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HEALTH SCIENCES

Resistance training increases insulin-induced vasodilation in the mesenteric artery of healthy rats

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Abstract: This study evaluated the ability of resistance training (RT) of moderate intensity to promote vascular changes in insulin-induced vasodilation in healthy animals. Wistar rats were divided into two groups: control (CON) and trained (eight weeks of training, performing 3 sets with 10 repetitions at 60% of maximum intensity). Forty-eight hours after the last session of the RT, the animals were sacrificed and vascular reactivity to insulin in the absence and presence of LY294002 (phosphatidylinositol 3-kinase inhibitors (PI3K), L-NAME (nitric oxide synthase (NOS) inhibitors) and BQ123 (endothelin A antagonist (ET-A) receptor). In addition, phenylephrine (Phe)-induced vasoconstriction in the absence and presence of L-NAME was also evaluated. The RT group showed greater vasodilation in maximal response compared to the CON group. After PI3K inhibition, vasodilation was reduced in both groups. However, when the NOS participation was evaluated, the RT group showed contraction in relation to the CON group, which was abolished by BQ123. In addition, the RT group had an increase in nitrite levels compared to the CON group. When the Phe response was evaluated, there was a reduction in tension in the RT group compared to the CON group. The results suggest that RT improves vascular reactivity.

Key words: Strength training, insulin, nitric oxide, vascular reactivity.

INTRODUCTION

The vascular endothelium (EV) is involved in several functions, including maintenance of vascular tone, prevention of vascular smooth muscle proliferation, reduction in leukocyte adhesion and activation, inhibition of platelet aggregation, formation of thrombi and flow regulation of blood (Rajendran et al. 2013, Cahill & Redmond 2016). One of the main mechanisms responsible for maintaining endothelial function is nitric oxide (NO), which induces endothelium-dependent vasodilation by increasing intracellular calcium concentrations ($[Ca^{2+}]i$). On the other hand, insulin, in addition to playing an important role in regulating glucose homeostasis, also acts in maintaining vascular health (Muniyappa et al. 2008, Arce-Esquivel et al. 2013).

Some studies have shown that this hormone also acts in vascular modulation, playing an important role in controlling blood flow, directly participating in the maintenance of homeostasis and vascular tone, which can represent up to 25% of maximum vasodilation (Arce-Esquivel et al. 2013, Fontes et al. 2014, Mota et al. 2015). When insulin binds with its tyrosine kinase receptor in the endothelium, it stimulates the phosphorylation of the insulin receptor substrate (IRS-1), activating phosphatidylinositol 3-kinase

(PI3K), which stimulates Akt phosphorylation. Akt then activates endothelial nitric oxide synthase (eNOS) through the phosphorylation of its specific site, the serine1177 residue, thus promoting an increase in eNOS activity and, consequently, the production of NO (Muniyappa et al. 2008, Muniyappa & Sowers 2013).

In addition to the vasodilator pathway, insulin can also stimulate a second pathway, responsible for the production of a potent vasoconstrictor called endothelin-1 (ET-1), through the signaling pathway that involves mitogen-activated protein kinase (MAPK) in the endothelium vascular. This imbalance between the vasoconstrictor and vasodilator actions of insulin can cause changes in the control of vascular tone and blood flow adjustments allowing the appearance of endothelial dysfunction, an early event in the atherosclerotic process, and is related to the loss or attenuation of physiological vasodilation mediated by endothelium (Arce-Esquivel et al. 2013, Muniyappa & Sowers 2013).

Therefore, resistance training (RT), characterized by a muscle contraction performed by a given body segment against resistance, has been used for the therapeutic and preventive purposes of a series of pathophysiological conditions such as obesity (Westcott 2012), diabetes (Liu et al. 2019) and arterial hypertension (Faria et al. 2010), being a non-pharmacological strategy of great importance in the prevention and treatment of cardiovascular risk factors, including endothelial dysfunction (Macedo et al. 2016, Winzer et al. 2018). A study has shown that NO-dependent vasodilation is increased after a single session of resistance exercise in hypertensive rats by increasing intracellular calcium (Faria et al. 2010, 2017). On the other hand, other studies in healthy animals have shown an increase in insulin-induced vasodilation, after a single session of resistance exercise, through a signaling pathway that promotes hemodynamic effects without changes in intracellular calcium [Ca2+]i (Fontes et al. 2014, Mota et al. 2015, 2017).

Considering that insulin can help regulate cardiovascular homeostasis, through vasodilator actions, which stimulates the production of NO in the vascular endothelium and increased of the blood flow though the IR/PI3K signaling pathway (Fontes et al. 2014, Mota et al. 2015, 2017), which can contribute to the improvement of metabolic homeostasis and glucose uptake in skeletal muscle (Muniyappa et al. 2008, Muniyappa & Sowers 2013). The objective of this study was to evaluate the mechanisms involved in the insulin-induced vasodilation after RT moderate-intensity in Wistar rats.

MATERIAL AND METHODS

Animals

All procedures described in this study were performed according to the guidelines of the Sociedade Brasileira de Ciência Animal Laboratorial and were approved by the Ethics Committee on Animal Research of the Universidade Federal de Sergipe, Brazil. Sixteen male Wistar rats, three months old and weighing between 250 and 300 g, were obtained from the central vivarium of the physiology department of the Universidade Federal de Sergipe. These animals were transferred to the sectoral vivarium of the Laboratório de Farmacologia Cardiovascular (LAFAC/DFS/UFS) and kept in collective cages (5 animals / cage), with controlled temperature (23 ± 2 °C) and a 12-hour light and dark cycle. They received commercial rodent food (Nuvilab®) and filtered water ad libitum. The rats were weighed and randomly distributed into two groups of eight animals: (1) Control (CON) and (2) RT. In addition, body weight assessment was performed every 2 weeks. All procedures described in this study

were performed according to the guidelines of the Conselho Nacional de Controle de Experimentação Animal (CONCEA) and approved by the Animal Research Ethics Committee of the Universidade Federal de Sergipe, Brazil (protocol number 75/2015).

Resistance exercise protocol

The CON and RT groups were submitted to an adaptation period of one week (5 days, 5 min per day at rest) in a squat machine for resistance exercise developed by Tamaki (Tamaki et al. 1992). Electrical stimulation (20 V, 0.3 s duration, at 3 s intervals) was applied on the tail of the rat through a surface electrode. After the adaptation period, both groups were subjected to a one repetition maximum test (1RM) to determine the maximum weight lifted by the rat in the exercise apparatus. This test consists of increasing the load on the equipment, in which 1RM was defined as the highest maximum load lifted by the animal, in which it was possible to perform the knee extension movement completely in the exercise apparatus. The 1RM test was repeated every 2 weeks in attempt to maintain the desired intensity. The RT group was subjected to a RT protocol which consists in 3 sets of 10 repetitions with a 180 s resting period between each set with the intensity of 60% of 1RM, three times per week (alternate days) for 8 weeks. CO group was subjected to a fictitious exercise consisting in a similar procedures and electrical stimulation as RT group, however, without physical effort.

Vascular reactivity studies

Endothelium-dependent vasodilation was assessed using rat superior mesenteric artery rings prepared as described in (Araujo et al. 2020). The rats were euthanized forty-eight hours after the last exercise session., and superior mesenteric artery was removed, stripped from connective and fatty tissues and sectioned into rings (1–2 mm). The rings were suspended from fine stainless-steel hooks, connected to a force transducer (Letica, Model TRI210; Barcelona, Spain) coupled to an amplifier-recorder (BD-01, AVS, SP, Brazil) with cotton threads in organ baths containing 10 mL of Tyrode's solution (composition in mM: NaCl 158.3, KCl 4.0, CaCl2 2.0, NaHCO3 10.0, C6H12O6 5.6, MgCl2 1.05 and NaH2PO4 0.42). This solution was continually gassed with carbogen (95% O2 and 5% CO2) and maintained at 37°C under a resting tension of 0.75 g for 60 min (stabilization period). During this time, the nutrient solution was changed every 15 min to prevent the interference from metabolites.

The functionality of the endothelium was assessed by the ability of acetylcholine (ACh, 1 μM) to induce more than 75% relaxation of phenylephrine induced (Phe, 1 μM) pre-contraction. Changes in vascular reactivity were then assessed by obtaining concentration-response curves for insulin (10^{-13}–10^{-6} M). These same curves were obtained after incubation for 30 min in the following inhibitors: LY294002, to evaluate the role of the PI3K pathway (inhibitor of PI3K; 50 μM); N^{ω}-nitro-l-arginine methyl ester (L-NAME), to evaluate the role of NO (inhibitor of nitric oxide synthase; 100 μM); L-NAME + BQ123 (a selective ETA receptor antagonist; 1 μM), to evaluate the role of endothelin-1. Phe-induced vasoconstriction (10^{-6} M) was also assessed in the absence or presence of L-NAME. Contractile responses were plotted as a percentage of the contraction Phe-induced by. Vasoconstriction Phe-induced was expressed as maximal tension developed (grams).

In addition, the area under the curve (AUC), and the variation of the area under the curve (dAUC) of endothelium vasodilation in the control and experimental groups was calculated with the following inhibitors: LY294002, L-NAME

and L-NAME + BQ123. These values indicate whether the magnitude of the effect of the vasodilation is different among the CO and RT groups).

Determination of plasma nitrite levels

Forty-eight hours after the end of the RT protocol, the animals were euthanized by exsanguination. Blood samples were collected and centrifuged at 5,000 g for 10 min at 4ºC and stored at -80°C until they were analyzed plasma nitrite levels.

NO production was determined indirectly by measuring the nitrite (NO_2-) levels based on the Griess reaction. Briefly, 100 µl of each plasma sample were incubated with 100 µl of the Griess reagent (1% sulfanilamide in 2.5% H3PO4/0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% H3PO4, 1:1) at room temperature for 10 min. The absorbance was measured at 490 nm in a microplate reader, and NO_2- concentration was determined from a standard NO_2- curve generated using NaNO2.

Statistical analysis

All data are expressed as mean ± SEM. The maximum response (Rmax) was calculated by a non-linear regression analysis of each individual concentration response curve. The AUC was calculated from the graph of the individual concentration-response curve. The differences in area under the concentration-response curves (dAUC) were expressed between the presence and absence of inhibitors and were expressed as a percentage of the AUC of the corresponding control situation. Significant differences between groups were determined using the Student's t-test followed by the Bonferroni post-test to compare the difference of the area under the curve (dAUC) and nitrite. One-way ANOVA followed by the Bonferroni post-test, was used to compare the body weight, 1RM, 1RM/body weight ratio and Phe-induced vasoconstriction and two-way ANOVA, followed by the Bonferroni post-test, was used to compare the concentration-response curves obtained in the mesenteric rings. For all these procedures, the statistical program GraphPad Prism version 5.00 (GraphPad software, San Diego, CA, USA) was used and p values <0.05 were considered statistically significant.

RESULTS

The body weight of the animals was similar between the groups at the beginning of the study, and with no difference at the end of the eight weeks, even the RT group presenting lower values compared to the CON group. However, the animals in the RT group increased their strength levels at the end of eight weeks, and also when compared to the initial and final CON group data. In addition, when normalizing the

Table I. Body weight, 1RM and 1RM/body weight ratio initial and final.

Group		CON	RT
Body weight (g)	Initial	277.6 ± 6.6	284.9 ± 11.7
	Final	323.0 ± 7.1	301.0 ± 5.7
1RM	Initial	1520.0 ± 53.3	1.533 ± 37.2
	Final	1880 ± 64.6***	2188 ± 65.4 $^{***,\#\#,\&\&\&}$
1RM/ Body weight ratio	Final	5.4 ± 0.2	5.3 ± 0.1
	Final	5.7 ± 0.2	7.5 ± 0.$^{2***,\#\#\#,\&\&\&}$

Control (CON) and resistance training (RT). The data represent the mean ± SEM, (n = 10). Statistical differences were determined by one-way ANOVA, followed by the Bonferroni post-test, it was used body weight, one repetition maximum test (1RM) and 1RM/ body weight ratio. ***p < 0.001 *vs.* CO Initial; $^{\#\#}p$ < 0.01, $^{\#\#\#}p$ < 0.001, vs. CO final; $^{\&\&\&}p$ < 0.001 *vs.* RT initial.

levels of force by weight, it was observed that the animals in the RT group supported a greater weight in relation to the CON at the end of the eight weeks (Table I).

Insulin-induced vasodilation was greater in the RT group compared to the CON group (Rmax = 27.2 ± 2.5 vs 15.5 ± 1.6%; p <0.001; Fig. 1). After that, to assess the participation of PI3K in insulin-induced vasodilation, a PI3K inhibitor (LY294002) was used. After incubation with LY294002, a reduction in vasodilation of the mesenteric artery was observed in the CON group (Rmax = 15.5 ± 1,6% to 6.7 ± 1.1%, p <0.001; Fig. 2a), same response was observed in the RT group (Rmax = 27.2 ± 2.5% para 5.3 ± 1.3%, p <0.001; Fig. 2a). dAUC variation values indicated the greater role of PI3K in insulin-induced vasodilation in the RT group (52.0 ± 2.5%; Fig. 2b) compared to the CON group (26.8 ± 3.2%; p <0.05; Fig. 2b).

In addition, to assess the participation of NO in insulin-induced vasodilation, a non-selective NOS inhibitor (L-NAME) was used. After incubation with L-NAME, reduced relaxation was observed in the CON group (Rmax = 15.5 ± 1.6% vs 3.6 ± 1.2%; p <0.001; Fig. 3a), whereas in the RT group, vasodilation was completely abolished, showing a contractile effect (Rmax = 27.2 ± 2.5% vs -1.9 ± 0.2%; p <0.001; Fig. 3a). The dAUC values indicated that NOS involvement is greater in the RT group (97.8 ± 7.0%; Fig. 3b) compared to the CON group (65.8 ± 3.2%; p <0.05; Fig. 3b).

To understand the participation of ET-1 in this response, a concentration-response curve was constructed in the presence of L-NAME + BQ123 (an antagonist of ETA receptors). The CON group showed no change in Rmax (3.6 ± 1.2% a 2.3 ± 0.7%, p> 0.05; Fig. 4a). However, in the RT group, vasoconstriction in the presence of L-NAME + BQ123 was inhibited (Rmáx = -1.9 ± 0.2 % to 2.2 ± 0.3%, p <0.05; Fig. 4b). In addition, the dAUC values between the groups revealed that after incubation with L-NAME + BQ123 it increased in the RT group (68.5 ± 1.1%; Fig. 4b) in relation to the CON group (43.1 ± 4.5 %; p <0.001; Fig. 4b).

Considering the in vitro findings, where an increase in vasodilation was observed in the RT

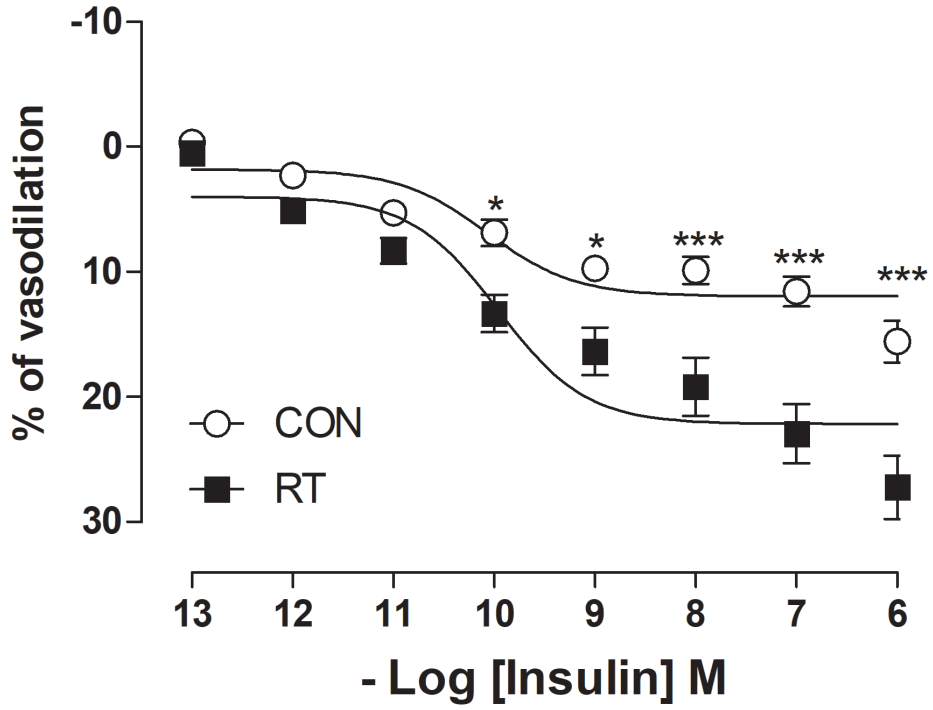

Figure 1. Concentration-response curves for insulin (10^{-13} - 10^{-6} M) in isolated rings of the superior mesenteric artery of a rat with functional endothelium and pre-contracted with Phe (1μM). Rings obtained from animals of the Control group (CON) and Resistance training (RT). The data represent the mean ± SEM for 8 - 10 experiments in each group. *p <0.05, ***p <0.001 vs RT.

group, we evaluated the nitrite levels. The RT group had an increase in the levels of NO_2- (1.9 ± 0.07 %; Fig. 5a) in relation to the CON group (1.6 ± 0.08 %; p <0.01; Fig. 5a).

Regarding the response to the vasoconstrictor induced by Phe, there was an increase in the development of tension in the CON group compared to the RT (0.95 ± 0.03g vs 0.55 ± 0.05g, p <0.05; Fig. 5b). In addition, after incubation with L-NAME, the response to Phe-induced vasoconstriction was enhanced in all groups; however, the developed tension was lower in the RT group (0.99 ± 0.1g) compared to the CON group (1.30 ± 0.05 g, p <0.05; Fig. 5b).

DISCUSSION

In the present study, the effect of moderate-intensity RT on insulin-induced vasodilation was evaluated, which can lead to less risk for individuals and better health benefits (Braith & Stewart 2006). The main results indicate that the eight-week moderate-intensity RT was able to: (1) increased the insulin-induced PI3K/eNOS response, (2) reduce the vasoconstrictor response to phenylephrine and (3) increase nitrate levels.

Insulin plays an important role in maintaining vascular homeostasis, stimulating the release of substances that act to control

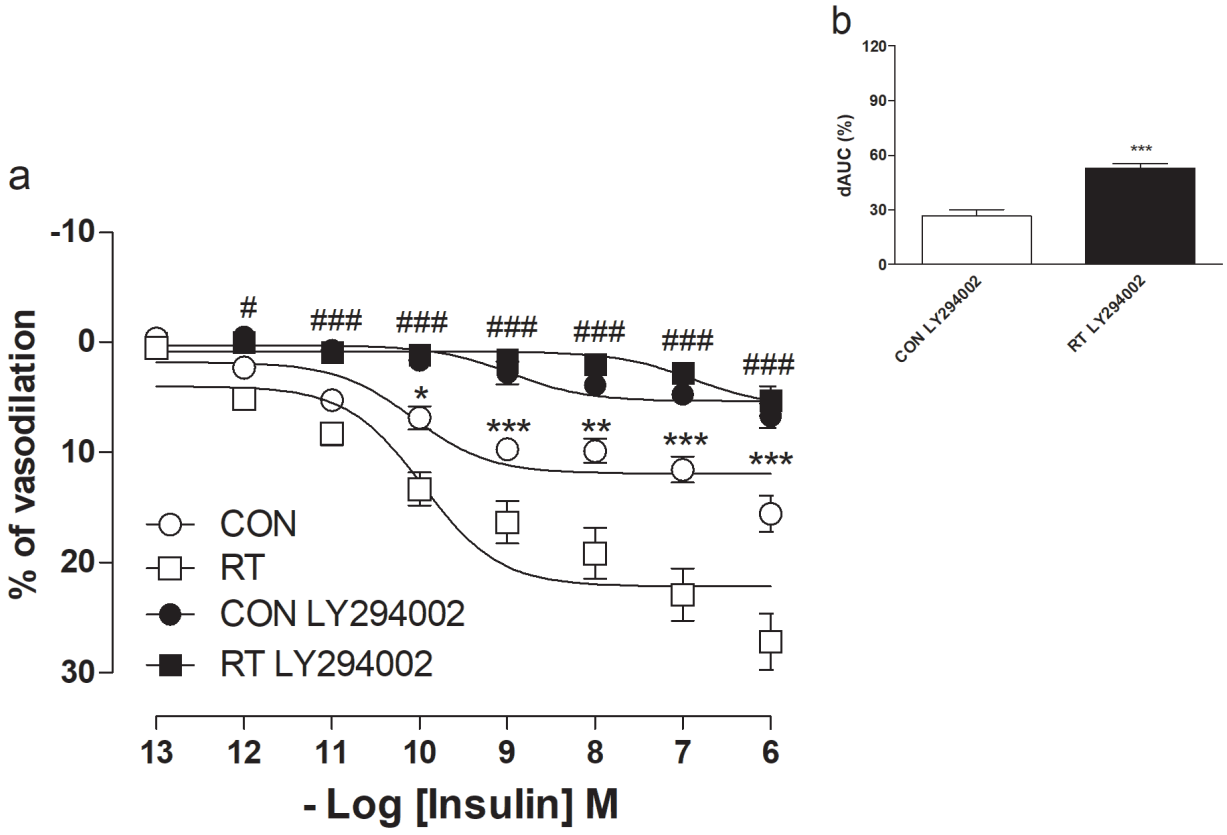

Figure 2. Concentration-response curves for insulin (10^{-13} - 10^{-6} M) in isolated rings of the rat superior mesenteric artery with functional endothelium and pre-contracted with Phe (1µM). (a) Rings obtained from animals of the Control group (CON) and Resistance training (RT) in the absence and presence of LY294002 (50µM). (b) Variation of the area under the curve (dAUC) between the presence and absence of LY294002. The data represent the mean ± SEM for 8 - 10 experiments for each group. (a): *p <0.05, **p <0.01, ***p <0.001, CON vs CON LY294002. $^{\#}p$ <0.05, $^{\#\#\#}p$ <0.001, RT vs. RT LY294002; (b): ***p <0.001 CON LY294002 vs. RT LY294002.

vascular tone. However, endothelial cells may show a reduction in insulin sensitivity, altering the vasodilator response, which may contribute to the appearance of vascular dysfunction (Arce-Esquivel et al. 2013, Muniyappa & Sowers 2013). In this study, insulin-mediated vasodilation was increased in the RT group compared to CON, which may be associated with increased NO production. To our knowledge, this is the first study to demonstrate that RT is able to promote an increase in insulin-induced vasodilation in the superior mesenteric artery of rats.

One of the main mechanisms responsible for this greater vasodilation after RT, may be related to increased shear stress (tension on the vessel wall, which converts mechanical stimuli into chemical stimuli) during exercise sessions, and which can interact with insulin, being able to increase the bioavailability of endothelium-dependent NO, by increasing the expression and activity of the eNOS protein via PI3K/Akt (Arce-Esquivel et al. 2013, Fontes et al. 2014, Mota et al. 2015). In addition, some studies have shown that physical training may be important for vascular sensitivity to insulin in pathologies or risk factors present, such as type 2 diabetes mellitus and insulin resistance, enabling the increase of insulin-mediated vasodilation in the arteries and arterioles (Martin et al. 2012, Mikus et al. 2012).

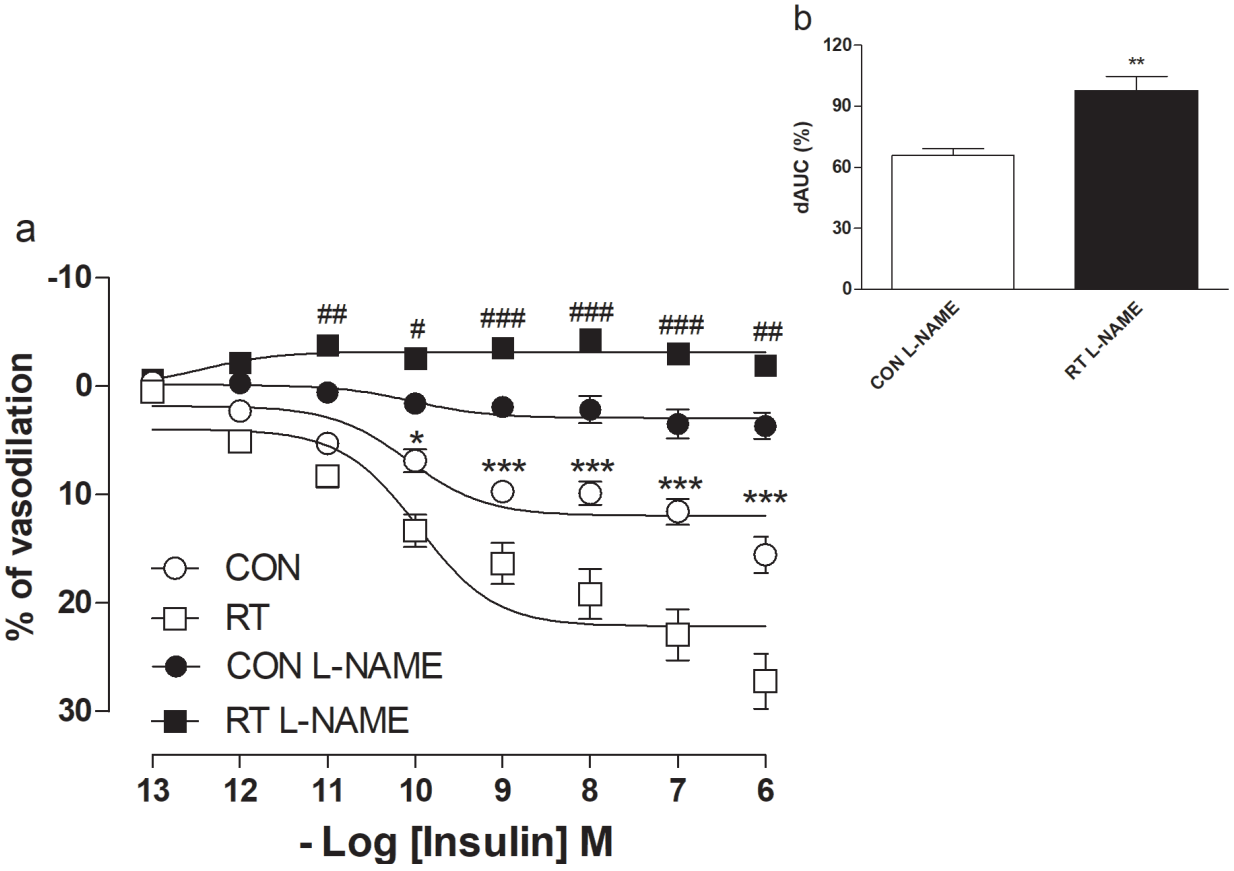

Figure 3. Concentration-response curves for insulin (10^{-13} - 10^{-6} M) in isolated rings of the rat superior mesenteric artery with functional endothelium and pre-contracted with Phe (1μM). (a) Rings obtained from animals of the Control group (CON) and Resistance training (RT) in the absence and presence of L-NAME (100μM). (b) Variation of the area under the curve (dAUC) between the presence and absence of L-NAME. The data represent the mean ± SEM for 8 - 10 experiments for each group. (a): $^{*}p$ <0.05, $^{***}p$ <0.001, CON vs CON L-NAME. $^{\#}p$ <0.05, $^{\#\#}p$ <0.01, $^{\#\#\#}p$ <0.001 RT vs RT L-NAME; (b): $^{**}p$ <0.01 CON L-NAME vs. TR L-NAME.

The physiological effect of insulin can represent up to 25% of maximum vasodilation in different vascular beds, playing an important role in maintaining homeostasis and vascular tone (Padilla et al. 2011, Mikus et al. 2012, Cadore et al. 2014). This happens through the activation of the PI3K/eNOS signaling pathway, resulting in an increase in NO production. However, most studies have examined the effects of infusion of endothelial agonists, such as ACh, to improve vasodilation, where this pathway is characterized by increased [Ca^{2+}]i release, allowing a greater connection with calmodulin, activating the eNOS and therefore increasing NO production (Mallat et al. 2017). Thus, we tried to evaluate the vascular effect induced by insulin in the presence of LY294002 (PI3K inhibitor) and it was found that in both groups there was an attenuation of vasodilation. However, the RT group showed a higher dAUC indicating a greater role for PI3K in insulin-induced vasodilation.

This may have been motivated by the increase in shear stress promoted by exercise, triggered by mechanoreceptors present in endothelial cells, which directly activate G proteins, ion channels and increased activity of enzymes,

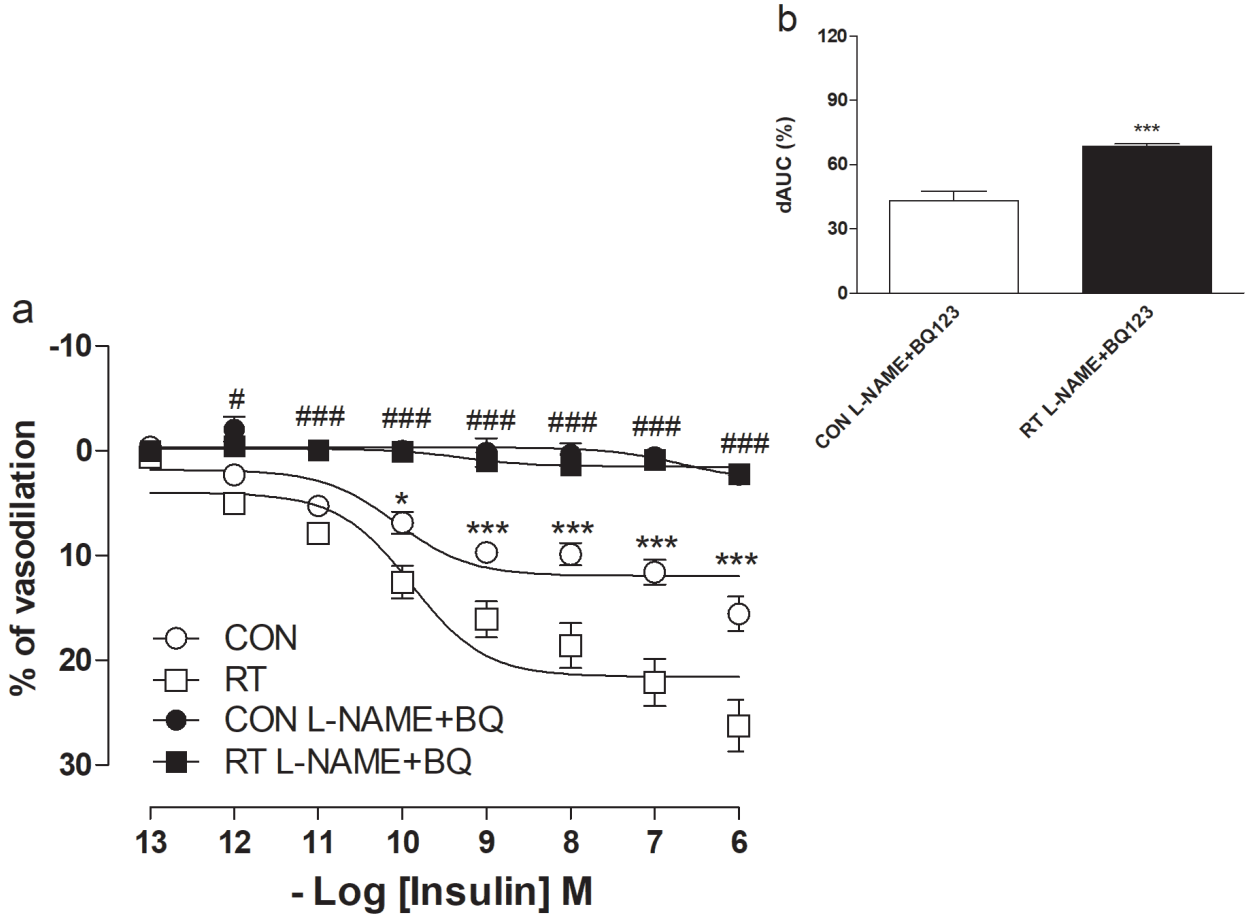

Figure 4. Concentration-response curves for insulin (10^{-13}- 10^{-6} M) in isolated rings of the rat superior mesenteric artery with functional endothelium and pre-contracted with Phe (1μM). (a) Rings obtained from animals of the Control group (CON) and Resistance training (RT) in the absence and presence of L-NAME + BQ123. (b) Variation of the area under the curve (dAUC) between the presence and absence of L-NAME + BQ123. The data represent the mean ± SEM for 8 - 10 experiments for each group. A: *p <0.05, ***p <0.001 CON vs. CON L-NAME + BQ123. $^{\#}p$ <0.01, $^{\#\#\#}p$ <0.001 RT vs. RT L-NAME + BQ123; B: ***p <0.001 CON L-NAME + BQ123 vs. RT L-NAME + BQ123.

such as PI3K, stimulating phosphorylation and Akt activation resulting in increased eNOS activity and subsequent NO production (Muniyappa & Sowers 2013). In addition, during exercise sessions, shear stress remains high, caused by an increase in the metabolic demands of muscle contraction, which allows a greater blood flow to active muscle tissue (Padilla et al. 2011), causing an increase in NO bioavailability, probably involving the activation of the PI3K/eNOS signaling pathway, thus favoring greater vasodilation (Fontes et al. 2014).

NO plays a key role in controlling vascular tone, acting as the main responsible for vasodilation in active vascular beds (Muniyappa & Sowers 2013). Regarding the participation of NO in vasodilation, not only attenuation was observed, but also a reversal of the concentration-response curve after inhibition of eNOS in the RT group compared to the CON group, demonstrating that the RT may be able to promote an increase in phosphorylation of serine1177 and expression of eNOS, thus allowing greater bioavailability of NO, and therefore vasodilation (Chen et al. 2016). Some other factors may also be involved in eNOS activation, such as shear stress and changes in chemical signaling (hormones, cytokines, adipokines) that are present during and after exercise, where these events can contribute to a synergistic effect with the PI3K/eNOS pathway, promoting important systemic benefits in endothelial cells (Padilla et al. 2011). In addition, we speculate that these events may have contributed to a greater release of NO, in view of this, we evaluated the NO_2- levels, substrates that derived from NO.

High levels of NO_2- after exercise may represent a greater bioavailability of NO, since 85% of plasma levels of nitrites and nitrates (NOx) seem to be related to the formation of NO (Lundberg et al. 2009). In fact, in our study there was a significant increase in NO_2- levels in the RT group compared to CON, which shows a possible increase in NO bioavailability after training.. The literature has shown that during moderate-intensity resistance exercise there is a laminar pattern shear stress, increasing the activity of eNOS and, consequently, increasing the bioavailability of NO (Bussell 2002, Boeno et al. 2019). This was also seen in the studies by Willoughby et al. (2011) and Boeno et al. (2019) who demonstrate an increase in the levels of nitrites and nitrates (NOx) after resistance exercise of moderate intensity (Willoughby et al. 2011, Boeno et al. 2019). Thus, RT increases the PI3K/eNOS/NO signaling pathway, while other

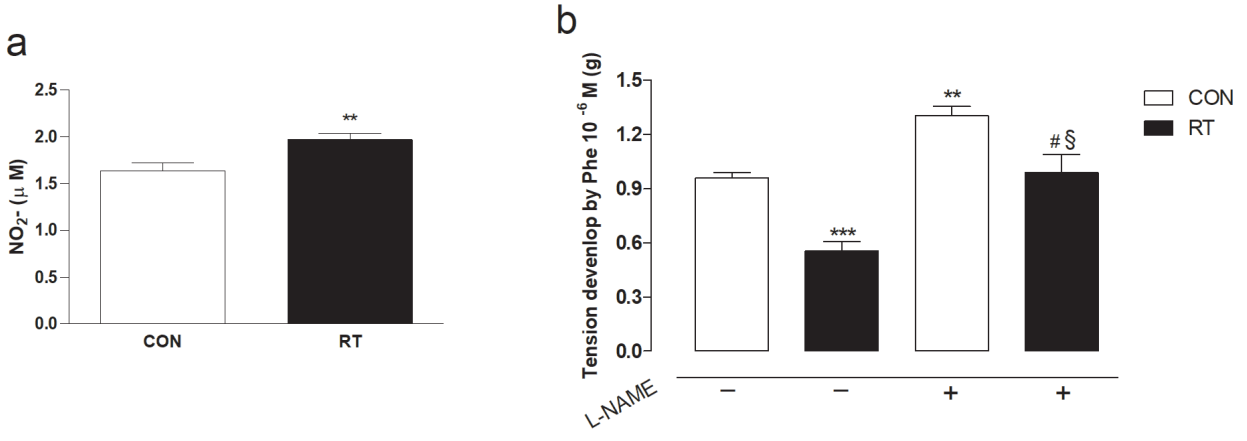

Figure 5. (a) Indirect NO production by measuring nitrite levels. (b) Tension developed by phenylephrine (Phe) (1 μM) evaluated in the mesenteric artery of rats in the Control (CON) group and resistance training (RT) in the absence or presence of L-NAME (100 μM). Values are expressed as mean ± S.E.M for 8-10 experiments in each group. (a): **p <0.01 CON vs. RT; (b): **p <0.01 vs CON; #p <0.01 vs DEX + RT without L-NAME (-); §p <0.05 vs. CON pre-incubated with L-NAME (+).

insulin-stimulated signaling branches, such as the MAPK/ET-1 pathway, appear to remain unchanged.

The literature states that insulin can induce vasoconstriction by an endothelium-dependent mechanism through activation of the MAPK/ET-1 pathway (Muniyappa & Sowers 2013). This vasoconstriction may be due to a predominance of MAPK/ET-1 over the PI3K/eNOS/NO pathway (Muniyappa & Sowers 2013). Then, to confirm that the RT was also acting on the activation of the MAPK/ET-1 pathway, BQ123 + L-NAME was used simultaneously, with the aim of inhibiting both insulin signaling pathways. In this condition, insulin-induced vasoconstriction was inhibited, suggesting that RT also appears to increase activation of the MAPK/ET-1 vasoconstrictor pathway. Studies have shown that ET-1 production may increase during and after resistance exercise promoting unfavorable effects on arterial walls (Okamoto et al. 2008, Boeno et al. 2019). On the other hand, regular resistance exercise can reduce the plasma concentration of ET-1 in healthy individuals, being an important component of prevention and treatment of the increase in cardiovascular diseases.(Maeda et al. 2004).

These results suggest an important contribution of the ET-1/ETA vasoconstrictor mechanism not only at rest, but also in response to exercise. In addition, the release of ET-1 through the insulin-stimulated pathway may be important for the control of vascular tone, since some studies show that the release of this vasocontrictor in healthy conditions is important to maintain the balance between the MAPK/ET-1 and PI3K/eNOS pathways. (Mikus et al. 2012). In addition, some authors have reported that during physical exercise the release of ET-1 has the function of improving the redistribution of blood flow to the exercised tissues and also the effect of ET-1 is compensated for by stimulated NO production (Muniyappa et al. 2008, Muniyappa & Sowers 2013, Janus et al. 2016).

In addition, one of the main causes of increased vasoconstriction may be related to hypersensitivity to Phe, due to the loss of NO-dependent vasodilation (Faria et al. 2017, Araujo et al. 2020). However, knowing that vascular tone is the result of the balance between vasodilator and vasoconstrictor factors, we found a decrease in contractile responses to Phe in the RT group compared to the CON group. Thus, RT may have contributed to the reduction of the contractile response due to the increase in NO bioavailability, probably involving the activation of the PI3K/eNOS signaling pathway (Fontes et al. 2014, Mota et al. 2015, 2017), favoring greater vasodilation due to the increase in the endothelial bioavailability of the NO in the mesenteric artery of rats (Macedo et al. 2016) as can be confirmed in the results of NO_2-.

CONCLUSION

The results of the present study allow us to suggest that 8-week RT resistance training was able to increase the PI3K/eNOS vasodilator pathway response, which is due may be, in part, to a greater production of NO, due to the elevation of nitrite (NO_2-) levels found.. In addition, a slight increase in the MAPK/ET-1 vasoconstrictor pathway was observed, however without promoting losses in the vasodilation of these animals induced by insulin. Together, these results demonstrate that resistance training is able to promote important vascular adjustments that act directly in favor of better control of vascular tone. Therefore, our results suggest that moderate-intensity RT can may be an important non-pharmacological tool for the prevention and treatment of endothelial dysfunction, which can reduce development cardiovascular diseases, such as myocardial infarction, stroke and hypertension."

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REFERENCES

ARAUJO JES, MACEDO FN, OLIVEIRA DPM, BRITTO RM, QUINTANS JSS, BARRETO RSS, SANTOS MRV, QUINTANS-JUNIOR LJ & BARRETO AS. 2020. Resistance training prevents the reduction of insulin-mediated vasodilation in the mesenteric artery of dexamethasone-treated rats. An Acad Bras Cienc 92: 20200316.

ARCE-ESQUIVEL AA, BUNKER AK, MIKUS CR & LAUGHLIN MH. 2013. Insulin Resistance and Endothelial Dysfunction: Macro and Microangiopathy. http://www.intechopen.com/books/type-2-diabetes/insulin-resistance-and-endothelial-dysfunction-macro-and-microangiopathy. Acesso em 26 de Setembro de 2017.

BOENO FP, FARINHA JB, RAMIS TR, MACEDO RCO, RODRIGUES-KRAUSE J, QUEIROZ JN, LOPEZ P, PINTO RS & REISCHAK-OLIVEIRA A. 2019. Effects of a Single Session of High- and Moderate-Intensity Resistance Exercise on Endothelial Function of Middle-Aged Sedentary Men. Front Physiol 10: 777.

BRAITH RW & STEWART KJ. 2006. Resistance Exercise Training Its Role in the Prevention of Cardiovascular Disease. Circulation 113: 2642-2650.

BUSSELL K. 2002. Legless — but still the way forward. Nat Rev Drug Discov 1: 331.

CADORE EL, PINTO RS, BOTTARO M & IZQUIERDO M. 2014. Strength and Endurance Training Prescription in Healthy and Frail Elderly. Aging Dis 1-5(3): 183-195.

CAHILL PA & REDMOND EM. 2016. Vascular endothelium – Gatekeeper of vessel health. Atherosclerosis 248: 97-109.

CHEN X, AN X, CHEN D, YE M, SHEN W, HAN W, ZHANG Y & GAO P. 2016. Chronic Exercise Training Improved Aortic Endothelial and Mitochondrial Function via an AMPKα2-Dependent Manner. Front Physiol 21(7): 631.

FARIA TO, ANGELI JK, MELLO LGM, PINTO GC, STEFANON I, VASSALLO DV & LIZARDO JHF. 2017. A Single Resistance Exercise Session Improves Aortic Endothelial Function in Hypertensive Rats. Arq Bras Cardiol 108(3): 228-236.

FARIA TO, TARGUETA GP, ANGELI JK, ALMEIDA EAS, STEFANON I, VASSALLO DV & LIZARDO JHF. 2010. Acute resistance exercise reduces blood pressure and vascular reactivity, and increases endothelium-dependent relaxation in spontaneously hypertensive rats. Eur J Appl Physiol 110(2): 359-366.

FONTES MT, SILVA TLBT, MOTA MM, BARRETO AS, ROSSONI LV & SANTOS MRV. 2014. Resistance exercise acutely enhances mesenteric artery insulin-induced relaxation in healthy rats. Life Sci 94(1): 24-29.

JANUS A, SZAHIDEWICZ-KRUPSKA E, MAZUR G & DOROSZKO A. 2016. Insulin Resistance and Endothelial Dysfunction Constitute a Common Therapeutic Target in Cardiometabolic Disorders. Mediators Inflamm 2016: 1-10.

LIU Y, YE W, CHEN Q, ZHANG Y, KUO CH & KORIVI M. 2019. Resistance Exercise Intensity is Correlated with Attenuation of HbA1c and Insulin in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis. Int J Environ Res Public Health 16(1): 140.

LUNDBERG JO ET AL. 2009. Nitrate and nitrite in biology, nutrition and therapeutics. Nat Chem Biol 5(12): 865-869.

MACEDO FN ET AL. 2016. Increased Nitric Oxide Bioavailability and Decreased Sympathetic Modulation Are Involved in Vascular Adjustments Induced by Low-Intensity Resistance Training. Front Physiol 28(7): 265.

MAEDA S, MIYAUCHI T, IEMITSU M, SUGAWARA J, NAGATA Y & GOTO K. 2004. Resistance exercise training reduces plasma endothelin-1 concentration in healthy young humans. J Cardiovasc Pharmacol 44(1): 443-446.

MALLAT RK, JOHN CM, KENDRICK DJ & BRAUN AP. 2017. The vascular endothelium: A regulator of arterial tone and interface for the immune system. Crit Rev Clin Lab Sci 54(7-8): 458-470.

MARTIN JS, PADILLA J, JENKINS NT, CRISSEY JM, BENDER SB, RECTOR RS, THYFAULT JP & LAUGHLIN MH. 2012. Functional adaptations in the skeletal muscle microvasculature to endurance and interval sprint training in the type 2 diabetic OLETF rat. J Appl Physiol 113(8): 1223-1232.

MIKUS CR ET AL. 2012. Voluntary Wheel Running Selectively Augments Insulin-Stimulated Vasodilation in Arterioles from White Skeletal Muscle of Insulin-Resistant Rats. Microcirculation 19(8): 729-738.

MOTA MM ET AL. 2015. Endothelium adjustments to acute resistance exercise are intensity-dependent in healthy animals. Life Sci 1(142): 86-91.

MOTA MM ET AL. 2017. Effects of a Single Bout of Resistance Exercise in Different Volumes on Endothelium Adaptations in Healthy Animals. Arq Bras Cardiol 108(5): 436-442.

MUNIYAPPA R, IANTORNO M & QUON MJ. 2008. An integrated view of insulin resistance and endothelial dysfunction. Endocrinol Metab Clin North Am 37(3): 685-711.

MUNIYAPPA R & SOWERS JR. 2013. Role of insulin resistance in endothelial dysfunction. Rev Endocr Metab Disord 14(1): 5-12.

OKAMOTO T, MASUHARA M & IKUTA K. 2008. Relationship between plasma endothelin-1 concentration and cardiovascular responses during high-intensity eccentric and concentric exercise. Clin Physiol Funct Imaging 28(1): 43-48.

PADILLA J, SIMMONS GH, BENDER SB, ARCE-ESQUIVEL AA, WHYTE JJ & LAUGHLIN MH. 2011. Vascular Effects of Exercise: Endothelial Adaptations Beyond Active Muscle Beds. Physiology (Bethesda) 26(3): 132-145.

RAJENDRAN P, RENGARAJAN T, THANGAVEL J, NISHIGAKI Y, SAKTHISEKARAN D, SETHI G & NISHIGAKI I. 2013. The Vascular Endothelium and Human Diseases. Int J Biol Sci 9(10): 1057-1069.

TAMAKI T, UCHIYAMA S & NAKANO S. 1992. A weight-lifting exercise model for inducing hypertrophy in the hindlimb muscles of rats. Med Sci Sports Exerc 24(8): 881-886.

WESTCOTT WL. 2012. Resistance Training is Medicine: Effects of Strength Training on Health. Curr Sports Med Rep 11(4): 209-216.

WILLOUGHBY DS, BOUCHER T, REID J, SKELTON G & CLARK M. 2011. Effects of 7 days of arginine-alpha-ketoglutarate supplementation on blood flow, plasma L-arginine, nitric oxide metabolites, and asymmetric dimethyl arginine after resistance exercise. Int J Sport Nutr Exerc Metab 21(4): 291-299.

WINZER EB, WOITEK F & LINKE A. 2018. Physical Activity in the Prevention and Treatment of Coronary Artery Disease. J Am Heart Assoc 7(4): e007725.

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