Low-dose Estrogen Is as Effective as High-dose Treatment in Rats With Postmenopausal Hypertension

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Abstract: This study was conducted to test the hypothesis that 17β-estradiol therapy improves redox balance by decreasing reactive oxygen species production and increasing nitric oxide (NO) bioavailability, favoring Akt pathway activation and resulting in a better autonomic vascular control. Ovariectomized female Wistar rats were divided into 4 groups: (1) vehicle (VL) and animals treated with a pellet of 17β-estradiol for 21 days; (2) low dose (LE; 0.05 mg); (3) medium dose (ME; 0.2 mg); and (4) high dose (HE; 0.5 mg). Arterial pressure and its sympathetic nervous system modulation were evaluated by spectral analysis. Nitric oxide synthase and NADPH oxidase (Nox) activities, H_2O_2 concentration, redox status (GSH/GSSG), protein expression of Trx-1 and p-Akt/Akt were evaluated in the aorta, whereas NO metabolites were measured in the serum. Estrogen-treated groups showed a significant decrease in arterial pressure and sympathetic vascular drive. Redox status was significantly improved and NADPH oxidase and H_2O_2 were decreased in all estrogen-treated groups. Estrogen also induced an enhancement in NO metabolites, nitric oxide synthase activity, and Akt phosphorylation. This study demonstrated that estrogen treatment to ovariectomized rats induced cardioprotection, which was evidenced by reduced blood pressure variability and vascular sympathetic drive. These effects were associated with an improved redox balance and Akt activation, resulting in an enhanced NO bioavailability.

Key Words: estrogen therapy, ovariectomized rats, blood pressure variability, hydrogen peroxide, thioredoxin, NADPH oxidase

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INTRODUCTION

Menopause is characterized by a natural decline in estrogen production, being responsible for physiological and biochemical changes involved in the development of cardiovascular diseases,[1] representing the main cause of mortality and morbidity in women older than 50 years.[2]

Menopause is also associated with oxidative stress, a condition in which there is an overproduction and/or inadequate removal of reactive oxygen species (ROS). A primary source of ROS in the vascular wall is NADPH oxidase,[3] a membrane-associated enzyme that generates superoxide anion (O_2^-).[4] There is substantial evidence showing that the excessive generation of O_2^- by NADPH oxidase contributes to oxidative stress associated with cardiovascular diseases[5]; whereas, estrogens are known by their cardioprotective effects, which are responsible for the inhibition of NADPH oxidase.[6]

Endogenous antioxidant systems, such as thioredoxin and glutathione (GSH), act against oxidative stress.[7] The thioredoxin family comprises at least 3 isoforms: thioredoxin-1 (Trx-1), thioredoxin-2, and sperm thioredoxin,[8] which are essential for cell survival and the maintenance of cellular thiol redox balance.[7] GSH, an important antioxidant, is a ROS scavenger. The GSH/GSSG ratio is a relevant couple that represents the redox balance of cells.[9] Redox balance is important in the modulation of the levels of phosphorylation and, consequently, the activation of some intracellular proteins.[10]

It is known in the literature that estrogen antioxidant actions are also mediated by protein kinase B (Akt) activation, an important redox-sensitive signaling protein.[11] When activated, Akt phosphorylates a range of intracellular substrates that regulate growth, metabolism, and survival.[12]

Estrogen is able to increase endothelial nitric oxide synthase (eNOS) activity through Akt pathway that enhances nitric oxide (NO) release.[13] In fact, estrogen-mediated eNOS activation was also demonstrated in cultured human endothelial cells.[14] In addition, NO is necessary to modulate both responses to increases in arterial pressure and the sympathetic and parasympathetic nervous systems,[15] inhibiting the sympathetic nerve system activity,[16] and contributing to the regulation of vascular tone.

It is very well established in the literature that NO deficiency and the imbalance in the autonomic nervous system induce an increase in arterial pressure and its variability. The increased systolic arterial pressure variability (SAPV) is an important determinant for cardiovascular risk, which is independent from absolute arterial pressure levels.[17] Sympathetic overactivity is involved in the pathophysiological mechanism underlying abnormal SAPV,[18] which may be

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assessed through the spectral analysis, an important tool for noninvasive evaluation of the integrity of neurocardiac function,[19] and a higher SAPV means that the mechanisms responsible for arterial pressure control are less effective.[20,21] The prevalence of hypertension and cardiovascular disease increases significantly in postmenopausal women probably because estrogen deprivation may induce autonomic impairment[22]; however, estrogen therapy improves autonomic cardiovascular control.[23] According to Ong et al,[24] 75% of postmenopausal women from the United States are affected by essential hypertension, corroborating other studies that demonstrate that aging and estrogen withdrawal after menopause are accompanied by increases in blood pressure.[25]

Because estrogen therapy improves quality of life for women,[26] it is commonly used for controlling menopausal symptoms. However, at high doses, estrogen has been associated with an increased risk of uterine and breast cancer.[27,28] According to Lambrinoudaki et al,[29] the lowest effective estrogen dose should be used. Moreover, studies by the Women's Health Initiative and Heart and Estrogen/Progestin Replacement Study have demonstrated that, in addition to the type of hormone used (in this case, nonhuman horse estrogens and progestins), the number of years since menopause is crucial to determine the benefits of hormone treatment.

Thus, the aim of this study was to test whether an early administration of a physiological dose of 17β-estradiol would be as effective as supraphysiological doses of this hormone in the modulation of aortic oxidative stress and autonomic blood pressure control in ovariectomized rats.

We tested the hypothesis that estrogen therapy is able to improve redox balance by decreasing ROS production and increasing NO bioavailability, favoring prosurvival Akt pathway activation, and resulting in better autonomic vascular control.

METHODS

Drugs and Reagents

Ketamine hydrochloride was purchased from König Lab SA (São Paulo, Brazil) and xylazine, from Virbac do Brasil IP (São Paulo, Brazil). Estradiol and all other drugs/reagents were purchased from Sigma Chemical Co (St Louis, MO).

Animals and Groups

All procedures were approved by the institutional animal ethics committee. In total, 40 female Wistar rats (weight, 200–230 g) from the Federal University of Rio Grande do Sul were studied. They were kept at 20–22°C, 12:12 hour dark–light photoperiod, and submitted to bilateral ovariectomy under ketamine hydrochloride (80 mg/kg intraperitoneally) and xylazine anesthesia (16 mg/kg intraperitoneally).[30] After 1 week, each animal was given silastic capsules filled with 17β-estradiol diluted in sunflower oil (under ketamine hydrochloride and xylazine anesthesia), whereas the vehicle group was given only sunflower oil.[31] All animals had ad libitum access to water and regular rodent chow, and the experiments were conducted in accordance with institutional guidelines and the Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services, NIH Publication No. 86–23). The animals were divided into 4 experimental groups: (1) vehicle (VL; n = 10); (2) low dose (LE; n = 10); animals treated with a dose designed to be near the physiological 17β-estradiol dose (0.05 mg per pellet for 21 days)[32]; (3) medium dose (ME; n = 10), animals treated with 40% of the high dose of 17β-estradiol (0.2 mg per pellet for 21 days); and (4) high dose (HE; n = 10), animals treated with 17β-estradiol (0.5 mg per pellet for 21 days).[32]

Serum Hormone Determination

Serum estradiol was measured by electrochemiluminescence (Roche Diagnostics) at Weinmann Clinical Analysis Laboratory. Briefly, this test uses the principle of competitive assay using a polyclonal antibody against the 17β-estradiol.

Cardiovascular Evaluations

Twenty-four hours before, the experiment under ketamine hydrochloride (80 mg/kg intraperitoneally; König Lab SA) and xylazine anesthesia (16 mg/kg intraperitoneally; Virbac do Brasil), a catheter (PE-10) filled with saline was implanted into the carotid artery for arterial pressure measurement. This catheter was connected to a strain-gauge transducer (Narco Bio-Systems Miniature Pressure Transducer RP 1500) for direct measurements of mean arterial pressure (MAP) and heart rate (HR). MAP signals were recorded for 20 minutes with a microcomputer equipped with an analog-to-digital converter board (Windaq, 2-kHz sampling frequency; Dataq Instruments, Inc, Akron, OH). After the cardiovascular evaluations, rats were killed by decapitation, exsanguinated, and the uterus was harvested and weighed. The blood was collected by exsanguination to measure hormone and total nitrites concentrations, and the aorta was excised to Western blot and biochemical measurements.

Autonomic Evaluation

Time series of systolic arterial pressure (SAP, systograms) were obtained from blood pressure records. Stationary fragments with about 300 beats were selected, and spectral analysis was performed by an autoregressive model. The spectral bands for rats [low frequency (LF): 0.2–0.75 Hz; high frequency (HF): 0.75–3.0 Hz] were defined according to previous references,[33] being LF band related to sympathetic modulation.[34,35] HF represents respiratory sinus arrhythmia and is a reliable indicator of parasympathetic efferent activity in the heart, but in vessels, it does not have a physiological significance. Systogram spectra for each stationary fragment were evaluated quantitatively, and SAPV values were obtained.

NADPH Oxidase Activity

Superoxide production by aorta ring segments was measured using 5 μmol/L lucigenin-enhanced chemiluminescence, as previously described,[36] expressed as counts per minute per mg of dry weight of the vessel (cpm/g dw).

Hydrogen Peroxide Measurement

The assay was based on the horseradish peroxidase–mediated oxidation of phenol red by H_2O_2, resulting in the formation of a compound that absorbs at 610 nm. Aorta rings were incubated for 30 minutes at 37°C in phosphate buffer 10 mmol/L (140 mmol/L of NaCl and 5 mmol/L of dextrose), and the supernatants were transferred into tubes with 0.28 mmol/L phenol red and 8.5 U/mL horseradish peroxidase. After 25 minutes of incubation, 1 mol/L of NaOH was added, and it was read at 610 nm with the results expressed in nmoles H_2O_2/g tissue.[37]

Determination of Reduced and Oxidized Glutathione

To determine oxidized and reduced glutathione concentration, the aorta was deproteinized with 2 mol/L perchloric acid, centrifuged for 10 minutes at 1000*g*, and the supernatant was neutralized with 2 mol/L potassium hydroxide. The reaction medium contained 100 mmol/L phosphate buffer (pH 7.2), 2 mmol/L nicotinamide dinucleotide phosphate acid, 0.2 U/mL glutathione reductase, and 70 μmol/L 5,5′ dithiobis (2-nitrobenzoic acid). To determine reduced glutathione, the supernatant was neutralized with 2 mol/L potassium hydroxide. Then, 70 μmol/L 5,5′ dithiobis (2-nitro benzoic acid) was added, and the absorbance values were measured at 420 nm.[38]

Western Blot Analysis

Aortic samples were homogenized in ice-cold RIPA buffer (150 mmol/L NaCl; 50 mmol/L Tris-HCl; 5 mmol/L EDTA; 40 1% Nonidet-P; 0.5% deoxycholate; 10 mmol/L NaF; 10 mmol/L sodium pyrophosphate; 100 mmol/L phenylmethylsulfonyl fluoride; 2 μg/mL aprotinin; and 2 μg/mL leupeptin).[39] Aortic extracts were mixed with sample loading buffer and separated under reducing conditions on 12% SDS–polyacrylamide gel. Proteins were electrotransferred onto polyvinylidene fluoride (PVDF) membranes (Immuno-Blot 0.2 μm, Bio-Rad). The membranes were processed for immunodetection using mouse anti-thioredoxin-1 (Trx-1) (12 kDa), rabbit anti-total Akt1 polyclonal antibody, and rabbit anti-phospho-Akt1 (ser657; 60 kDa) (Santa Cruz Biotechnology, Santa Cruz, CA), and the bound primary antibodies were detected using rabbit anti-mouse or goat anti-rabbit HRP–conjugated secondary antibodies, and membranes were revealed for chemiluminescence. The autoradiographies generated were quantitatively analyzed for the protein levels with an image densitometer (ImageMaster VDS CI, Amersham Biosciences Europe). The molecular weights of the bands were determined by reference to a standard molecular weight marker (RPN 800 rainbow full range Bio-Rad), and Trx-1 results were normalized by Ponceau red method.[40]

NOS Activity

NOS activity was assessed by measuring the NO-induced conversion of oxyhemoglobin (HbO_2) to methemoglobin oxyhemoglobin as previously described by Valdez et al.[41] The reaction medium was composed of: $CaCl_2$ 1.8, KCl 2.7, $MgCl_2$ 0.23, NaCl 137, NaH_2PO_4 3.6, glucose 5.0, HEPES 10 (units in mmol/L), pH 7.4, containing 2 μmol/L HbO_2 and 1 mmol/L L-arginine.

NO Metabolites

Total nitrites were determined in the serum according to Granger et al. To convert nitrite to nitrate, samples were incubated with enzymatic cofactors and nitrate reductase (1.75 U/mL; Sigma, St Louis, MO) for 30 minutes at room temperature. The reaction was finished by the addition of Griess reagent, and the colored final product obtained was determined spectrophotometrically at 542 nm.[42]

Statistical Analysis

Data are shown as mean ± SD. Statistical analysis was performed using 1-way analysis of variance followed by Student–Newman–Keuls post hoc test. The Pearson correlation was used to assess the association among variables. $P < 0.05$ was considered significant.

RESULTS

Ovariectomy and Estradiol Treatment

To confirm the effectiveness of estrogen treatment, hormonal and morphometric measurements were performed. As expected, 17β-estradiol treatment increased significantly ($P < 0.001$) its serum concentration (LE = 124 ± 3; ME = 600 ± 19; HE = 1818 ± 39 pg/L) and uterine weight (LE = 0.29 ± 0.11; EM = 0.61 ± 0.06; HE = 0.86 ± 0.15 g) compared with VL (estradiol concentration = 61 ± 8 pg/L; uterine weight = 0.15 ± 0.02 g). Body weight also decreased by estradiol treatment (LE = 222 ± 13; ME = 214 ± 14; HE = 216 ± 9 g) as compared with the vehicle group (VL = 247 ± 15 g; $P < 0.05$).

Hemodynamic and Autonomic Evaluations

MAP and diastolic arterial pressure (DAP) were significantly ($P < 0.05$) decreased in the estrogen-treated groups when compared with the VL group, and there was no difference among the estrogen-treated groups. HR and SAP did not show any differences among groups. LF, which is associated with the vascular sympathetic modulation, decreased only in LE and HE groups. SAPV was significantly ($P < 0.05$) decreased in all estrogen-treated groups when compared with the VL group, and there was no difference among the estrogen-treated groups. HF did not show any differences among groups (Table 1).

NADPH Oxidase Activity

NADPH oxidase activity (in cpm/g dw) was significantly lower ($P < 0.01$) in all estrogen-treated groups (LE = 22,838 ± 3636; ME = 17,195 ± 7090; HE = 7470 ± 2850) when compared with the VL group (37,750 ± 9900; Fig. 1A). Moreover, this enzyme activity was lower in the HE group when compared with LE ($P < 0.05$), which seems to be dose dependent.

Hydrogen Peroxide Concentration

H_2O_2 concentration (in nmol/g tissue), measured in aortas, was significantly ($P < 0.01$) lower in all treated groups

TABLE 1. Hemodynamic and Autonomic Evaluations in Ovariectomized Rats Treated With 3 Different Doses of Estrogen

	VL (n = 5)	LE (n = 5)	ME (n = 5)	HE (n = 5)
MAP (mm Hg)	118 ± 3	105 ± 6*	109 ± 4*	104 ± 4*
DAP (mm Hg)	105 ± 4	94 ± 9*	94 ± 4*	90 ± 2*
SAP (mm Hg)	141 ± 2	128 ± 14	139 ± 5	134 ± 9
HR (bpm)	360 ± 42	338 ± 46	349 ± 32	338 ± 28
SAPV (mm Hg^2)	11.54 ± 3.64	4.26 ± 1.19*	5.22 ± 1.58*	5.24 ± 2.18*
LF (mm Hg^2)	3.60 ± 2.21	0.59 ± 0.25*	2.01 ± 1.13	1.00 ± 0.46*
HF (mm Hg^2)	3.17 ± 2.33	1.32 ± 0.65	1.16 ± 0.3	1.78 ± 1.18

*$P < 0.05$ versus VL.
Data are mean ± SD.

(LE = 0.69 ± 0.05; ME = 0.44 ± 0.10; HE = 0.2 ± 0.03) when compared with the VL group (1.46 ± 0.39; Fig. 1B). Moreover, this parameter was lower in the HE group when compared to LE ($P < 0.05$), which seems to be dose dependent.

Determination of Reduced and Oxidized Glutathione

The treatment with estrogen increased significantly ($P < 0.05$) GSH and decreased GSSG in comparison with the VL group (Table 2). The redox status (GSH/GSSG) was significantly increased in LE and HE groups ($P < 0.05$) when compared with VL.

Trx-1 and p-Akt/total Akt Protein Expression

Estrogen treatment decreased significantly Trx-1 expression ($P < 0.05$) (LE = 80 ± 20; ME = 62 ± 20; HE = 80 ± 14) as compared with VL (VL = 150 ± 36; Fig. 2). The expression p-Akt protein did not differ among groups (Fig. 3A). Total Akt protein expression was significantly reduced in ME and HE groups as compared with VL ($P < 0.05$; Fig. 3B). The ratio p-Akt/total Akt was increased in all estrogen-treated groups (LE = 109 ± 19; ME = 94 ± 6; HE = 128 ± 9) as compared with VL (66 ± 13; $P < 0.05$; Fig. 3C). The ratio p-Akt/total Akt was significantly higher in HE than in ME ($P < 0.05$).

NO Measurements

Total nitrites (in μmol/L) and NOS activity (in nmol NO/mg protein min) were significantly higher in all groups treated with estrogen ($P < 0.05$) (total nitrites: LE = 0.231 ± 0.034; ME = 0.237 ± 0.068; HE = 0.215 ± 0.023; and NOS: LE = 1.4735 ± 0.3159; ME = 1.5488 ± 0.2529; HE = 1.1894 ± 0.1002), when compared with VL (total nitrites: 0.126 ± 0.04; and NOS: 0.8358 ± 0.0457; Figs. 3B, C, respectively), and there was no difference among the treated groups. This result does not seem to show a dose effect, and a negative correlation between total nitrites and SAPV ($R = -0.81$; $P < 0.001$) was also found (Fig. 4).

DISCUSSION

The major finding of this study was to demonstrate that estrogen administration, even in the lowest dose, to ovariectomized hypertensive rats was able to improve redox balance and NO bioavailability in the aorta, which was associated to an enhanced activation of the prosurvival signaling protein Akt. These effects were accompanied by a decrease in sympathetic activity to blood vessels, resulting in reduced blood pressure and its variability.

The effectiveness of the model used in this study was demonstrated by the elevation in the serum estrogen levels and uterine weight and reduction in the body weight in the estrogen-treated rats. Paigel et al[43] observed serum estrogen levels in ovariectomized rats similar to those observed by us.

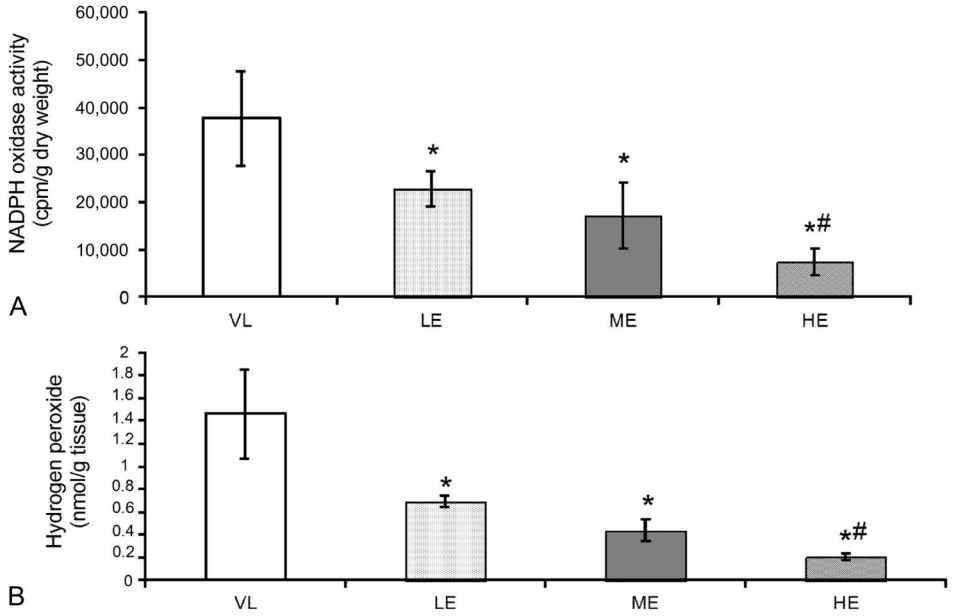

FIGURE 1. A, NADPH oxidase activity (cpm/g dw; VL, n = 4; LE, n = 4; ME, n = 5; HE, n = 5) in aorta, and (B) hydrogen peroxide concentration (nmol/g tissue; VL, n = 5; LE, n = 5; ME, n = 4; HE, n = 5) in aorta. *$P < 0.01$ versus VL group; #$P < 0.05$ versus LE group. Data are mean ± SD.

TABLE 2. Reduced and Oxidized Glutathione Levels in Aorta of Ovariectomized Rats Treated With 3 Different Doses of Estrogen

	VL (n = 5)	LE (n = 4)	ME (n = 4)	HE (n = 6)
GSH (pmol/mg protein)	2.60 ± 2.20	5.60 ± 0.10*	7.00 ± 0.10*	5.60 ± 1.0*
GSSG (pmol/mg protein)	9.40 ± 3.70	1.8 ± 1.0*	3.0 ± 0.30*	2.60 ± 2.10*
GSH/GSSG (redox status)	0.67 ± 0.27	1.76 ± 0.51*	1.41 ± 0.35*	1.71 ± 0.65*

*$P < 0.05$ versus VL group.
Data are mean ± SD.

Moreover, the ovary-intact rats from Paigel's[43] study had estrogen concentrations similar to those found in our LE group, suggesting that the low estrogen dose reached physiological levels.

Regarding blood pressure data, our findings are in agreement with the study of Flues et al,[44] showing very similar values of systolic and diastolic pressure for ovariectomized rats. In our study, the lowest dose of estradiol used was able to reduce DAP significantly to values close to those found in the studies of Flues et al[44] and Silva et al[45] for intact animals in the same magnitude as the higher doses. It suggests that probably the basal tonus of the vessels was already reached with the lowest dose of estrogen.

We also found a significant decrease in MAP in all treated groups, although no differences were found in HR and SAP among the groups. These results are in agreement with studies by He et al[46] and Masi et al,[47] who did not find changes in HR and SAP after estrogen treatment either. Our results are in agreement with previous studies, showing a decrease in the blood pressure after estrogen treatment both in subjects[48] and experimental models.[46] We also found that the decrease in MAP was due to reduction in DAP, indicating that estrogen induced a decrease in total peripheral resistance, which was not a dose-dependent effect. These data are in accordance with Magness and Rosenfeld,[49] who demonstrated a fall in systemic vascular resistance with estrogen administration. It is very well established in the literature that estrogen has a vasodilatory role,[46,50,51] and that the estrogen-induced vasorelaxation is mediated by NO.[14] We also found a decrease in SAPV in all estrogen-treated groups without showing differences related to doses.

Czarnecka et al,[52] studying postmenopausal hypertensive women, found a sympathetic nervous system overactivity, suggesting the participation of this system in the pathogenesis of hypertension in this group of patients. Similarly, in our study, the hypertensive ovariectomized rats also showed this result and the estrogen treatment, even in the lower dose, reverted the blood pressure. This result is in accordance with Head et al[53] who observed a lower SAPV in the presence of estrogen. Our findings indicate an improvement in the arterial pressure control, probably due to a decrease in sympathetic modulation in the vessels. This conclusion is reinforced by the fact that LF component of SAPV, associated to the vascular sympathetic modulation, was reduced after estrogen treatment. The present finding also agrees with that of El-Mas and Abdel-Rahman[54] who observed reduced LF oscillations of SAP in rats treated with estrogen. HF does not have a physiological significance in the vessels, and it was not significantly different among the groups. Our results highlight the estrogen effectiveness to decrease sympathetic nervous system participation and arterial pressure variability, even in a low dose. These results may have strong impact on occurrence of cardiovascular events because patients with normal mean SAP, but high variability, are at increased risk.[55]

This reduction of sympathetic drive was associated with a significant decrease in NADPH oxidase activity in all animals treated with estrogen, in a dose-dependent way, suggesting a reduction in superoxide anion production by the aorta. This finding is in agreement with the study of Wagner et al[56] using a pharmacological dose of estrogen. Our results are also in consonance with the findings of Gao et al,[57] which showed that NADPH oxidase–derived ROS play an important role in the modulation of sympathetic activity. Aortic hydrogen peroxide concentration was also shown to be reduced dose dependently in the estrogen-treated rats. Similar result was observed in the study of Dantas et al.[58]

A decreased ROS production may improve the cellular redox balance. In fact, GSH/GSSG ratio, an important redox balance index,[9] was significantly increased after estrogen treatment with all doses, indicating that there was a reduction

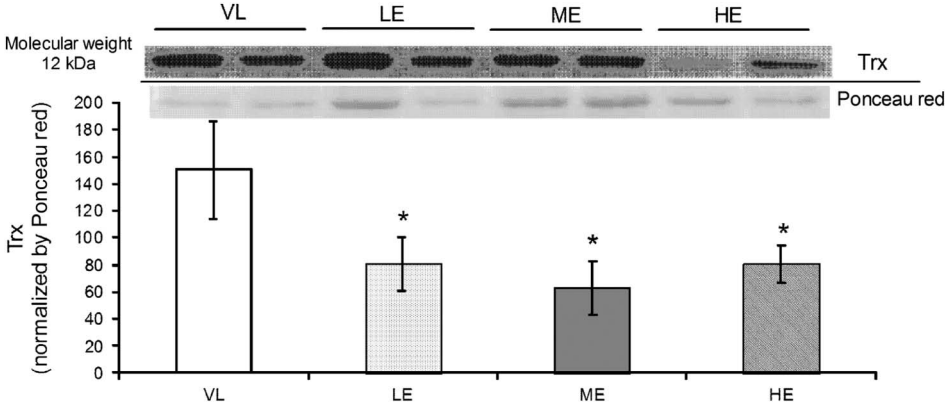

FIGURE 2. Western blot analysis in aorta homogenates using Trx-1 antibody, 1 representative gel showing 2 bands for each experimental group (n = 4 in each group). *$P < 0.01$ versus VL group. Data are mean ± SD.

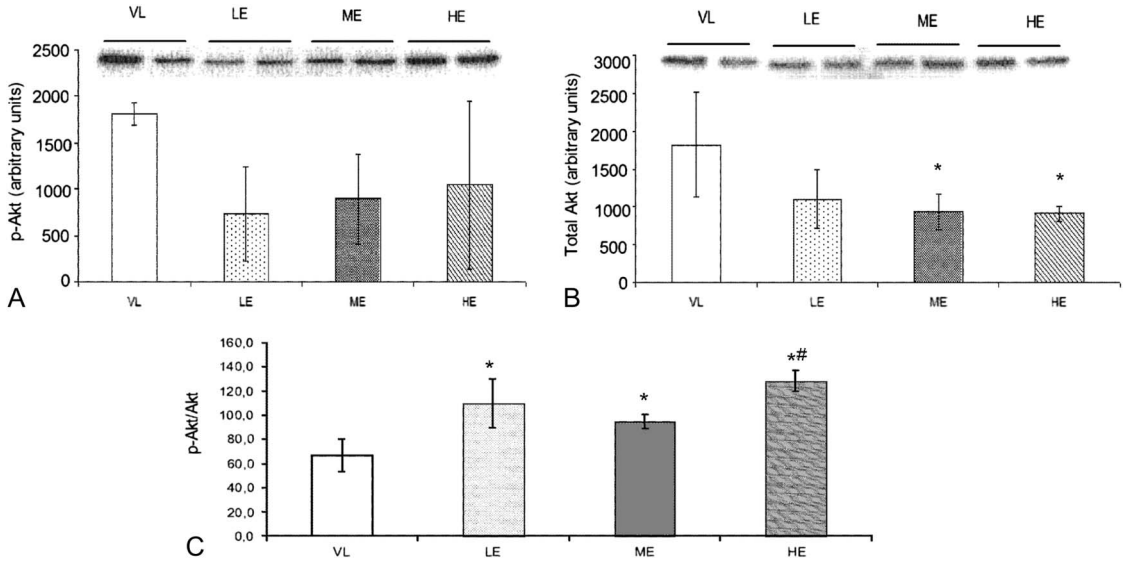

FIGURE 3. Western blot analysis in aorta homogenates using p-Akt (ser657) (p-Akt (ser657)/Akt ratio) antibody. One representative gel showing 2 bands for each experimental group (n = 4 in each group); (A) p-Akt, (B) total Akt, and (C) p-Akt (ser657)/Akt ratio. *$P < 0.05$ versus VL group; #$P < 0.05$ versus ME group. Data are mean ± SD.

in oxidative stress in treated groups independently of the dose. It is important to highlight that, although the higher dose of estrogen has promoted less ROS production than the others, it did not determine a better redox balance. Also, another important marker of imbalance redox is the Trx-1,[59] which increases its levels during oxidative stress in endothelial cells.[7,60] Indeed, in all treated groups, the presence of estrogen protected against oxidative stress and reduced Trx-1 expression.[61]

Cellular redox balance exerts an important role in the modulation of many intracellular signaling pathways. Among the numerous redox-sensitive signaling pathways is Akt, which can be activated by the binding of estrogen to its membrane receptor.[62] According to the literature, estrogen activates eNOS through the Akt pathway, promoting NO production.[62]

We observed a significant increase in NO metabolites and in NOS activity in all treated groups, independently of the dose, suggesting that, in this protocol, there is an association of estrogen, Akt activation, and increased NO bioavailability. These results may be due to the influence of NO bioavailability directly on blood vessel function and brain centers

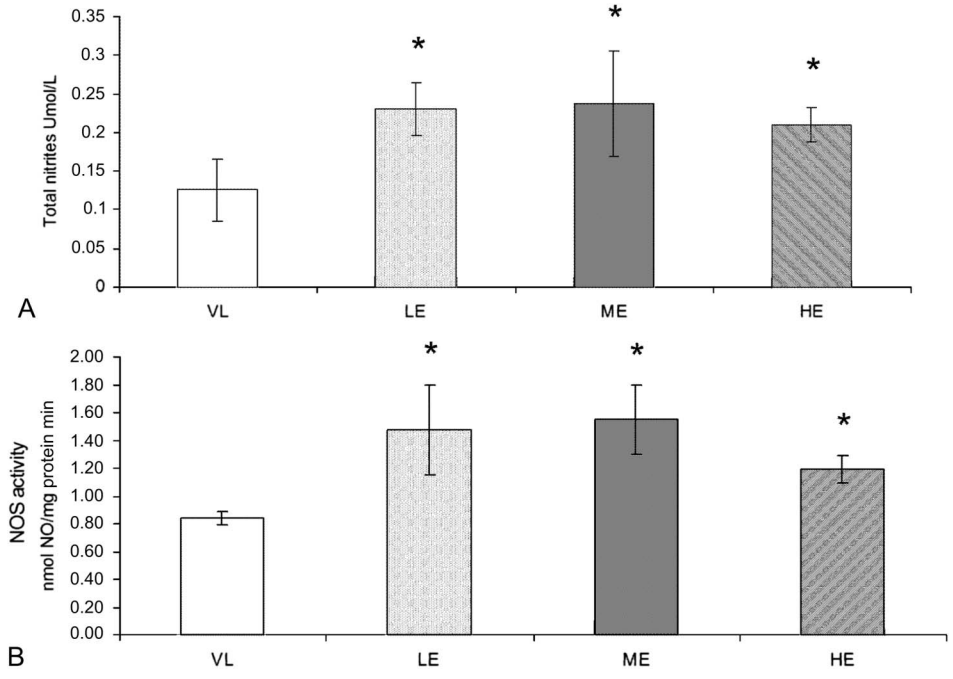

FIGURE 4. Serum total nitrites concentration (A) and NOS activity (B); VL, n = 6; LE, n = 8; ME, n = 6; HE, n = 8. *$P < 0.05$ versus VL group; #$P < 0.05$ versus ME group. Data are mean ± SD.

involved in autonomic nervous system regulation,[63] determining vascular tone through interactions between endothelial control and neural mechanisms. These beneficial effects of estrogen were achieved even with the lower dose used in this study, showing a negative correlation between total nitrites and SAPV. This finding is in consonance with a previous study that demonstrated that NO has a role in the reduction of sympathetic tonus to the blood vessels, contributing to the improvement in the blood pressure control.[15]

Our data indicate a cardiovascular protection provided by a low dose of estrogen, which can cause an increase in NO bioavailability. This lower dose might be safer than high doses,[64,65] being a potential therapeutic approach to minimize the menopause symptoms.

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