Research, Society and Development, v. 14, n. 12, e43141250241, 2025
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v14i12.50241

Ovariectomized hypertensive rats submitted to exercise and estrogen therapy present improved levels of angiotensin receptors in the aorta

Ratas hipertensas ovariectomizadas submetidas a exercício e terapia estrogênica apresentam níveis melhorados de receptores de angiotensina na aorta

Ratas hipertensas ovariectomizadas sometidas a ejercicio y terapia estrogénica presentan niveles mejorados de receptores de angiotensina en la aorta

Received: 11/23/2025 | Revised: 12/02/2025 | Accepted: 12/03/2025 | Published: 12/05/2025

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Abstract

The renin-angiotensin system (RAS) and the antioxidant system play integral and interconnected roles in finely regulating cardiovascular function during exercise training and estrogen level alterations. This study aimed to investigate whether training modulates RAAS receptors and antioxidant proteins in a manner similar to 17β-estradiol (E2) therapy in the aorta of ovariectomized spontaneously hypertensive rats (SHR). Animals were divided into Sham (SH), Ovariectomized (OVX), OVX+ET (OE2), OVX+swimming (OSW) and OVX+ET+swimming (OE2+SW) groups. ET entailed the administration of 5 µg 17β-estradiol three times per week. Swimming was performed for one hour/day, five times per week. Two days after the last treatment and/or training session, systolic blood pressure was assessed. Protein content from isolated aorta was analyzed by western blot for the RAS receptors (angiotensin AT_1-receptor - AT_1R, angiotensin AT_2-receptor - AT_2R, and Mas receptor - Mas), endothelial nitric oxide synthase (eNOS), total and phosphorylated Protein Kinase B at Ser^{473} (p-Akt^{Ser473}), Superoxide Dismutase 2

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(SOD 2) and ß-actin. The results showed that AT_1R was increased only in the OVX group, while angiotensin AT_2R and Mas increased in both OSW and OE2+SW. In addition, ET therapy increased both eNOS and SOD 2 levels in SH, OE2 and OE2+SW. Interestingly, p-Akt^{Ser473} levels increased similarly in the same groups observed for eNOS. This study provided mechanistic evidence suggesting that both physical and hormonal interventions act via the protective RAS by stimulating the p-Akt^{Ser473}-eNOS and SOD 2 pathways, which can be associated with vasodilation and potential antioxidant capacity in estrogen deficiency conditions.
Keywords: Renin-angiotensin system (RAS); Endothelial nitric oxide synthase (eNOS); Superoxide Dismutase 2 (SOD 2).

Resumo

O sistema renina-angiotensina (SRA) e o sistema antioxidante exercem funções integradas na regulação cardiovascular, especialmente diante do treinamento físico e de alterações nos níveis de estrogênio. Este estudo teve como objetivo investigar se o treinamento modula receptores do SRA e proteínas antioxidantes de forma semelhante à terapia com 17β-estradiol (E2) na aorta de ratas espontaneamente hipertensas ovariectomizadas (SHR). Os animais foram distribuídos em cinco grupos: Sham (SH), Ovariectomizado (OVX), OVX+E2 (OE2), OVX+natação (OSW) e OVX+E2+natação (OE2+SW). A terapia hormonal consistiu em 5 µg de E2, três vezes por semana; o treinamento em natação foi realizado uma hora por dia, cinco vezes por semana. Dois dias após o último procedimento, avaliou-se a pressão arterial sistólica. Amostras de aorta foram analisadas por western blot para os receptores AT1R, AT2R e Mas, além de eNOS, proteína quinase B total e fosforilada (p-AktSer473), SOD2 e β-actina. Observou-se aumento de AT1R apenas no grupo OVX, enquanto AT2R e Mas aumentaram nos grupos OSW e OE2+SW. A terapia com E2 elevou eNOS e SOD2 nos grupos SH, OE2 e OE2+SW, padrão também observado para p-AktSer473. Os achados sugerem que intervenções físicas e hormonais estimulam vias protetoras do SRA, especialmente p-AktSer473-eNOS e SOD2, favorecendo vasodilatação e capacidade antioxidante em condições de deficiência estrogênica.
Palavras-chave: Sistema renina-angiotensina (SRA); Óxido nítrico sintase endotelial (eNOS); Superóxido Dismutase 2 (SOD 2).

Resumen

El sistema renina-angiotensina (SRA) y el sistema antioxidante cumplen funciones integradas en la regulación cardiovascular, especialmente frente al entrenamiento físico y a las alteraciones en los niveles de estrógeno. Este estudio tuvo como objetivo investiga si el entrenamiento modula los receptores del SRA y proteínas antioxidantes de manera similar a la terapia con 17β-estradiol (E2) en la aorta de ratas hipertensas espontáneas ovariectomizadas (SHR). Los animales se dividieron en cinco grupos: Sham (SH), Ovariectomizado (OVX), OVX+E2 (OE2), OVX+natación (OSW) y OVX+E2+natación (OE2+SW). La terapia hormonal consistió en 5 µg de E2 tres veces por semana; el entrenamiento de natación se realizó una hora diaria, cinco veces por semana. Dos días después del último procedimiento, se evaluó la presión arterial sistólica. Las aortas aisladas se analizaron mediante western blot para los receptores AT1R, AT2R y Mas, además de eNOS, Akt total y fosforilada (p-AktSer473), SOD2 y β-actina. Se observó un aumento de AT1R solo en el grupo OVX, mientras que AT2R y Mas aumentaron en los grupos OSW y OE2+SW. La terapia con E2 elevó eNOS y SOD2 en los grupos SH, OE2 y OE2+SW, patrón también visto para p-AktSer473. Los resultados sugieren que las intervenciones físicas y hormonales activan vías protectoras del SRA, especialmente p-AktSer473-eNOS y SOD2, favoreciendo la vasodilatación y la capacidad antioxidante en condiciones de deficiencia estrogénica.
Palabras clave: Sistema renina-angiotensina (SRA); Óxido nítrico sintasa endotelial (eNOS); Superóxido Dismutasa 2 (SOD 2).

1. Introduction

Absolute numbers of deaths caused by cardiovascular diseases (CVD) are traditionally higher in men compared to women. However, this scenario has changed in the past two decades, with CVD death records averaging 27.7% in men against 30.6% in women (Ritchie, Spooner, & Roser, 2018; Wenger, Speroff, & Packard, 1993). A key reason for such impact in women is the sudden deficiency in estrogen levels after menopause onset or bilateral ovariectomy, which considerably induces or aggravates pathological cardiovascular conditions, particularly hypertension (Regitz-Zagrosek & Kararigas, 2017; Burt et al., 1995; Barton & Meyer, 2009).

Clinical decision-making for post-menopause women counseled to hormone therapy requires evaluating the potential benefits of this intervention in face of its potential risks.

Late initiation of estrogen therapy has been associated with higher coronary risk, a side effect not observed in early-stage treatment (Manson & Bassuk, 2018), while short-term estrogen therapy might be an alternative for recently menopausal women with moderate to severe symptoms but a healthy cardiovascular system (Manson & Bassuk, 2018; Martin & Manson, 2008). Despite the potential benefits of short-term estrogen therapy, it is not indicated for more than 4–5 years due to reduced menopausal symptoms over time and increased breast cancer risk after long periods of hormone therapy (Manson & Bassuk, 2018; Martin & Manson, 2008).

Estrogen levels influence the hormonal pathways of cardiovascular control such as the renin-angiotensin system (RAS), being capable of downregulating the vasoconstrictor axis [Angiotensin II/Angiotensin-converting enzyme/Angiotensin AT1-receptor (Ang II)/ACE/AT1R] and upregulating the vasodilator axis [Angiotensin-(1-7)/ACE2/Angiotensin AT2-receptor] (Gersh et al., 2021).

Exercise training is considered an effective method to prevent or refrain from CVD progression. It can lower blood pressure in humans (Higashi et al., 1999) and in male spontaneously hypertensive rats (SHR) (Endlich et al., 2011), while the same effect is controversial for female SHR (Coimbra et al., 2008; Marques et al., 2006).

We previously showed that ovariectomized SHR presented increased Ang II-mediated vasoconstriction responses in isolated aortic rings. However, when subjected to swim training or associated with ET, these animals showed weaker vasoconstriction responses to Ang II compared to the controls (Endlich et al., 2017). In addition, Ang-(1-7)-induced vasodilation was greatly enhanced in ovariectomized SHR submitted to either swim training alone or combined with ET (Endlich et al., 2017).

There is molecular evidence for the antioxidant effects of E2, which increases the bioavailability of endothelial nitric oxide (NO), a key molecule with potent vasodilation properties, subsequently reducing peripheral vascular resistance (Hernández et al., 2000). In ovariectomized SHR treated with ET, this vasoprotective effect has also been attributed to lower superoxide anion generation, a main free radical responsible for depleting NO in a superoxide dismutase (SOD 2)-independent mechanism (da Silva et al., 2022; de Almeida et al., 2018).

Despite the physiological effects of swim training and ET, angiotensin-induced altered vascular reactivity was neither evaluated at the molecular level of RAS receptors nor whether swim training modulates RAS receptors similarly to the ET mechanism.

This study evaluated the effects of 8 weeks of swim training combined with ET on the regulation of angiotensin receptors in the aorta of ovariectomized SHR and their participation in vasodilation mechanisms, particularly endothelial NO synthase (eNOS) and phosphorylated Protein Kinase B (p-AktSer473). Lastly, we sought to evaluate the antioxidant enzyme SOD 2 levels to verify adaptive responses to both treatments.

2. Methodology

An experimental study of a quantitative nature was conducted (Pereira et al., 2018) and using descriptive statatics (Shitsuka et al., 2014) and statistical analysis (Costa Neto & Bekman, 2009).

Female SHR at 10 weeks of age weighing between 120 and 140 grams were obtained from the Animal Facility of the Federal University of Espírito Santo (UFES) and housed in a humidity- and temperature-controlled room (60%; 22–24°C) under a 12h/12h light/dark cycle in collective cages (4 animals/cage) with free access to water and food. All procedures and experiments followed the UFES ethics committee (001/2010) and the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (2011).

At the time of ovariectomy, animals were randomly divided into the following groups: Sham (SH), ovariectomized (OVX), ovariectomized + 17β-estradiol therapy (OE2), ovariectomized + swimming (OSW), and ovariectomized + 17β-estradiol therapy + swimming (OE2+SW).

Ovariectomy

Ovariectomy was performed via a mid-abdominal incision after anesthesia with intraperitoneal (i.p.) injections of ketamine and xylazine (70 mg/kg and 10 mg/kg, respectively) (Zarrow, 2012). Surgery, but not ovariectomy, was performed in Sham-operated rats. All animals underwent surgery during the same period and started swim training or ET after seven days of recovery.

17ß-estradiol therapy (ET)

ET was administered via subcutaneous injection (0.1 mL) of 5 µg 17β-estradiol 3-benzoate (Sigma, St. Louis, MO) diluted in corn oil, three times per week, as previously described (Claudio et al., 2013; Saengsirisuwan et al., 2009). Animals that did not receive ET were administered the same volume of corn oil (vehicle). Uterine wet weights were used to assess the effectiveness of ovariectomy and ET (Endlich et al., 2017).

Swim training

Animals were subjected to forced swim training as previously described (Claudio et al., 2013). Briefly, a glass cylinder (20 cm in diameter) was filled with 60 cm of water (30–32°C) to prevent hind paws from touching the bottom. Swimming was performed regularly for 8 weeks after a 1-week adaptation period, consisting of an initial 20-minute continuous swimming session followed by 10-minute increments each day until reaching 60 minutes on the fifth day.

After adaptation, the training protocol was kept constant (60 min/day, 5 days/week), including two consecutive rest days. Immobility time was quantified during a 5-minute test period, and immobility was considered fatigue. After each session, rats were dried and returned to their cages. All animals rested 48 hours before euthanasia.

Blood pressure measurements

Systolic blood pressure (SBP) was assessed by non-invasive tail-cuff plethysmography in conscious rats. An electro-sphygmomanometer recorder (IITC Life Science) was used 48 hours after the end of the treatment period. Animals were placed in a restraining device on a thermostatically warmed plate (37°C). Three readings were taken per animal, and the mean value was considered (Buñag, 1973).

Aorta isolation and collection

Following SBP assessment, rats were anesthetized with ketamine and xylazine (70 mg/kg and 10 mg/kg, respectively) and euthanized by exsanguination. The thoracic aorta was removed, placed in cold Krebs–Henseleit solution, cut into rings, and stored at –80°C for further analysis.

Western Blot

Aortic rings were homogenized in lysis buffer (Nonidet P40 1%, sodium deoxycholate 0.5%, SDS 0.1%, pH 7.2, and a cOmplete™, EDTA-free Protease Inhibitor Cocktail – Roche, USA), and protein concentration was determined using the Bradford method (Claudio et al., 2017). A total protein amount of 30 µg (AT1R, Akt 1/2/3, and pAktSer473), 40 µg (AT2R,

SOD2, and eNOS), or 50 µg (MasR) was resolved on SDS-PAGE gels (10%) and transferred to a 0.45 µm nitrocellulose membrane (Merck Millipore, EUA), as described by Savergnini et al. (2013). The membranes were blocked in tris-buffered saline containing Tween 1% (TBS-T) and skim milk (5%) for 2 hours. After blocking, membranes were washed and incubated overnight at 4°C with one of the following antibodies: rabbit polyclonal anti-AT1R (1:1000; Millipore, Billerica, MA, USA), rabbit polyclonal anti-AT2R (1:500; Millipore, Billerica, MA, USA), rabbit polyclonal anti-Mas (1:300; Alomone, Jerusalem, Israel), rabbit polyclonal anti-Akt (1:1000; Abcam, Cambridge, MA, USA), rabbit polyclonal anti-p-AktSer473 (1:500; Abcam, Cambridge, MA, USA), rabbit polyclonal anti-SOD2 (1:1000; Santa Cruz Biotechnology, California, USA), rabbit monoclonal anti-eNOS (1:1000; BD, New Jersey, USA), and mouse monoclonal anti-β-actin (1:5000; Sigma Chemical, St. Louis, USA). Membranes were then washed with TBS-T and incubated with appropriate anti-rabbit or anti-mouse secondary antibodies (1:7000; Santa Cruz Biotechnology, CA, USA). Protein bands were detected and quantified using the Odyssey scanning system (Li-Cor, USA) and Image Studio 4.0 software (Li-Cor, USA). p-AktSer473 levels were corrected by total Akt levels. Relative protein expression was calculated by dividing the target protein values by β-actin, used as a loading control. Data were normalized to the mean values of the SH group. For Mas receptor detection, rat testicle tissue was used as a positive control.

Statistical analysis

Data are reported as mean ± SEM. One-way ANOVA followed by Tukey's post hoc test was used, with significance set at $p < 0.05$. Analyses were performed with GraphPad Prism 8 (GraphPad, La Jolla, CA, USA).

3. Results

Swim training prevented blood pressure increase in ovariectomized hypertensive rats independently of ET

Body weight (BW), uterine weight, and systolic blood pressure (SBP) are summarized in Table 1. All groups initiated the experimental protocol with similar body weights, but after 8 weeks, BW gain was significantly evident in the OVX and OSW groups, which were 55.7% and 52.3%, respectively. Moreover, 17ß-estradiol therapy (ET) prevented 75.4% uterine weight reduction compared to OVX. Swim training prevented the SBP increment compared to all sedentary groups, independently of ET. On the other hand, such differences were not observed between the SH, OVX, and OE2 groups. These data indicated that swimming alone was capable of preventing blood pressure increase caused by estrogen deficiency in ovariectomized hypertensive rats.

Table 1. Effects of ovariectomy, swimming, and/or estrogen therapy on uterine weight (UW), body weight (BW), and systolic blood pressure (SBP).

	SH (n=5)	OVX(n=5)	OE2(n=5)	OSW(n=5)	OE2+SW(n=5)
UW (mg)	301±23	70±7*#†	285±3	74±5*#†	294±14
BW initial (g)	130±2	131±2	127±5	130±2	132±3
BW final (g)	175±5	204±5*#†	163±2	198±2*#†	163±4
SBP (mmHg)	181±3	198±2	183±4	169±2§	172±3§

Data are expressed as mean±SEM. N=5 One-way ANOVA with post hoc Tukey's test. SH, Sham; OVX, ovariectomized; OE2, ovariectomized + 17ß-estradiol therapy; OSW, ovariectomized + swimming; OE2+SW, ovariectomized + 17ß-estradiol therapy + swimming. *P<0.05 *vs*. SH; §P<0.05 *vs*. OVX; #P<0.05 *vs*. OE2; †P<0.05 *vs*. OE2+SW. Source: Research data (2025).

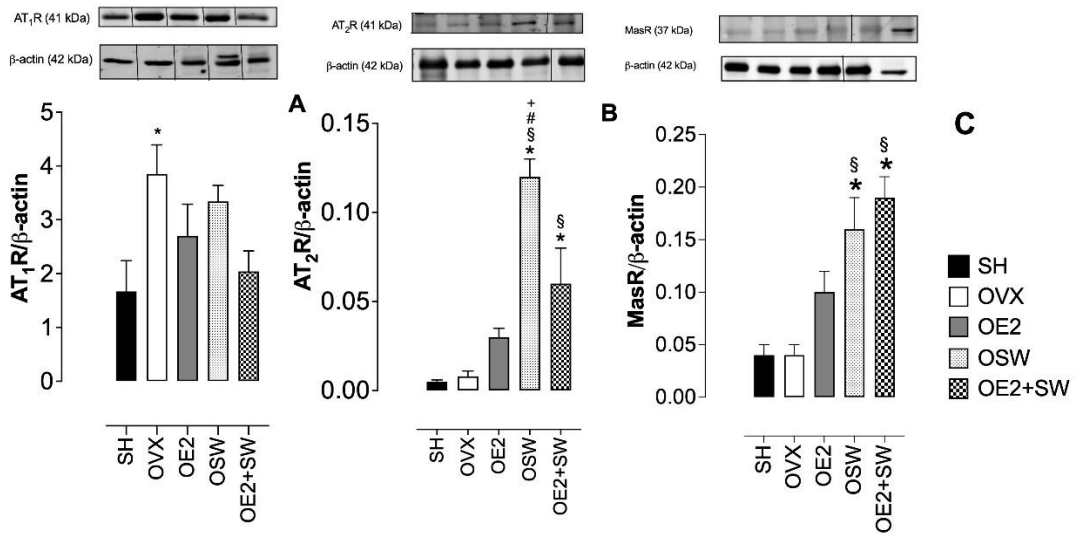
Swim training increased levels of Angiotensin receptors associated with vasodilation in ovariectomized hypertensive rats independently of ET

We evaluated different angiotensin receptors belonging to both classic (AT_1R) and protective (AT_2R and Mas) RAS axes in the aorta of ovariectomized SHR. AT_1R levels were significantly increased in the OVX compared to the SH group, while such difference was not observed in groups that received E2 and/or were subject to swim training (Figure 1A). This suggested a preventive effect in increasing levels of AT_1R by both treatment and training

In the opposite direction, swim training significantly increased AT_2R levels in the aorta of OSW animals compared to all other groups. However, it was not observed a synergic effect between swimming and ET evidenced by no differences between E2+SW in comparison to OE2 or OSW but increased AT_2R levels compared to SH or OVX groups (Figure 1B). Swim training also increased Mas levels independently of estrogen treatment as observed by comparing OSW to SH and OVX groups and no differences were observed when comparing OWS to the group that combined swim training with E2 treatment (OE2+SW) (Figure 1C).

Therefore, these data indicated that in ovariectomized SHR, reduced SBP observed in swimming groups might be dependent on the protective RAS axis activation, observed by significantly increased levels of AT_2R and Mas.

Figure 1. Representative figures of the angiotensin receptors (A) AT_1R, (B) AT_2R, and (C) Mas in the aorta of ovariectomized spontaneously hypertensive rats.



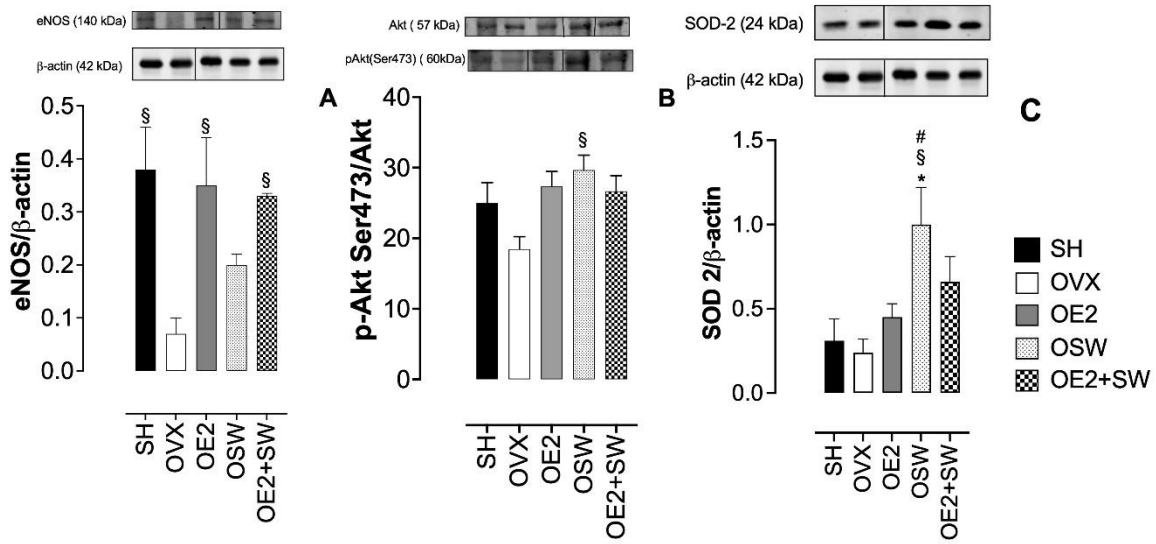
Rat testicle tissue sample was used as positive control for Mas receptor (Figure 1-C). Data are expressed as mean±SEM. One-Way ANOVA followed by post hoc Tukey's test. *p<0.05 *vs*. SH, §P<0.05 *vs*. OVX; #P<0.05 *vs*. OE2; +P<0.05 *vs*. OE2+SW. N=4. Images of blotted bands are displayed as a composition. All individual blot bands are shown in Supplementary Figure 1. Source: Research data (2025).

The vasodilator p-AKT^{Ser435}-eNOS pathway is upregulatedin ovariectomized hypertensive rats submitted to swim training or treated with estrogen

After observing changes in the levels of angiotensin receptors commonly involved in blood pressure regulation (Paz Ocaranza et al., 2020), we further explored the possible signaling of downstream pathways, such as the Akt–eNOS pathway, previously reported to be activated by both AT2R and Mas receptors (Buñag, 1973; Claudio et al., 2017; Savergnini et al., 2013) and inhibited by AT1R (Su et al., 2009). Protein levels of eNOS were significantly decreased in OVX animals, while

treatment with ET (OE2) and OE2+SW restored eNOS levels to those of the SH group. Swim training alone also tended to restore eNOS levels; however, statistical significance was only reached in the OE2 and OE2+SW groups compared to OVX (Figure 2A). Interestingly, a similar profile was observed for p-AktSer473, but in this case statistical significance was reached only in the OSW group compared to OVX (Figure 2B). Therefore, our data suggest a positive correlation between E2 treatment and/or swim training and the upregulation of the p-AktSer473–eNOS pathway.

Figure 2. Representative figures of the enzymes (A) eNOS, (B) p-Akt(Ser^{473})/Akt, and (C) SOD-2 in the aorta of ovariectomized spontaneously hypertensive rats.



Data are expressed as mean±SEM. One-Way ANOVA followed by the post hoc Tukey's test. *p<0.05 *vs*. SH, §P<0.05 *vs*. OVX; #P<0.05 *vs*. OE2. N=4. Images of blotted bands are displayed as a composition. All individual blot bands are shown in Supplementary Figure 2.
Source: Research data (2025).

Swim training increased the levels of superoxide dismutase (SOD 2) in ovariectomized hypertensive rats

Because SOD2 activity accelerates the reaction of superoxide anion into oxygen and hydrogen peroxide, maintaining the physiological functions of NO (Wang, Branicky, Noë, & Hekimi, 2018), we evaluated the effects of our experimental protocol on SOD2 levels. SOD2 presented significantly increased levels only in the OSW group when compared to the SH, OVX, and OE2 groups, as shown in Figure 2C.

4. Discussion

Dysregulation of RAS has a major role in hypertension development, particularly in postmenopausal women, considering the known inverse relationship between estrogen and RAS activity (Nogawa et al., 2001; Schunkert et al., 1997; Hinojosa-Laborde et al., 2004; Nickenig et al., 1998; Wassmann et al., 2001). Although estrogen therapy (ET) has beneficial cardiovascular effects by maintaining 17β-estradiol levels after menopause onset, side effects have been reported (Nabulsi et al., 1993; Chen et al., 2009; Boardman et al., 2015). Therefore, alternative therapies for physiological adaptations are sought in order to reduce and maintain blood pressure within a homeostatic range and prevent the development or worsening of CVDs. We hypothesized that swim training, associated or not with ET, would upregulate the protective RAS axis while downregulating the classic RAS axis in the aorta of ovariectomized SHR. This study showed that 8 weeks of swim exercise

were sufficient to prevent SBP increase, accompanied by a positive correlation between swim training, with or without ET, and upregulation of Mas and AT2R. Moreover, both interventions prevented the increase of AT1R levels.

Previous studies reported that ET alone did not prevent increased systolic blood pressure in SHR. In fact, Wassmann et al. (2001) also evaluated blood pressure in SHR submitted to ovariectomy and ET and found no difference between groups. In addition, others have also found no differences in blood pressure when comparing Sham to OVX SHR (Dalpiaz et al., 2013; Reckelhoff et al., 2000), indicating that ET alone may not be sufficient to restore normal blood pressure levels. Our data showed a reduction in SBP in ovariectomized SHR subjected to swim training, and this effect was not enhanced by combining ET with training. These data confirm the essential role of physical exercise in blood pressure control and reinforce previous studies from our group showing reduced blood pressure in ovariectomized SHR subjected to running or swimming (Claudio et al., 2017; Endlich et al., 2017).

Both ET and swim training were efficient in preventing AT1R upregulation in the aorta of ovariectomized SHR. In agreement with our findings, infarcted ovariectomized rats subjected to physical exercise also showed reduced AT1R levels in the left ventricle (de Almeida et al., 2018), while estrogen deficiency was shown to upregulate AT1R (Wassmann et al., 2001).

It is established that AT2R mediates vasodilation via NO production (Hannan et al., 2003; Siragy & Carey, 1997). Our data showed that swim training increased AT2R receptor levels independently of ET in OVX SHR. However, swim training per se was not capable of re-establishing eNOS levels, except when combined with ET (OE2+SW). OE2 alone also restored eNOS levels. Thus, in this case, estrogen directly affected the vessels by modulating eNOS levels, a mechanism associated with NO synthesis and consequent vasodilation (Caulin-Glaser et al., 1997; Lantin-Hermoso et al., 1997).

Besides AT2R, this study indicated increased Mas receptor levels independently of ET in ovariectomized SHR (OSW), an effect not potentiated by combining ET with swimming (OE2+SW). Similar to our findings, swim training has been shown to stimulate the ACE2/Ang-(1-7)/Mas axis at both mRNA and protein levels in SHR or 2K1C hypertensive rats (Filho et al., 2008; Shah et al., 2012; Silva et al., 2011). Moreover, Mas receptor activation is known to increase NO production via phosphorylation and activation of the p-AktSer473–eNOS pathway (Sampaio et al., 2007). Although we observed upregulation of Mas receptor and eNOS in OSW and OE2+SW compared to OVX, only OSW significantly activated Akt via phosphorylation at the Ser473 site.

We further observed significantly increased SOD2 levels only after swim training (OSW), whereas ET (OE2) alone or combined with swimming (OE2+SW) did not regulate this enzyme. This again corroborates previous studies highlighting the importance of exercise in regulating superoxide anion formation and antioxidant mechanisms (Claudio et al., 2017; de Almeida et al., 2018; de Oliveira et al., 2019).

5. Final Considerations

In this study, we sought to determine whether swim training, estrogen therapy (ET), or their combination could modulate angiotensin receptors and antioxidant proteins in the aorta of ovariectomized spontaneously hypertensive rats. Based on our experimental findings, we believe that the objectives outlined in the Abstract and Introduction were fully achieved. The interventions successfully influenced key components of the renin-angiotensin system (RAS) and oxidative pathways, confirming our initial hypothesis that both exercise and hormonal therapy could act on mechanisms related to blood pressure regulation and vascular protection.

Overall, the results demonstrated that swim training, ET, or the combination of both were beneficial for systolic blood pressure regulation in ovariectomized SHR. Importantly, we also provide mechanistic evidence indicating that these interventions act via the protective RAS axis by stimulating the p-AktSer473–eNOS and SOD2 intracellular pathways, which

are associated with vasodilation and enhanced antioxidant capacity. These findings reinforce the relevance of physical exercise and hormonal modulation as strategies capable of attenuating vascular dysfunction under estrogen-deficient conditions.

Future studies should explore longer training durations, different exercise intensities, and potential synergistic effects of other non-pharmacological approaches combined with hormonal therapy. Additionally, expanding the investigation to other vascular beds, cardiac tissue, or molecular targets may provide further insight into systemic adaptations. Long-term follow-up studies could also help determine the sustainability of these effects and support the development of comprehensive therapeutic strategies for postmenopausal hypertension.

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