNOS gene polymorphism

Table 2 shows systolic and diastolic

blood pressure values and plasma NO_x^- levels before and after 8 weeks of exercise training in postmenopausal women according to the eNOS genotypes. Aerobic physical exercise promoted a significant reduction in both systolic and diastolic blood pressure in all groups (-786T>C, G894T (Glu298Asp) and Intron 4b/a), which was genotype-independent. Regarding plasma NO_x^- levels, we observed that the presence of eNOS gene polymorphism did not alter the basal values in postmenopausal women. Furthermore, 8 weeks of exercise training did not affect the plasma NO_x^- levels in any of the three groups (-786T>C, G894T (Glu298Asp) and Intron 4b/a).

Table 3. Effect of 8 weeks aerobic exercise training on plasma lipids of postmenopausal women with or without polymorphism for the eNOS gene at positions -786T>C, Glu298Asp and Intron 4b/a.

	Before	After	Δ%	Before	After	Δ%
	-786T>C					
	TT (N = 13)			TC+CC (N = 36)		
Glycemia	86.1 ± 4.3	85.4 ± 3.6	-0.8	85.1 ± 2.3	81.8 ± 1.8	-3.8
TG	106 ± 14.4	99.9 ± 10.7	-5.6	111.8 ± 11.1	107 ± 7.7	-4.3
TC	213 ± 12.1	159.8 ± 14.4*	-24.9	206.6 ± 6.7	192 ± 5.8	-7.0
LDL-C	146.1 ± 13.3	82.8 ± 9.2*	-43.3	139.3 ± 6.8	116.8 ± 5.2*	-16.2
HDL-C	64.0 ± 3.9	74.6 ± 3.8*	16.5	65.4 ± 2.5	73.1 ± 2.2*	11.8
	Glu298Asp					
	GG (N = 27)			GT+TT (N = 22)		
Glycemia	83.9 ± 2.9	82.6 ± 2.5	-1.5	87.3 ± 2.6	83 ± 2	-5.0
TG	114.5 ± 13.5	111.7 ± 9.8	-2.4	105 ± 11.5	97 ± 7	-7.6
TC	207.8 ± 7.9	181.5 ± 7.2*	-12.7	208.8 ± 8.9	185.9 ± 7.6*	-11.0
LDL-C	140.5 ± 8.4	105 ± 7*	-25.3	141.9 ± 8.8	111.2 ± 7.0*	-21.6
HDL-C	67.4 ± 2.4	76.5 ± 2.8*	13.5	61.9 ± 2.4	69.5 ± 2.8*	12.2
	Intron 4b/a					
	bb (N = 31)			ba+aa (N = 18)		
Glycemia	85.4 ± 2.5	81.7 ± 1.9	-4.4	85.2 ± 3.6	85 ± 3.3	0.0
TG	97.9 ± 8.1	97.2 ± 6.9	-0.7	130 ± 18.9	117.9 ± 11.7	-9.3
TC	211.8 ± 7.4	180.12 ± 6.4*	-15.0	202.2 ± 9.5	189.2 ± 8.8	-6.4
LDL-C	143.2 ± 8	102.7 ± 5.8*	-28.3	137.4 ± 9.2	116.5 ± 8.8	-15.2
HDL-C	65.3 ± 2.3	74.0 ± 2.6*	13.4	64.7 ± 2.9	72.7 ± 2.9	12.3

Data are reported as means ± SEM in mg/dL. TT, GG, and bb = non-polymorphic; TC+CC, GT+TT and ba+aa = with polymorphism for eNOS gene; TG = triglycerides; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol. *P < 0.05, compared with before intervention (paired Student *t*-test).

Glycemia, lipid profile, physical training, and eNOS gene polymorphism

Exercise training for 8 weeks did not alter blood glucose or triglyceride levels in any of the groups studied. On the other hand, HDL-C levels were significantly increased in all groups in a similar fashion (ranging between 11.8 up to 16.5%, see Table 3 for more details). Regarding the influence of eNOS gene polymorphism on plasma total cholesterol and LDL-C levels in response to exercise training, we can see that women without polymorphism at position -786T>C and Intron 4b/a were more responsive to the beneficial effect of exercise training compared to those who carried genetic variations (Table 3 and Figure 2). On the other hand, the presence of eNOS gene polymorphism at position G894T (Glu298Asp) did not affect the beneficial effect of exercise training in reducing total cholesterol and LDL-C levels in postmenopausal women (Table 3 and Figure 2). Furthermore, trained postmenopausal women without eNOS gene polymorphism at position -786T>C and Intron 4b/a showed a decrease in atherogenic index compared to those who carried the mutant alleles. However, this reduction was not statistically significant (Figure 2).

Oxidative stress status, physical training and eNOS gene polymorphism

Plasma levels of SOD and MDA, markers of antioxidant and oxidant status, respectively, did not differ between groups under basal conditions. After 8 weeks of exercise training, we observed a significant increase in plasma SOD levels in all groups studied, which was genotype-independent. On the other hand, exercise training did not affect plasma MDA levels in postmenopausal women. All data are summarized in Table 4.

Discussion

The present study examined the effect of aerobic exercise training on the arterial blood pressure, lipid profile and redox state of postmenopausal women without or with eNOS gene polymorphism at position -786T>C, G894T (Glu298Asp) and Intron 4b/a. Our findings show that 8 weeks of physical training were effective in reducing systolic and diastolic blood pressure values in postmenopausal women, an effect that was genotype-independent. On the other hand, women without eNOS gene polymorphism at position -786T>C and Intron 4b/a showed a greater reduc-

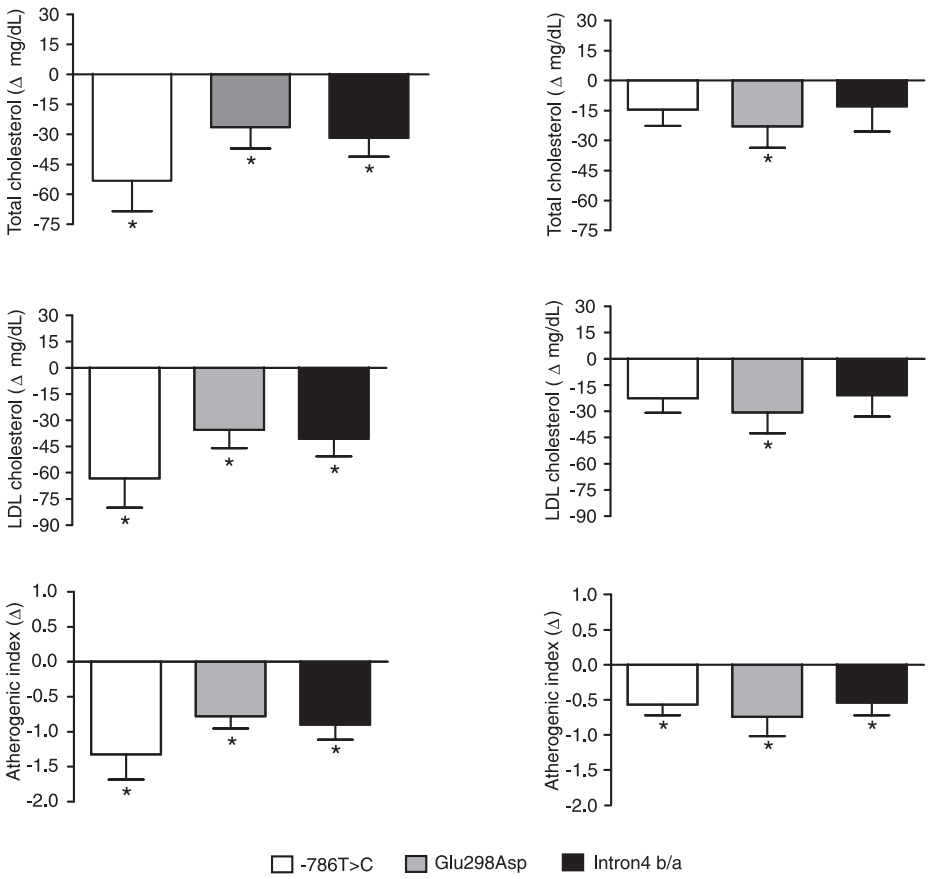


Figure 2. Effect of exercise training for 8 weeks on plasma total cholesterol and LDL cholesterol levels and atherogenic index of postmenopausal women without eNOS gene polymorphism (left panels) or with eNOS gene polymorphism (right panels) at positions -786T>C, Glu298Asp, and Intron 4b/a. Data are reported as means ± SEM for 49 women.

tion of total plasma cholesterol and LDL-C in response to 8 weeks of exercise training than those who carried the mutant allele.

The relationship between eNOS gene polymorphism and the incidence of CVD has been studied in different populations (9-11). However, the data for the three most studied eNOS polymorphisms, T-786C, G894T and Intron 4b/a, are not yet conclusive (12). Evidence has shown that physically active subjects have more longevity with less morbidity and mortality (23,24). Confirming previous studies, in the present investigation, we observed that a 8-week exercise training was effective in lowering both systolic and diastolic blood pressure in postmenopausal women. On the other hand, we found no relationship between the beneficial effects of 8 weeks of exercise training in lowering arterial blood pressure and the presence of the eNOS gene polymorphisms -786T>C, G894T (Glu298Asp) and Intron 4b/a in postmenopausal women. Interestingly, a previous study from our laboratory showed that women without eNOS polymorphism at position -786T>C were more responsive in lowering arterial blood pressure than those who carried allele C (14). The reason for this discrepancy might be the duration of the exercise training program. Indeed, in the present study, we employed only 8 weeks as compared to the previous one that was performed over a period of 24 weeks. Furthermore, previous studies have demonstrated that the volume of exercise training is more important in favoring health-promoting effects than its intensity (25,26).

Regarding lipid profile, the presence of eNOS polymorphism at position -786T>C and Intron 4b/a clearly influenced the beneficial effects of exercise training on the total cholesterol and LDL-C levels. Even though the atherogenic index did not differ significantly between the time before and after exercise training in both position -786T>C and Intron 4b/a, when comparing the TT and TC+CC groups and bb and ba+aa groups, we observed a tendency of this index to be lower in postmenopausal women with eNOS polymorphism

Table 4. Plasma superoxide dismutase activity (SOD, U/mL) and malondialdehyde levels (MDA, µM) before and after 8 weeks of aerobic exercise training by postmenopausal women with or without polymorphism for eNOS gene at position -786T>C, Glu298Asp and Intron 4b/a.

	Before	After	Δ%	Before	After	Δ%
	-786T>C					
	TT (N = 13)			TC+CC (N = 36)		
SOD	4.91 ± 0.53	5.83 ± 0.36*	18.7	4.84 ± 0.24	5.62 ± 0.30*	16.1
MDA	6.33 ± 0.53	5.77 ± 0.52	-8.8	5.60 ± 0.42	5.59 ± 0.29	-0.2
	Glu298Asp					
	GG (N = 27)			GT+TT (N = 22)		
SOD	5.06 ± 0.28	5.78 ± 0.30*	14.2	4.62 ± 0.37	5.54 ± 0.39*	-19.9
MDA	5.64 ± 0.33	5.26 ± 0.30	-6.7	5.98 ± 0.59	6.03 ± 0.36	0.8
	Intron 4b/a					
	bb (N = 31)			ba+aa (N = 18)		
SOD	4.94 ± 0.30	5.58 ± 0.33	13.0	4.73 ± 0.35	5.85 ± 0.32*	23.7
MDA	5.58 ± 0.42	5.91 ± 0.29	-5.4	6.15 ± 0.55	5.25 ± 0.42	-14.6

Data are reported as means ± SEM. TT, GG, and bb = non-polymorphic; TC+CC, GT+TT, and ba+aa = with polymorphism for eNOS gene. *P < 0.05, compared with before intervention (paired Student *t*-test).

compared to those who did not carry the mutant allele. The number of volunteers in the TT (N = 13) and ba+aa (N = 18) groups compared to the number of volunteers in the TC+CC (N = 36) and bb (N = 31) groups was probably the reason for this lack of difference. Regarding the magnitude of reduction of total cholesterol and LDL-C in women who carried the eNOS gene polymorphism at position G894T (Glu298Asp), we observed no changes between the GG and GT+TT groups. The reason for this is not clear at the present time. Indeed, few studies exist evaluating the influence of eNOS gene polymorphism on the lipid profile in human populations. To our knowledge, only two studies exist analyzing this issue and the results are contradictory. A previous study showed a positive relationship between the presence of the eNOS polymorphism at position G894T (Glu298asp) and alterations in lipid profile in men (27). In contrast, another study failed to find a relationship between eNOS polymorphism at position G894T (Glu298asp) and changing in blood flow in response to statins (28). Thus, the present study is the first to examine the influence of the three most studied eNOS gene polymorphisms [-786T>C, G894T (Glu298Asp) and Intron 4b/a] on the lipid profile. Considering the relevance of exercise training in preventing or improving the deleterious effects of chronic diseases such as dyslipidemia and atherosclerosis, our data support our hypothesis that eNOS polymorphism at position -786T>C and Intron 4b/a interferes with the beneficial effects of 8 weeks of exercise training on cardiometabolic diseases in postmenopausal women.

Regarding oxidative stress status, our data confirm previous results from our laboratory using an experimental model (29,30) showing that aerobic exercise promotes an improvement of antioxidant status by increasing plasma SOD levels, which in turn can ameliorate vascular and metabolic functions. In contrast, no change was found in thiobarbituric acid-reactive species concentrations in plasma measured as MDA in this particular population, showing that 8 weeks of exercise training did not modify the oxidant status. Furthermore, eNOS gene polymorphism does not influence the oxidative stress status under basal conditions. Our data agree with a previous study that showed that eNOS gene polymorphism at position -786T>C had no influence on the MDA levels of young subjects (31).

It is important to critically evaluate the limitations of our study. There are two limitations that need to be taken into account, i.e., the number of volunteers and the duration of the exercise training program. However, these limitations can be seen as an important window for future research under the same theme since the role of genetics and of physical exercise is relevant for the health of the population.

Thus, the novelty of the present study is that we examined the effect of the three most studied eNOS gene

polymorphisms on the cardiometabolic responses of postmenopausal women submitted to 8 weeks of exercise training. Our findings showed that the presence of eNOS gene polymorphism at position -786T>C and Intron 4b/a provoked a lower reduction in cholesterol levels in response to physical exercise suggesting that more attention should be given to this population regarding preventive actions related to plasma lipid control. Indeed, dyslipidemia is the primary cause of endocrine-metabolic disorders, which results in clinical outcomes such as myocardial infarction, stroke and peripheral artery disease and most postmenopausal women have lipid profile alterations (1). Therefore, preventive actions changing lifestyle for women's health are crucial since women live longer than men and effective prevention could decrease the high cost of the health care system for this population.

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