ORIGINAL ARTICLE

# Comparative study of nitric oxide production induced by selective estrogen receptors alpha and beta agonists in rats*

## *Comparação da produção de óxido nítrico mediada por agonistas seletivos de receptores estrogênicos alfa e beta em ratos*

Graciliano Ramos Alencar do Nascimento[1], Célia Akemi Kanashiro[1], Michelle Serock[2], Raouf Khalil[3], Maria Aparecida da Silva Pinhal[4]

**Received from the Departamento de Ciências Fisiológicas da Universidade Estadual de Ciências da Saúde de Alagoas, Maceió, AL, Brazil.*


## ABSTRACT

**BACKGROUND AND OBJECTIVES**: Nitric oxide (NO) is a potent vasodilator and estrogen-mediated vasodilation that increases NO production. The association of the vascular endothelium, gender and vasodilation induced by estrogen is due to the activation of two estrogen receptors, alpha (ERα) and beta (ERβ). The aim of this study was to compare NO production stimulating receptors ERα and ERβ with the use of selective agonists in thoracic aortas of rats. **METHODS**: Aortic rings were either treated with 17 β-estradiol (17-BE2); acetylcholine (Ach); 4,4',4-[4-propil-(1H)-pirazol-1,3,5-triyl]tris-phenol (PPT), and 2,3-Bis(4-hydroxyphenyl)-propionitrile (DPN), or left untreated, and the concentration of NO was determined by spectrophotometry method. **RESULTS**: The females presented a higher basal concentration of nitrite than males. PPT determined increased production of nitrite in both females and males, compared to 17-beta-estradiol (17-BE2). In males, the production of nitrite induced by DPN and PPT was higher than that induced by 17-BE2. The stimulation with 17-BE2 increased the production of nitrite in females compared to males. Regardless the gender, the stimulation of aortic rings by PPT caused a greater production of nitrite compared to that induced by 17-BE2. Interestingly, the stimulation of aortic rings from males with DPN provided an increase in the nitrite production compared to the levels induced by 17-BE2 incubation. **CONCLUSION**: The stimulation of estrogen receptor (ER) by PPT provides greater production of nitrite than 17-BE2 regardless of gender; in males, the stimulation of ER by DPN provides bigger production of nitrite than 17-BE2; the basal production of nitrite is higher in females compared to males.


**Keywords**: Estrogens; Adrenergic alpha-agonists; Adrenergic beta-agonists; Nitric oxide; Rats.


## RESUMO

**JUSTIFICATIVA E OBJETIVOS**: O óxido nítrico (NO) é um potente vasodilatador e o estrógeno promove vasodilatação aumentando a produção de NO. A associação entre endotélio vascular, gênero e vasodilatação induzida pelo estrógeno, é pela ativação de receptores estrogênicos, alfa (ERα) e (beta) ERβ. O objetivo deste estudo foi comparar a produção de NO estimulando receptores estrogênicos, ERα e ERβ, por agonistas seletivos em aorta torácica de ratos. **MÉTODOS**: Anéis aórticos foram tratados com 17 β-estradiol (17-BE2), acetilcolina (Ach), 4,4',4-[4-propil-(1H)-pirazol-1,3,5-triyl]tris-fenol (PPT) e 2,3-Bis(4-hidroxifenil)-propionitrila (DPN) ou não tratados e a determinação de NO foi feita por método espectrofotométrico. **RESULTADOS**: As fêmeas apresentaram produção constitutiva basal de nitrito mais elevada do que os machos. O PPT causou elevação na produção de nitrito em ambos os sexos, em relação ao observado com 17-beta estradiol (17-BE2). Nos machos, PPT e DPN, aumentaram a produção de nitrito comparada àquela induzida por 17-BE2. A estimulação com 17-BE2 causou maior produção de nitrito em fêmeas que em machos. A incubação com PPT determinou maior produção de nitrito comparada àquela induzida por 17-BE2, independente do gênero. Interessantemente, em machos, a estimulação das artérias com DPN acarretou em elevação na produção de nitrito comparada ao efeito causado por 17-BE2. **CONCLUSÃO**: A estimulação de receptor estrogênico (ER) pelo PPT determina maior produção de nitrito do que 17-BE2 independente do gênero; a estimulação de ER pelo DPN determina maior produção de nitrito do que 17-BE2 em machos; a produção basal de nitrito é mais elevada em fêmeas comparada aos machos.


**Descritores**: Estrogenios; Agonistas adrenérgicos alfa; Agonistas adrenérgicos-beta; Óxido nítrico; Ratos.

1. Universidade Estadual de Ciências da Saúde de Alagoas, Maceió, AL, Brazil.
2. Northeast University, Boston, MA, US.
3. Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, US.
4. Universidade Federal de São Paulo , São Paulo, SP, Brazil.

Submitted in April 23, 2013.
Accepted for publication in August 05, 2013.
Conflict of interests: None

**Correspondence to:**
Graciliano Ramos Alencar do Nascimento. M.D.
Departamento de Ciências Fisiológicas
Universidade Estadual de Ciências da Saúde de Alagoas-UNCISAL
Rua Jorge de Lima 113 – Trapiche da Barra
57010-300 Maceió, AL, Brazil.
E-mail: graciliano_alencar@hotmail.com

## INTRODUCTION

Nitric oxide (NO) is a potent vasodilator, relaxing vascular smooth muscle (VSM). NO is produced by the vascular endothelium by the transformation of L-arginine into L-citruline enzyme family nitric oxide synthase (NOS). There are three types of NOS: neuronal (nNOS, NOS I), induced (iNOS, NOS II), and endothelial (eNOS, NOS III)[1]. Currently, the IN-derived endothelial cells is considered essential for vascular homeostasis and has been the target for the prevention of cardiovascular diseases[2]. Estrogen promotes vasodilatation mediated by increased expression of eNOS via genomic as well as increased eNOS activity by non-genômica[3]. It has been found, in humans, a positive correlation between production of NO and plasma levels estrógeno[4-7]. Other researchers have demonstrated that in mice there was a greater release of endothelial NO in the arteries of females compared to males arteries, demonstrating the occurrence of differences in NO production in different gender[6,8,9].

The vascular endothelial (VE) is essential for the establishment of an association between gender and vasodilation induced estrógeno[8,10]. The EV human, including the umbilical vein (HUVEC), expresses two isoforms α and β for the estrogen receptor (ER), and actions of estrogen have been suggested as modulators of vascular physiology in different studies of animal cell cultures and humans[11]. Estrogen is a potent vasodilator and increases the flow in the brachial artery in women during the post-menopausa[12-14]. Cellular signaling is mediated by two estrogen receptors, ERα (NR2A1) and ERβ (NR3A2), both belonging to the family of nuclear receptors and factors transcrição[15]. These ERs have been identified in vascular endothelium and vascular smooth muscle cells and adventitial animal humanos[16-18]. ERβ tissues occurs more often than ERα, but both are present in the kidneys, and vessels. The ERβ is found predominantly in cells of VSM humana[19]. There are two selective agonists for estrogen receptors: 4,4 ', 4 "- [4-propyl-(1H)-pyrazole-1 ,3,5-triyl] tris-phenol (PPT) which has a 410 fold higher affinity ERα for compared to ERβ[20], while 2,3-bis (4-hydroxyphenyl) propionitrila (DPN) has 170 times higher affinity for ERβ than ERα[21]. Recently, using PPT or DPN, the researchers demonstrated that the vascular relaxation induced by PPT was dose-dependent ERα and similar to the effects induced by estradiol. On the other hand, observed that stimulation of ERβ by DPN had no effect, thus demonstrating the importance of ERα in vasos[22]. Other researchers have shown that activation of ERα by PPT promotes a protective effect on myocardial ischemia and reperfusion situations[23].

Thus, the present study aimed to compare the NO production in thoracic aortas of rats stimulated by agonists selective estrogen receptor α and β, and compare with production stimulated by 17-β estradiol (17-BE2).

## METHODS

We used 32 rings arteries thoracic aortas of adult virgin mice, male and female wistar coming the Center for Experimental Surgery and vivarium UNCISAL. They were divided into 4 groups stimulation with: acetylcholine (Ach) 17-β estradiol (17-BE2),4,4',4 "-[4-propyl-(1H)-pyrazole-1,3,5-triyl] tris-phenol (PPT) and 2,3-bis (4-hydroxyphenyl)-propionitrila (DPN). These groups were matched for males and females and dosages in triplicates. To obtain the rings, the animals were anesthetized with ketamine (1000mg/kg) and xylazine (14mg/kg) and then were euthanized with a solution of potassium chloride (KCl) 19% intracardiac route. Obtained rings, they were cleaning of the connective tissue immersed in 2mL of Krebs solution in a water bath at 37° C, aeration (95% O2 / 5% CO2) and allowed to equilibrate for 45 minutes. 50 µL were removed from each solution the test tube, and placed on plate spectrophotometer (for determining the value of basal production of NO tissue). In the remaining volume of the tube were added 18.5 µL agonist desired in the following concentrations ($10^{-3}$ M Ach, 17-BE2 $10^{-6}$ M, $10^{-6}$ M DPN, PPT $10^{-6}$ M) as the experimental groups. The tissues were incubated with various agonists for 20 minutes. After the time of the stimulus, were again removed 50µL of solution from each tube and placed on plate spectrophotometer (for determining the amount of production of NO after stimulation). Then were added to Griess reagents (Griess Reagent System) brand Promega (Promega Corporation, Madison, WI, USA) to determine the quantitative NO[24]. The absorbance reading was performed in a spectrophotometer (535nm). All experiments were performed in triplicate. After reading the absorbance data were placed in Excell program for calculation for determination of NO production. Folders was used a standard curve of nitrite, to allow calculation of the amount of nitrite solution being analyzed using the absorbance data. For quantification of nitrite per mg of tissue, the rings were weighed after being dried with paper towel, and the results were expressed as nitrite concentration by dry weight of the tissue (pmoL nitrite/mg dry tissue). Data on production of nitrite were analyzed using Student's t-test

When necessary, we used Analysis of Variance one or two ways to test the validity of hypothesis ($p<0.05$). The data were presented using the mean value (X) and standard deviation (SD).

This study was approved by the Ethics Committee in Research of the Universidade Estadual de Ciências da Saúde of Alagoas, protocol 52-A/2007.

## RESULTS

**Effect of Agonist on nitrite production in rat aortae**

Rings of thoracic aortas obtained from rats after equilibration in Krebs solution were stimulated with agonists 17-BE2 $10^{-6}$M, $10^{-3}$M Ach, PPT $10^{-6}$M and $10^{-6}$M DPN for 20 minutes and nitrite production were determined spectrophotometrically.

It was found that all agonists caused a significant increase ($p<0.05$) in nitrite production compared with their respective baseline values (Table 1, Figure 1).

It can also be seen from the data presented in table 1 that already exist significant differences between the basal levels of nitrite production between males and females.

**Agonist effect on nitrite production in rat aortas**

For comparative purposes, the production of nitrite was measured in rings of thoracic aortas obtained from rats. The tissues, after stabilization with Krebs solution, were incubated for 20 minutes with the same agonists and at the same concentrations

Table 1 - Production of nitrite (pmoL/mg dry tissue) in rings of thoracic aortas of male and female rats.

| Agonists | Nitrite concentration (pmol / mg dry tissue) | | | |
|---|---|---|---|---|
| | Females | | Males | |
| | Basal | Stimulated | Basal | Stimulated |
| 17-BE2 $10^{-6}$ M | 1162,0 ± 173,7 | 1416,2 ± 194,4* | 1148,4 ± 138,8† | 1252,4 ± 120,4* |
| Ach $10^{-3}$ M | 845,2 ± 41,0 | 1257,1 ± 306,6* | 414,9 ± 17,3† | 433,9 ± 91,1* |
| PPT $10^{-6}$ M | 592,3 ± 41,4 | 882,7 ± 241,6* | 216,6 ± 44,7† | 362,3 ± 85,6* |
| DPN $10^{-6}$ M | 901,2 ± 134,7 | 1093,5 ± 332,9* | 217,2 ± 82,9† | 374,7 ±± 158,2* |

* Significant difference (p<0.05) with the respective control baseline.
† Significant difference (p<0.05) between males and females.

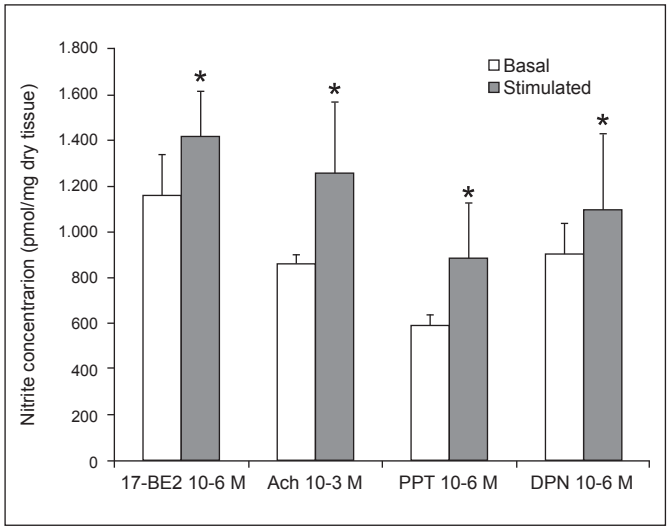


Figure 1 - Effect of agonist on the production of nitrite in rats. Aortic arteries were isolated and incubated with various agonists for 20 minutes and nitrite production was determined spectrophotometrically at 535 nm wavelength.

The data correspond to the mean and SD values of triplicate experiments performed on rings (4 independent experiments). *Significantly different from its baseline (p<0.05, Student's t-test).

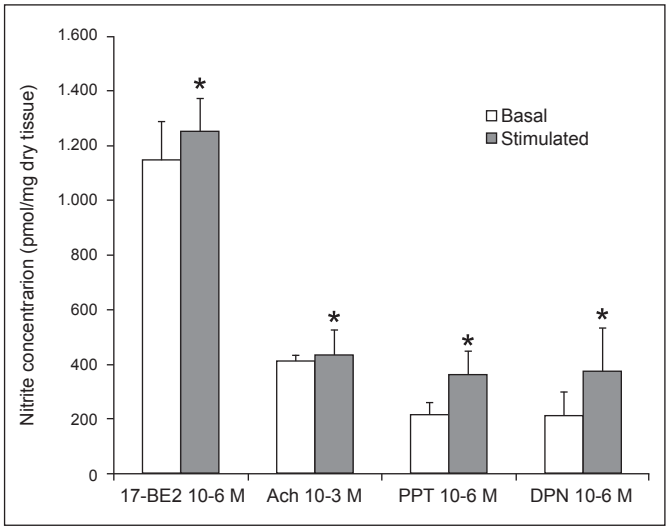


Figure 2 - Agonist effect on nitrite production in males. Isolated rat aortic arteries were incubated with various agonists for 20 minutes and nitrite production was determined spectrophotometrically at 535 nm wavelength.

Data represent mean and SD values of triplicate experiments performed on rings (4 independent experiments). *Significantly different from its baseline (p<0.05 Student's t- test).

used previously in tissues from females. It was observed that all agonists caused statistically significant increases (p<0.05) in nitrite production when faced with their respective baseline values (Figure 2, Table 1).

**Comparison of production of nitrite induced by various agonists females**

The effects on nitrite production stimulated by different agonists, 17-BE2 $10^{-6}$M, Ach $10^{-3}$M, PPT $10^{-6}$M and DPN $10^{-6}$M, in females are shown in figure 3 and table 1. As can be seen in table 1, there is a lot of variation between the baseline absolute numerical values for the different groups, thus facilitating the statistical analysis, the data were normalized taking into account the baseline control value as 100% (Figure 3).

It was found that nitrite production in female aortas showed greater efficacy when challenged with Ach or PPT, increase of around 50% above their baseline (p<0.05) (Figure 3). On the other hand, when the stimuli in the production of nitrite induced by 17-BE2 and DPN were compared to their respective controls standardized baseline was observed that although there is a tendency to increase the production of nitrite, these increases were not statistically significant (p>0.05) (Figure 3, Table 1).

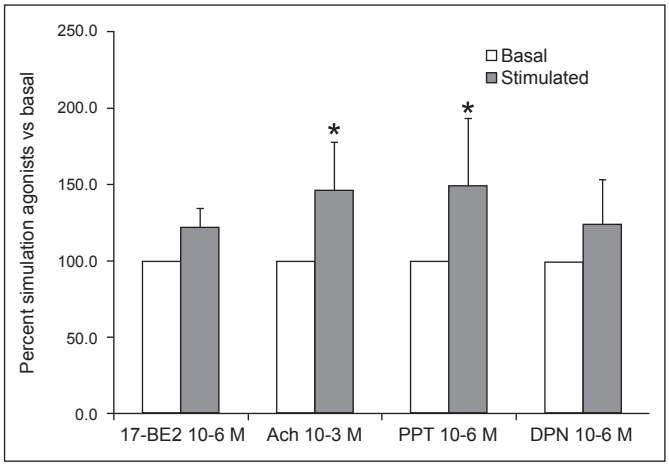


Figure 3 - Effect of various agonists on the production of nitrite in females. Isolated rat aortic arteries were incubated with various agonists for 20 min. and nitrite production was determined spectrophotometrically at 535 nm wavelength.

Data correspond to the percentage difference and SD of nitrite production stimulated by agonists after normalization of the baseline between the experimental groups and their respective basal made with triplicate rings (4 independent experiments). *Significant difference between 17-BE2 *vs* Ach and 17-BE2 *vs* PPT (p<0.05 one-way ANOVA, Tukey test).

**Comparison of production of nitrite induced by various agonists in males**

For purposes of statistical comparison of the effect of different agonists (17 BE2-$10^{-6}$M, $10^{-3}$M Ach, PPT $10^{-6}$M and DPN $10^{-6}$M) in rings of thoracic aortas obtained from mice was performed to normalize the data so identical to that performed with the information obtained in females. In males, it was observed that stimulation induced by Ach showed a significant difference ($p<0.05$) compared to stimulation with 17-BE2 (Figure 4). Interestingly, stimulation of the aortas of rats with estrogen receptor specific agonists, PPT and DPN caused a significantly greater nitrite production compared to that produced by the presence of ACh ($p<0.05$) (Figure 4). There was no significant difference between the specific agonists of estrogen receptors.

**Comparison of production of nitrite induced by various agonists in males and females**

Using the normalized data obtained from the analysis done by different stimuli agonists comparison was made between the production of nitrite females and males. In thoracic aortas are females, it was observed that the 17-BE2 stimulated more intensely nitrite production compared to males ($p<0.05$) (Figure 5). On the other hand, in thoracic aortas of male the DPN determined higher nitrite production than in females ($p<0.05$) (Figure 5). No significant differences were observed in males and females for the production of nitrite induced by Ach and PPT.

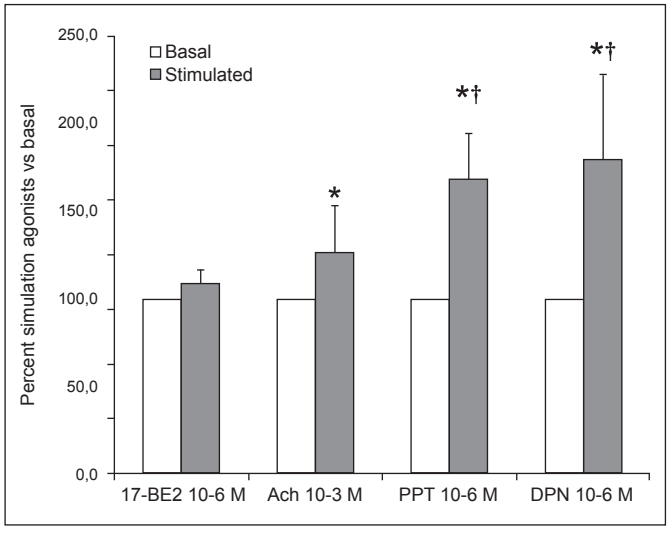


Figure 4 - Effect of various agonists on the production of nitrite in rats. Isolated aortic arteries of rats were incubated with various agonists for 20 minutes and nitrite production was determined spectrophotometrically at 535 nm wavelength.

Data correspond to the percentage difference and SD of nitrite production stimulated by agonists after normalization of the baseline between the experimental groups and their respective basal made with triplicate rings (4 independent experiments). *Significantly different 17-BE2. † Significantly different agonists Ach and 17-BE2 ($p<0.05$ one-way ANOVA, Tukey test).

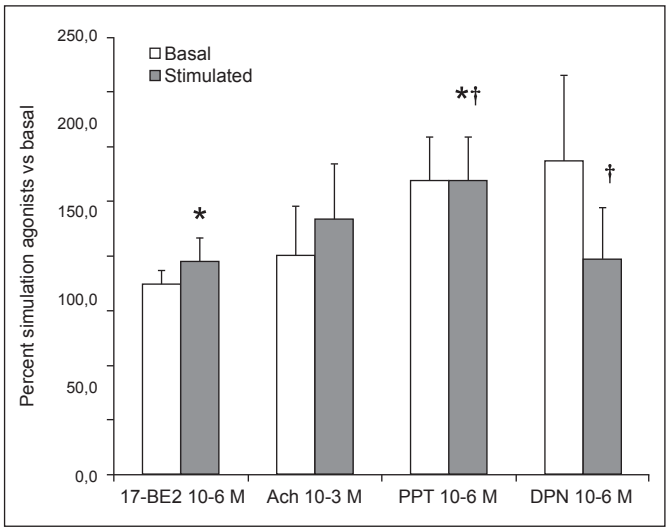


Figure 5 - Comparison of agonist effect on nitrite production in males from females. Isolated rat aortic arteries were incubated with various agonists for 20 minutes and nitrite production was determined spectrophotometrically at 535nm wavelength.

Data correspond to the percentage difference and SD of nitrite production stimulated by agonists after normalization of the baseline between the experimental groups and their respective basal made with triplicate rings (four independent experiments). *Significant difference between 17-BE2 (male *vs*. female) ($p<0.05$). † Significant difference between DPN (male vs. female) ($p<0.05$).

## DISCUSSION

Estrogen, in addition to being considered one of the key hormones involved in the growth, development and function of the female reproductive system, also has an effect on the cardiovascular system. Thus, the study of signaling mechanisms involved in the secretion and metabolism of estrogens, contributes to a better understanding of the observed changes in estrogenic activity that occurs in adults and in postmenopausal women. Human studies have shown a correlation between the production of NO and the levels estrogênicos[4,6,7,21] and estrogen-mediated vasodilation was attributed by both pathways, genomic and non-genomic, through the activation of cells endoteliais[3]. According Orshal and Khalil[19] and women have lower cardiovascular risk than men in the premenopausal period, however after menopause this risk becomes equal. This result implies that estrogen may be acting as a protective agent. In the present study, the determination of nitrite clearly shows a basal level higher in females than in males, or that females have a constitutive level of NO production larger than the males. Extrapolating these findings to humans, this might explain the cardiovascular protection observed by researchers. Reslan et al.[35] observed in his study with rats differentiated distribution of estrogen receptors in different vascular beds in the abdominal aorta, mesenteric and renal function, indicating that specific ER agonists could produce vasodilation in specific vascular beds without affecting other vessels within the vascular system.

Other studies have also shown that endothelial release of NO was higher in arteries of females compared with males arteries, it can be said that sex differences cause different productions NO[6,8,9].

Thus, we need a better investigation of signaling mediated by nuclear estrogen receptors, ERα and ERβ, which have been identified in the endothelium, VSM and adventitial cells of humans and animals. The estrogen receptor ERβ is found in various tissues, whereas the receiver ERα has a predominance of smooth muscle vasos[16-18].

Therefore, in an attempt to further elucidate the signaling pathway involved in estrogenic action, this study used selective agonists for estrogen receptors, 4,4 ',4"-[4-propyl-(1H)-pyrazole-1,3,5-triyl] tris-phenol, PPT with 410 times greater affinity for the ERα compared to ERβ[20] and 2,3-bis(4-hydroxyphenyl) propionitrila, DPN 170 times higher affinity for ERβ than to ERα[21], a non-specific agonist to ERs, 17-β estradiol, as well as Ach a standard agonist for NO production without acting on estrogen receptors.

The results of this study revealed that stimulation of the rings of thoracic aortas of females with PPT resulted in a significant increase in nitrite production compared to stimulation by the agonists caused 17-BE2 and DPN, thus demonstrating that stimulation of ERα established higher nitrite production. This observed difference between the PPT and other agonists of ERs have support, because the use of Ach agonist control, had the same performance in the production of nitrite, confirming the results found in the study by Traupe et al.[26] and cell culture endothelial cells.

When analyzing the results from stimulation of thoracic aortas rings of males is also observed that stimulation with PPT determines higher nitrite production when compared to the 17-BE2, but these, stimulation by DPN has also led to increased production of nitrite compared the 17-BE2. However, when comparing the production of nitrite induced by PPT and DPN not observed significant differences.

This result demonstrates that the isolated estrogen receptor stimulation determines the same magnitude in nitrite production in males. These results may be best seen for interpretation when the comparative analysis is made between males and females, which shows that the stimulation with determines a higher 17-BE2 nitrite production in females than in males, and that the DPN specific agonist ERβ determines higher nitrite production in males than in females.

It has been reported that ERα is found predominantly in VSM[19] which would explain the increased production of nitrite in the rings of thoracic aortas of females and males, when these receptors are activated. This result is also supported by the study of Darblade and et al.[27] who observed that estrogen induced vascular relaxation and increased NO production in mice expressing only the ERα functional. According Kublickiene et al.[28] and ERα can be considered the principal estrogen receptor associated with the stimulation of endothelial NO synthase.

Studies in animals suggest that ER subtypes act cooperatively to improve vascular condition. Responses to E2, as kinase activation in vivo and arterial vasodilation, are absent in mice "knockout" (KO) for both ERα as for ERβ, indicating that both ER subtypes cooperate for functions vasculares[29], which would justify action of PPT and DPN in aortic rings of males. Several studies suggest the beneficial effect of stimulation of ERα, for example, Christian et al.[30] and investigating the expression of ERα and ERβ in coronary arteries of women during the menopausal and post-treated or not with therapy hormone replacement concluded that increased expression of ERβ was associated with advanced atherosclerosis and calcification, regardless of age or hormonal status. Zhai et al.[31] and Wang et al.[32] found in mice with genetic deletion for ERα, that they lost the cardioprotective role of estrogen in the lesions caused by ischemia and reperfusion. Additional studies have shown that ERα is the prime mediator of the reduction aterosclerose[33].

In the present study it was observed that the agonist that determined higher nitrite production was the DPN in males, which acts on receptors ERβ, which may explain the fact that men are prone to a higher cardiovascular risk than women, on the other hand, in females, the increase in nitrite production by stimulation with the PPT increases the greater cardiovascular protection that sex. These results are corroborated by the study of Christian et al.[30] probably indicating that menopausal women have increased expression of ERβ, which would explain the failure of hormone replacement therapy, even at low doses, immediate or early menopause. On the other hand other studies have reported the "hypothesis of time" where the beginning still in the Peri menopause, the combination of estrogen and progestogenic could maximize therapeutic effects and minimize side effects in restoring hormonal[36].

This inference is supported by the study[34] and cross showed that the expression of ERβ vascular wall is increased in women with cardiovascular disease, whereas expression of ERα prevails in control individuals, suggesting that selective stimulation also ER subtype may have an effect on the cardiovascular status of the woman. Especially considering that the logo of a woman's life changes occur in the expression of ER subtypes of vascular endothelium and VSM, and changes in vascular architecture that can also associated with this failure of hormone replacement therapy, and increased disease cardiovasculares[37]. On the other hand Simino et al.[38] Assessing the antiatherosclerotic effect of E2 in hormone replacement therapy, noted that the transdermal route compared to the oral route, you get excellent therapeutic success. In our study was stimulated vascular rings in vitro, so leading to a more specific evaluation of the action of the agonist and E2 receptor subtype-specific, with no other actors with stimulating action of NO production.

## CONCLUSION

The results of this study suggest that: 1) stimulation of ER by PPT determines higher nitrite production than the 17-BE2 regardless of gender, 2) stimulation of ER by DPN determines higher nitrite production than in males 17-BE2 and 3) The basal production of nitrite is higher in females compared to males.

## REFERENCES

1. Wilcox JN, Subramanian RR, Sundell CL, Tracey WR, Pollock JS, Harrison DG, et al. Expression of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. Arterioscler Thromb Vasc Biol. 1997;17(11):2479-88.
2. Dusse LM, Silva RM, Vieira LM, das Graças Carvalho M. Does plasma nitrite determination by the Griess reaction reflect nitric

oxide synthesis? Clin Chim Acta. 2005;362(1-2):195-7.
3. Rahimian R, Chan L, Goel A, Poburko D, van Breemen C. Estrogen modulation of endothelium-derived relaxing factors by human endothelial cells. Biochem Biophys Res Commun. 2004;322(2):373-9.
4. Darkow DJ, Lu L, White RE. Estrogen relaxation of coronary artery smooth muscle is mediated by nitric oxide and cGMP. Am J Physiol. 1997;272(6 Pt 2):H2765-73.
5. Meyer MC, Cummings K, Osol G. Estrogen replacement attenuates resistance artery adrenergic sensitivity via endothelial vasodilators. Am J Physiol. 1997;272(5 Pt 2):H2264-70.
6. Knot HJ, Lounsbury KM, Brayden JE, Nelson MT. Gender differences in coronary artery diameter reflect changes in both endothelial Ca2+ and ecNOS activity. Am J Physiol. 1999;276(3 Pt 2):H961-9.
7. Geary GG, Krause DN, Duckles SP. Estrogen reduces mouse cerebral artery tone through endothelial NOS- and cyclooxygenase-dependent mechanisms. Am J Physiol Heart Circ Physiol. 2000;279(2):H511-9.
8. Kauser K, Rubanyi GM. Gender difference in endothelial dysfunction in the aorta of spontaneously hypertensive rats. Hypertension. 1995;25(4 Pt 1):517-23.
9. Wellman GC, Bonev AD, Nelson MT, Brayden JE. Gender differences in coronary artery diameter involve estrogen, nitric oxide, and Ca(2+)-dependent K+ channels. Circ Res. 1996;79(5):1024-30.
10. do Nascimento GR, Barros YV, Wells AK, Khalil RA. Research into Specific Modulators of Vascular Sex Hormone Receptors in the Management of Postmenopausal Cardiovascular Disease. Curr Hypertens Rev. 2009;5(4):283-306.
11. Mendelsohn ME. Mechanisms of estrogen action in the cardiovascular system. J Steroid Biochem Mol Biol. 2000;74(5):337-43. Review.
12. Chen Z, Yuhanna IS, Galcheva-Gargova Z, Karas RH, Mendelsohn ME, Shaul PW. Estrogen receptor alpha mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. J Clin Invest. 1999;103(3):401-6. Erratum in: J Clin Invest 1999;103(9):1363.
13. Pare G, Krust A, Karas RH, Dupont S, Aronovitz M, Chambon P, et al. Estrogen receptor-alpha mediates the protective effects of estrogen against vascular injury. Circ Res. 2002;90(10):1087-92.
14. Kublickiene K, Svedas E, Landgren BM, Crisby M, Nahar N, Nisell H, et al. Small artery endothelial dysfunction in postmenopausal women: in vitro function, morphology, and modification by estrogen and selective estrogen receptor modulators. J Clin Endocrinol Metab. 2005;90(11):6113-22.
15. Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, et al. Estrogen receptors: how do they signal and what are their targets. Physiol Rev. 2007;87(3):905-31. Review.
16. Mendelsohn ME. Genomic and nongenomic effects of estrogen in the vasculature. Am J Cardiol. 2002;90(1A):3F-6F. Review.
17. Zhu Y, Bian Z, Lu P, Karas RH, Bao L, Cox D, et al. Abnormal vascular function and hypertension in mice deficient in estrogen receptor beta. Science. 2002;295(5554):505-8.
18. Mendelsohn ME, Karas RH. Molecular and cellular basis of cardiovascular gender differences. Science. 2005;308(5728):1583-7. Review.
19. Orshal JM, Khalil RA. Gender, sex hormones, and vascular tone. Am J Physiol Regul Integr Comp Physiol. 2004;286(2):R233-49. Review.
20. Stauffer SR, Huang Y, Coletta CJ, Tedesco R, Katzenellenbogen JA. Estrogen pyrazoles: defining the pyrazole core structure and the orientation of substituents in the ligand binding pocket of the estrogen receptor. Bioorg Med Chem. 2001;9(1):141-50.
21. Meyer MC, Cummings K, Osol G. Estrogen replacement attenuates resistance artery adrenergic sensitivity via endothelial vasodilators. Am J Physiol. 1997;272(5 Pt 2):H2264-70.
22. Bolego C, Cignarella A, Sanvito P, Pelosi V, Pellegatta F, Puglisi L, et al. The acute estrogenic dilation of rat aorta is mediated solely by selective estrogen receptor-alpha agonists and is abolished by estrogen deprivation. J Pharmacol Exp Ther. 2005;313(3):1203-8.
23. Booth EA, Obeid NR, Lucchesi BR. Activation of estrogen receptor-alpha protects the in vivo rabbit heart from ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol. 2005;289(5):H2039-47.
24. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982;126(1):131-8.
25. Darkow DJ, Lu L, White RE. Estrogen relaxation of coronary artery smooth muscle is mediated by nitric oxide and cGMP. Am J Physiol. 1997;272(6 Pt 2):H2765-73.
26. Traupe T, Stettler CD, Li H, Haas E, Bhattacharya I, Minotti R, et al. Distinct roles of estrogen receptors alpha and beta mediating acute vasodilation of epicardial coronary arteries. Hypertension. 2007;49(6):1364-70.
27. Darblade B, Pendaries C, Krust A, Dupont S, Fouque MJ, Rami J, et al. Estradiol alters nitric oxide production in the mouse aorta through the alpha-, but not beta-, estrogen receptor. Circ Res. 2002;90(4):413-9.
28. Kublickiene K, Fu XD, Svedas E, Landgren BM, Genazzani AR, Simoncini T. Effects in postmenopausal women of estradiol and medroxyprogesterone alone and combined on resistance artery function and endothelial morphology and movement. J Clin Endocrinol Metab. 2008;93(5):1874-83.
29. Guo X, Razandi M, Pedram A, Kassab G, Levin ER. Estrogen induces vascular wall dilation: mediation through kinase signaling to nitric oxide and estrogen receptors alpha and beta. J Biol Chem. 2005;280(20):19704-10.
30. Christian RC, Liu PY, Harrington S, Ruan M, Miller VM, Fitzpatrick LA. Intimal estrogen receptor (ER)beta, but not ERalpha expression, is correlated with coronary calcification and atherosclerosis in pre- and postmenopausal women. J Clin Endocrinol Metab. 2006;91(7):2713-20.
31. Zhai P, Eurell TE, Cooke PS, Lubahn DB, Gross DR. Myocardial ischemia-reperfusion injury in estrogen receptor-alpha knockout and wild-type mice. Am J Physiol Heart Circ Physiol. 2000;278(5):H1640-7.
32. Wang M, Crisostomo P, Wairiuko GM, Meldrum DR. Estrogen receptor-alpha mediates acute myocardial protection in females. Am J Physiol Heart Circ Physiol. 2006;290(6):H2204-9.
33. Egan KM, Lawson JA, Fries S, Koller B, Rader DJ, Smyth EM, et al. COX-2-derived prostacyclin confers atheroprotection on female mice. Science. 2004;306(5703):1954-7.
34. Cruz MN, Agewall S, Schenck-Gustafsson K, Kublickiene K. Acute dilatation to phytoestrogens and estrogen receptor subtypes expression in small arteries from women with coronary heart disease. Atherosclerosis. 2008;196(1):49-58.
35. Reslan OM, Yin Z, do Nascimento GR, Khalil RA. Subtype--specific Estrogen Receptor-mediated Vasodilator Activity in the Cephalic, Thoracic, and Abdominal Vasculature of Female Rat. J Cardiovasc Pharmacol. 2013;62(1):26-40.
36. Reslan OM, Khalil RA. Vascular effects of estrogenic menopausal hormone therapy. Rev Recent Clin Trials. 2012 Feb;7(1):47-70. Review.
37. Masood DE, Roach EC, Beauregard KG, Khalil RA. Impact of sex hormone metabolism on the vascular effects of menopausal hormone therapy in cardiovascular disease. Curr Drug Metab. 2010;11(8):693-714. Review.
38. Sumino H, Murakami M. [Investigation of atherosclerosis in postmenopausal women: alteration of atherosclerosis-associated factors and vascular atherosclerosis by oral and transdermal estrogen replacement]. Rinsho Byori. 2013;61(3):256-62. Japanese.