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HEALTH SCIENCES

Resistance training prevents the reduction of insulin-mediated vasodilation in the mesenteric artery of dexamethasone-treated rats.

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Abstract: This study evaluated whether resistance training (RT) could prevent glucocorticoid-induced vascular changes. Wistar rats were divided into groups: control (CO), dexamethasone (DEX), and Dexamethasone+RT (DEX+RT). On the eighth week, dexamethasone was administered in the DEX and DEX+RT groups. Thereafter, the animals were sacrificed and blood samples were used to assess the lipid profile, glucose and insulin. Vascular reactivity to insulin and phenylephrine (Phe) were evaluated. The DEX+RT group presented an improvement in the lipid profile, fasting glucose, and insulin levels compared to the DEX group. In addition, vasodilation was reduced in the DEX group compared to the CO group, and was increased in the DEX+RT group. After inhibition of phosphatidylinositol 3-kinase, DEX group showed contraction, in which it was in the DEX + RT group. When nitric oxide synthase (NOS) participation was evaluated, the DEX group presented a contraction compared to the CO group, with no contractile effect in the DEX+RT group. Moreover, vasoconstriction caused by NOS inhibition was abolished by BQ123 (endothelin receptor antagonist). In respect Phe response, there was an increase in tension in the DEX group compared to the CO group, being reduced in the DEX+RT group. The results suggest that RT prevented damage to vascular reactivity.

Key words: Glucocorticoid, insulin, nitric oxide, resistance training, vascular reactivity.

INTRODUCTION

Glucocorticoids (GC) have been used to treat some of conditions due to their antiallergic and anti-inflammatory properties. However, a single dose and/or chronic use can lead to various side effects such as changes in lipid, protein and carbohydrate metabolism, resulting in metabolic disorders such as, dyslipidemia, hyperglycemia, hyperinsulinemia and insulin resistance (Coderre et al. 2007, Barel et al. 2010). This has been documented in glucocorticoid clinical trials, and during glucocorticoid treatment - particularly when treatment is in conjunction with mental stress, and patients with Cushing's disease (Wang 2005). These changes in glucose and insulin concentrations can be partially explained through damage to the insulin signaling pathway in both hepatic and extrahepatic cells (Brown et al. 2007, Geer et al. 2014). This can promote insulin resistance (IR), which is considered a risk factor for cardiovascular diseases, such as myocardial infarction, atherosclerosis (Laakso & Kuusisto 2014) and hypertension (Goodwin & Geller 2012, Hattori et al. 2013). Also, this can lead to peripheral vascular disease, due to the damage caused to the vascular endothelium, increasing

cardiovascular morbidity and mortality (Laakso & Kuusisto 2014).

Although insulin is considered a hormone that acts primarily on skeletal muscle, adipose tissue and the liver in respect of the control of glucose homeostasis, studies indicates that it also participates directly in the maintenance of homeostasis and vascular tone (Arce-Esquivel et al. 2013, Fontes et al. 2014, Mota et al. 2015). The insulin signaling pathways regulates endothelial production of NO through binding to its receptor tyrosine kinase, resulting in the phosphorylation of the insulin receptor substrate (IRS-1), which then binds and activates phosphatidylinositol 3-kinase (PI3K), stimulating Akt activity. Akt directly phosphorylates eNOS at Ser1177, resulting in increased eNOS activity and subsequent NO production (Muniyappa et al. 2008, Muniyappa & Sowers 2013). However, GC treatment can cause IR, raising insulin concentrations, which stimulate the MAPK-dependent pathway leading to secretion of the vasoconstrictor endothelin-1 (ET-1) from the vascular endothelium.

This imbalance between the vasoconstrictor and vasodilator actions of insulin associated with IR is an important factor in the vascular pathophysiology of IR and endothelial dysfunction (Muniyappa et al. 2008, Arce-Esquivel et al. 2013, Muniyappa & Sowers 2013). Exercise has been an important non-pharmacological tool in the prevention and treatment of cardiovascular risk factors, among them endothelial dysfunction (Winzer et al. 2018). The literature has shown that acute and chronic aerobic exercise improve the insulin signaling pathway, which is involved not only in glucose metabolism but also in vascular modulation (Tjønna et al. 2011, Mitranun et al. 2014).

In recent years, resistance training (RT) has been considered essential for maintaining several aspects of health, and is associated with important benefits, such as increased functional capacity (Marcos-Pardo et al. 2019), muscle mass (Cadore 2014), strength (Lopez et al. 2018), improved body composition (Arnarson et al. 2014) and reduced hypertension, obesity and diabetes (Westcott 2012). Despite this, studies are inconsistent regarding the effects of RT on vascular function. Some have demonstrated that RT increased NO-dependent vasodilation (Faria et al. 2010), while others indicate that RT has no effect and does not reduce vascular function (Westcott 2012, Miyachi 2013, Ashor et al. 2014).

On the other hand, previous studies have shown that insulin-induced vasodilation is enhanced after acute RT, in which, is a signaling pathway that promotes hemodynamic effects without changes intracellular calcium (Fontes et al. 2014, Mota et al. 2015). Therefore, given that it has been shown that RT can change the metabolic effects of insulin through the IR/PI3K signaling pathway, the objective of this study was to evaluate whether resistance training (RT) could prevent the side effects of dexamethasone on insulin-induced vasodilatation, since GCs can inhibit the PI3K/Akt/eNOS signaling pathway, reducing the insulin response.

MATERIALS AND METHODS

Animals

Twenty-four male Wistar rats (300-350g) were obtained from the Central Animal Facility of the Universidade Federal de Sergipe. Rats were kept in collective cages (five animals/cage), in a temperature-controlled room (22 ± 2°C) with a 12 h light/12 h dark cycle, and received commercial rodent chow (Presence®) and filtered water ad libitum. The rats were weighed weekly from the beginning to the end of the study using a digital electronic scale. All procedures described in this study were performed according to the guidelines of the Brazilian Society of Laboratory

Animal Science, and were approved by the Ethics Committee on Animal Research of the Universidade Federal de Sergipe, Brazil (protocol number 75/2015).

Experimental groups

The rats were weighed and distributed randomly into three groups of eight animals: (1) control group (CO), sedentary throughout the 8-week, receiving a daily injection of saline (2 ml/kg/day, i.p.) during the last week; (2) Dexamethasone (DEX), sedentary throughout the 8-week, receiving a daily injections of DEX (2 ml/kg/day, i.p., dissolved in saline) during the last week and (3) Dexamethasone + resistance training (DEX+RT), eight weeks exercise training, receiving daily injections of DEX (2 ml/kg/day, i.p., dissolved in saline) during the last week. This dosage of dexamethasone (2 mg/kg) used was based on a previously published study (Perry et al. 2003). Dexamethasone or saline (as a control) were injected between 2 pm and 3 pm.

Resistance training protocol

CO, DEX and DEX+RT animals underwent a five-day adaptation period (5 days, 5 min per day in rest position) in a customized squat apparatus for RT, as developed by 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, the groups were subjected to a one maximal repetition test (1RM) to determine the maximum weight lifted by the rat in the exercise apparatus. The 1RM test was repeated every 2 weeks in attempt to maintain the desired intensity. The DEX+RT group was subjected to a RT protocol which consists in 3 sets of 10 repetitions with an intensity of 60% of the maximum load established in the 1RM test, three times per week (alternate days) for 8 weeks. CO and DEX group were subjected to a fictitious exercise consisting in a similar procedures and electrical stimulation as DEX+RT group, however, without physical effort. In the eighth week of resistance training, was administered dexamethasone (DEXA, 2.0 mg/kg) for 7 days daily, through intraperitoneal injection in the DEX and DEX + RT groups and CO group, 0.9% NaCl was injected.

Measurement of metabolic parameters

Forty-eight hours after the end of the RT protocol, eight-hour fasting plasma glucose levels were measured using blood obtained through caudal puncture and a glucometer (Accu-Chek Advantage II, Roche, São Paulo, SP, Brazil). After measuring fasting glucose, the animals were anesthetized with isoflurane and euthanized by exsanguination. Blood samples were collected and centrifuged at 5,000 g for 10 min at 4°C and stored at -80°C until they were analyzed. Blood samples were used to measure insulin, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and triglyceride (TG) concentrations utilizing a commercial kit (Bioclin, Belo Horizonte, MG, Brazil). Levels of low-density lipoprotein cholesterol (LDL-c) were obtained using the Friedwald calculation (Friedewald et al. 1972).

Vascular reactivity studies

Following animal sacrifice, the 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); L-NAME, to evaluate the role of NO (inhibitor of nitric oxide synthase; 100 μM); L-NAME + BQ123, to evaluate the role of endothelin-1 (a selective ETA receptor antagonist; 1 μM). 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 induced by Phe. Vasoconstriction induced by Phe 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, DEX and DEX + RT groups.

Statistical analysis

All data are expressed as mean ± S.E.M. Significant differences between groups were determined using two-way ANOVA, followed by Bonferroni's post hoc test, to compare the concentration-response curves obtained in the mesenteric rings. One-way ANOVA, followed by Bonferroni's post hoc test, was used to compare the dAUC and Phe-elicited vasoconstriction. All statistical comparisons were made using GraphPad Prism 5.1 (GraphPad Software Inc., San Diego, CA, USA) and values of $p<0.05$ were considered to be statistically significant.

RESULTS

Body weight and metabolic parameters

Body weights and metabolic parameters are shown in Table I. The body weight of the animals was similar in all groups at the beginning of the study. Final body weight was significantly reduced in the DEX and DEX+RT groups compared to baseline ($p<0.05$) and to the CO group ($p<0.001$). Moreover, in the DEX group fasting glucose ($p<0.001$), insulin ($p<0.01$), total cholesterol TC ($p<0.01$), and low-density lipoprotein (LDL) ($p<0.01$) increased, and high-density lipoprotein (HDL) ($p<0.01$) decreased compared with the CO group. However, in the DEX + RT group there was no increase in fasting glucose ($p<0.05$), insulin ($p<0.01$), TC ($p<0.001$), LDL ($p<0.001$), and there was a decrease in HDL ($p<0.05$). No significant differences were observed in triglyceride levels.

Endothelial mechanisms and changes in the insulin-induced vasodilation signaling pathway

Insulin-induced vasodilatation was reduced in the DEX group compared to CO group (R_{max} = 7.9 ± 1.4 vs 20.6 ± 2.6 %; $p<0.001$; Fig. 1a). However, in the DEX+RT group insulin-induced vasodilatation was enhanced compared to the DEX group (R_{max} = 23.6 ± 1.5% vs 7.9 ± 1.4; $p<0.001$; Fig. 1a) and similar to the CO group (R_{max} = 20.6 ± 2.6 %; $p>0.05$; Fig. 1a). To evaluate PI3K participation in the vasodilatation induced by insulin, an inhibitor of PI3K (LY294002), was used. After incubation with LY294002, a reduction of relaxation was observed in the CO group (R_{max} = 20.6 ± 2.6% to

Table I. Bodyweight in the first and eighth week, fasting glucose, insulin, TG, TC, LDL and HDL.

Group		CO	DEX	DEX+RT
Body weight (g)	Initial	335.83 ± 4.6	335.33 ± 1.9	332,1 ± 3,2
	Final	333,8 ± 3,0	267,1 ± 10,7***$^{\&}$	267,9 ± 9,8***$^{\&}$
Fasting glucose (mmol/L)	Final	5.2 ± 0.1	6.2 ± 0.2**	5.4 ± 0.1$^{\#}$
Insulin (ng/ml)	Final	2.1 ± 0.8	10.5 ± 1.5**	2.33 ± 1.1$^{\#\#}$
TGs (mmol/L)	Final	1.05 ± 0.05	1.23 ± 0.02	1.25 ± 0.07
TC (mmol/L)	Final	4.1 ± 0.1	6.3 ± 0.3***	3.6 ± 0.3$^{\#\#\#}$
LDL (mmol/L)	Final	2.62 ± 0.19	5.12 ± 0.3***	2.12 ± 0.3$^{\#\#\#}$
HDL-c (mmol/L)	Final	1.35 ± 0.09	0.94 ± 0.04*	1.32 ± 0.05$^{\#}$

Control (CO), dexamethasone (DEX) and dexamethasone + resistance training (DEX+RT). The data represent the mean ± SEM, (n = 6). Statistical differences were determined by one-way ANOVA, followed by the Bonferroni post-test, it was used for fasting glucose, triglycerides (TG), total cholesterol (CT), low density lipoproteins (LDL) and high-density lipoprotein cholesterol (HDL-c). **p < 0.01 and ***p < 0.001 *vs.* CO; body weight $^{\&}$p < 0.05, DEX and DEX+RT initial *vs.* final; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01, $^{\#\#\#}$p < 0.001, vs. DEX.

7.4 ± 0.9%, p<0.001; Fig. 2a), whereas in the DEX group the vasodilation was totally abolished, showing a slight contractile effect (Before: R_{max} = 7.9 ± 1.4% vs after: R_{max} = -1.5 ± 0.5%, p<0.001; Fig. 2a). Reduced vasodilation was observed in the DEX+RT group after incubation with LY294002, however the contractile effect was abolished (R_{max} = 23.6 ± 1.5% vs after: R_{max} = 8.5 ± 1.3%, p<0.001; Fig. 2a). The dAUC values indicated an enhanced role of PI3K in the vasodilatation induced by insulin in the CO and DEX+RT groups (67.1 ± 1.1% and 70.8 ± 5.6%; Fig. 2b) compared to the DEX group (42.8 ± 7.0%; p<0.05; Fig. 2b).

Furthermore, to evaluate NO participation in the vasodilatation induced by insulin, a non-selective inhibitor of NOS (L-NAME), was used. After incubation with L-NAME a reduction of relaxation was observed in the CO group (R_{max} = 20.6 ± 2.6% to 3.6 ± 1.2%, p<0.001; Fig. 3a), whereas in the DEX group the vasodilation was totally abolished, showing a slight contractile effect (Before: R_{max} = 7.9 ± 1.4% vs after: R_{max} = -2.3 ± 0.4%, p<0.001; Fig. 3a). In the DEX+RT group vasodilation reduced after incubation with L-NAME, however the contractile effect was abolished (R_{max} = 23.6 ± 1.5% vs after: R_{max} = 4.6 ± 1.1%, p<0.001; Fig. 3a). A comparison of dAUC values indicated that the involvement of NOS is higher in the CO and DEX+RT group (100.0 ± 5.9% and 100.0 ± 7.0%; Fig. 3b) compared to the DEX group (54.1 ± 3.0%; p<0.05; Fig. 3b).

In order to understand the participation of ET-1 in this response, a concentration-response curve in the presence of L-NAME + BQ123 (an antagonist of ETA receptors) was constructed. The CO group showed no change in R_{max} (3.6 ± 1.2% to 2.3 ± 0.7%, p>0.05; Fig. 4a), a similar response was observed in the DEX+RT group (R_{max} = 4.6 ± 1.1% to 3.5 ± 1.2%, p>0.05; Fig. 4a). However, in the DEX group vasocontraction in

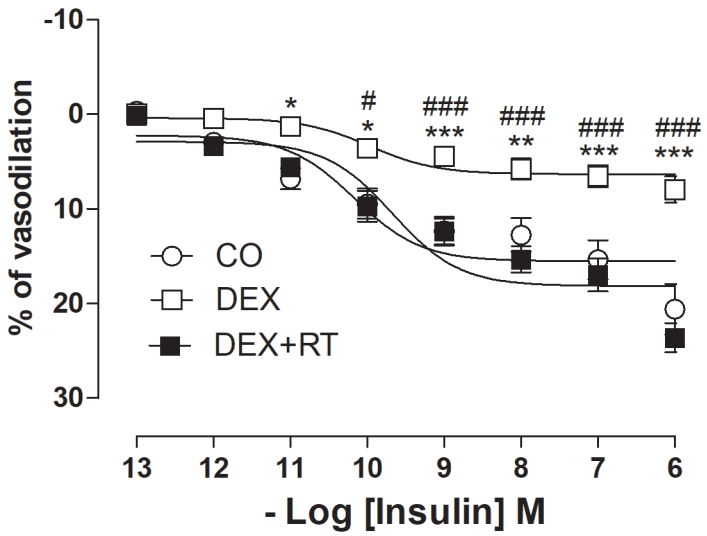

Figure 1. Cumulative concentration-response curve to insulin in intact segments obtained from the superior mesenteric artery of Wistar rats and pre-contracted with phenylephrine (1 µM) in the control (CO), dexamethasone-treated (DEX) and dexamethasone + resistance training (DEX+RT) groups. The results are expressed as the mean ± SEM for 8–10 experiments in each group. *p< 0.05, **p< 0.01 ***p<0.001, CO vs. DEX; #p< 0.05, ##p< 0.01, ###p< 0.001, DEX vs. DEX+RT.

the presence of L-NAME + BQ123 was inhibited (R_{max} = -4.9 ± 0.7% to 0.1 ± 1.3%, p<0.05, Fig. 4a). Moreover, dAUC values between groups revealed that there was an increased effect on the rings after incubation with L-NAME + BQ123 in the CO and DEX+RT groups (49.8 ± 4.0% and 43.6 ± 5.8%, respectively; Fig. 4b) compared to the DEX group (25.6 ± 3.9%; p<0.05; Fig. 4b).

Phenylephrine-induced vasoconstrictor response

Phe-induced vasoconstriction response increased tension in the DEX group compared to CO group (1.18 ± 0.07g vs 0.62 ± 0.06g, p<0.05) as shown in the Fig. 5. However, Phe-induced vasoconstriction decreased in the DEX+RT group compared with the DEX group (0.62 ± 1.1g vs 1.18 ± 0.07g, p<0.05), Fig. 5. Furthermore, after incubation with L-NAME, Phe-induced vasoconstriction increased the response in all groups, however, the developed tension was smaller in the CO and DEX+RT groups (1.02 ± 0.5g vs 1.12 ± 0.1g, p>0.05) than in the DEX group (1.57 ± 0.05g, p<0.05), Fig. 5.

DISCUSSION

In the present study, the effect of RT on preventing the side effects of glucocorticoids on insulin-induced vasodilatation was evaluated. The main results of the eight-week RT protocol were that RT: (1) prevented impairment of insulin-mediated vasodilatation; (2) increased insulin-induced vasodilation via the PI3K/Akt/eNOS pathway; (3) reduced ET-1-induced vasoconstriction, and (4) reduced vasoconstrictor responsiveness to phenylephrine.

Although widely used in the treatment of inflammation and allergies, chronic treatment with dexamethasone (synthetic glucocorticoid) can cause several side effects, such as glucose intolerance (Pauli et al. 2006), alterations in free fatty acid metabolism (Qi et al. 2004), hyperglycemia (Rhee et al. 2004) and hyperinsulinemia (Barel et al. 2010). In this study, TC and LDL-c increased, and HDL-c decreased, showing changes in lipid metabolism. High doses of GC increase the breakdown of TGs and the release of glycerol and free fatty acids (FFA) by lipolysis. Glycerol contributes to increased hepatic gluconeogenesis, promoting increased

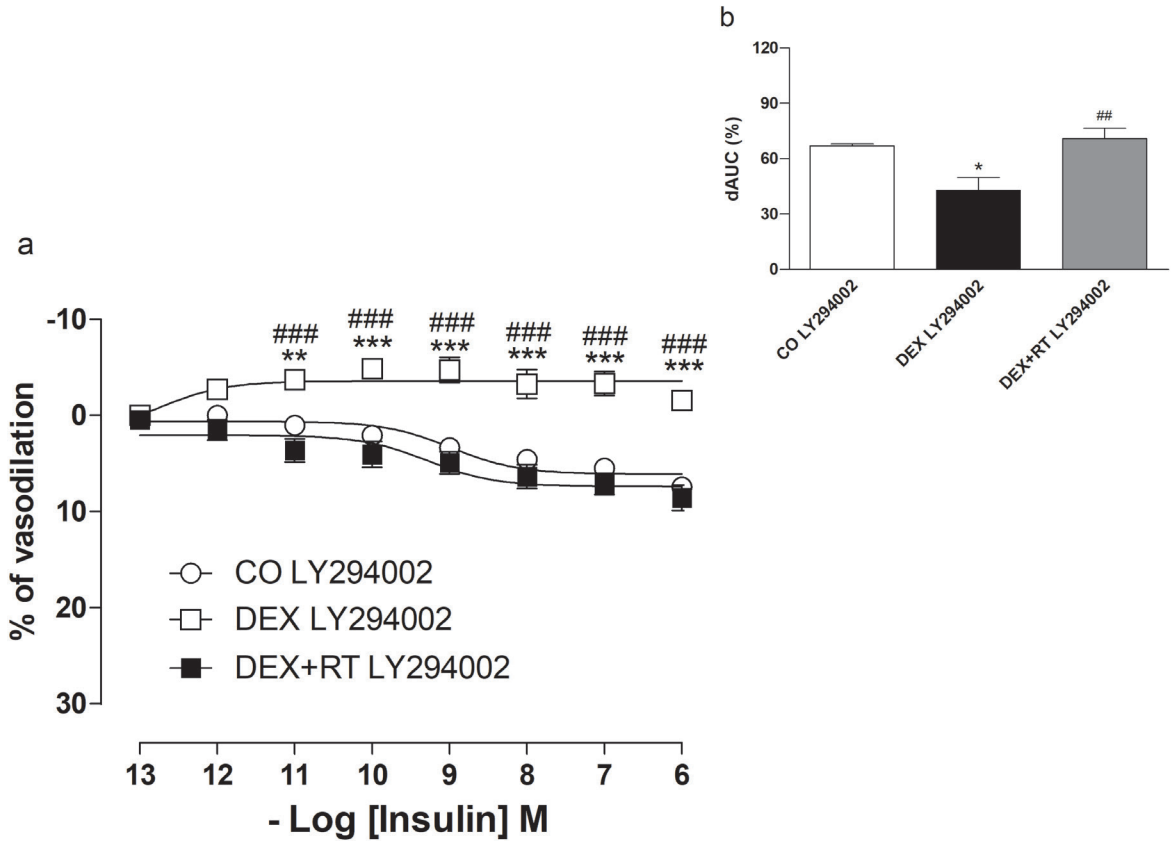

Figure 2. Cumulative concentration-response curve to insulin in intact segments obtained from the superior mesenteric artery of Wistar rats and pre-contracted with phenylephrine (1 μM) in the control (CO), dexamethasone-treated (DEX) and dexamethasone + resistance training (DEX+RT) groups, in the presence of LY294002 (50 μM) (a), and the difference in the area under the concentration–response curve (dAUC) for the response to insulin is shown for the CO, DEX and DEX+RT groups (b). The results are expressed as the mean ± SEM for 8–10 experiments in each group. **p< 0.01 ***p< 0.001, CO vs. DEX; ###p< 0.001, DEX vs. DEX+RT.

blood glucose concentration. Besides, FFA becomes the primary substrate in the energy formation process, thus making glucose a secondary energy substrate, increasing blood glucose (Vegiopoulos & Herzig 2007). GCs also impairs the plasma lipid profile by elevating total TC and LDL-c, and reducing HDL-c concentrations (Burén et al. 2008, Rafacho et al. 2008a). However, RT was able to prevent significant changes in plasma lipid profiles, preventing the onset of dexamethasone-induced dyslipidemia.

These changes in lipid profile caused by GCs may lead to increased glucose concentration, and reduce intracellular insulin signal transduction (Geer et al. 2014). In the present study, there was an increase in glucose and insulin concentration in the dexamethasone-treated animals, these changes being prevented with RT. It has been suggested that glucocorticoid may promote changes in glucose metabolism, without necessarily increasing fasting glucose (Pauli et al. 2006, Barel et al. 2010), causing changes only in insulin tolerance. Although we did not assess

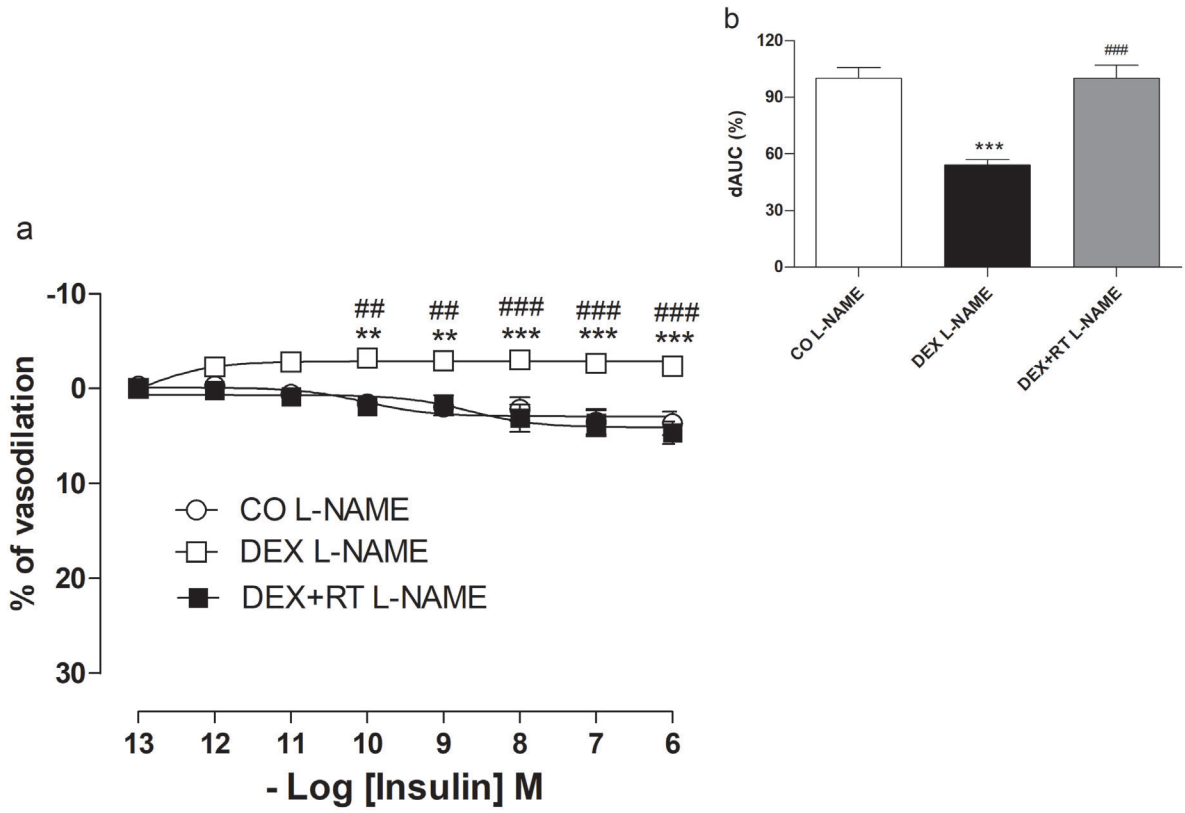

Figure 3. Cumulative concentration-response curve to insulin in intact segments obtained from the superior mesenteric artery of Wistar rats and pre-contracted with phenylephrine (1 μM) in the control (CO), dexamethasone-treated (DEX) and dexamethasone + resistance training (DEX+RT) groups, in the presence of L-NAME (100 μM) (a), and the difference in the area under the concentration–response curve (dAUC) for the response to insulin is shown for the CO, DEX and DEX+RT groups (b). The results are expressed as the mean ± SEM for 8–10 experiments in each group. *p< 0.05, **p< 0.01 ***p< 0.001, CO vs. DEX; #p< 0.05, ##p< 0.01, ###p< 0.001, DEX vs. DEX+RT.

insulin sensitivity, we can suggest that insulin sensitivity reduced in the animals in the DEX group, as elevated serum insulin and glucose concentrations were observed only in the dexamethasone-treated animals. IR may also contribute to cause hyperglycemia and decrease the insulin-induced vasodilator response, increasing its alternative vasoconstriction pathway; this promotes reduced blood flow and glucose uptake by blood vessels, which can lead to endothelial dysfunction (Muniyappa et al. 2008).

In this study, vasodilatation insulin-mediated was reduced in the DEX group. GCs increases muscle protein breakdown and adipose tissue (Cain & Cidlowski 2017). These changes contribute to altering the lipid profile, impairing the action of insulin and, subsequently, its signal transduction (Rafacho et al. 2014). This decrease in insulin sensitivity may lead to an imbalance between the vascular actions of insulin via PI3K/Akt/eNOS, decreasing NO bioavailability and, consequently, tissue responsiveness to insulin (Janus et al. 2016). However, the DEX+RT avoided damage to vasodilation caused by GCs. To our knowledge, this is the first study to observe a protective effect for RT on insulin-mediated vascular responsiveness after GCs treatment.

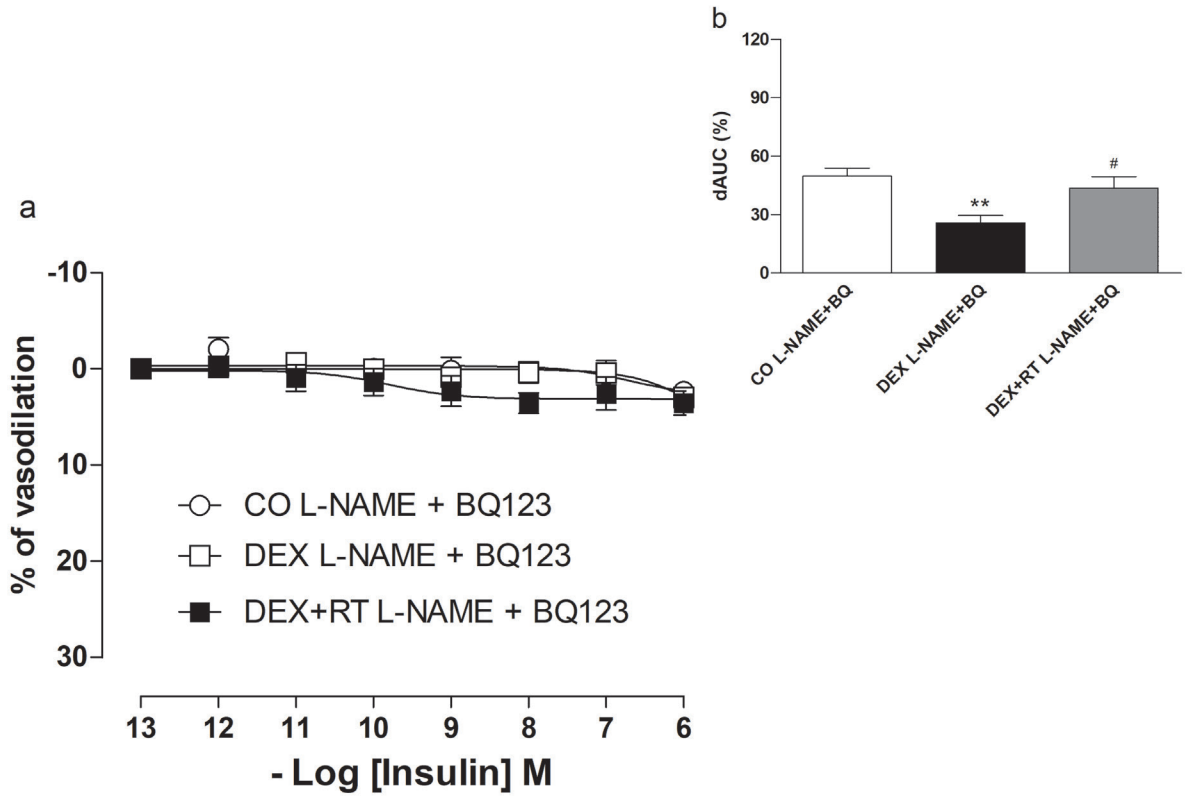

Figure 4. Cumulative concentration-response curve to insulin in intact segments obtained from the superior mesenteric artery of Wistar rats and pre-contracted with phenylephrine (1 µM) in the control (CO), dexamethasone-treated (DEX) and dexamethasone + resistance training (DEX+RT) groups, in the presence of L-NAME + BQ123 (100 µM and 1 µM, respectively) (a), and the difference in the area under the concentration–response curve (dAUC) for the response to insulin is shown for the CO, DEX and DEX+RT groups (b). The results are expressed as the mean ± SEM for 8–10 experiments in each group. *p< 0.01, CO vs. DEX; #p< 0.05, DEX vs. DEX+RT.

The literature has shown that physical training is important to improve vascular sensitivity to insulin in pathologies or risk factors such as T2DM and insulin resistance, enabling increased insulin-mediated vasodilation in arteries and arterioles (Martin et al. 2012, Mikus et al. 2012). This increase in vasodilation caused by RT in the present study may be related to shear stress (tension on vessel walls converting mechanical stimuli into chemical stimuli), which may interact with insulin, and favor increased expression and activity of eNOS protein via endothelium-dependent PI3K/Akt increasing NO bioavailability (Arce-Esquivel et al. 2013, Fontes et al. 2014, Mota et al. 2015).

Insulin participates directly in the maintenance of homeostasis and vascular tone, which can represent up to 25% of maximal vasodilation (Padilla et al. 2011, Mikus et al. 2012, Cadore 2014). The physiological effect of insulin on the different vascular beds comprise vasodilation, combined with an increase in NO production through activation of the PI3K/eNOS signaling pathway. GCs can promote disturbance of the insulin-mediated vasodilator response through reduced tyrosine-phosphorylated IR and total IRS-1 proteins, decreasing activity of

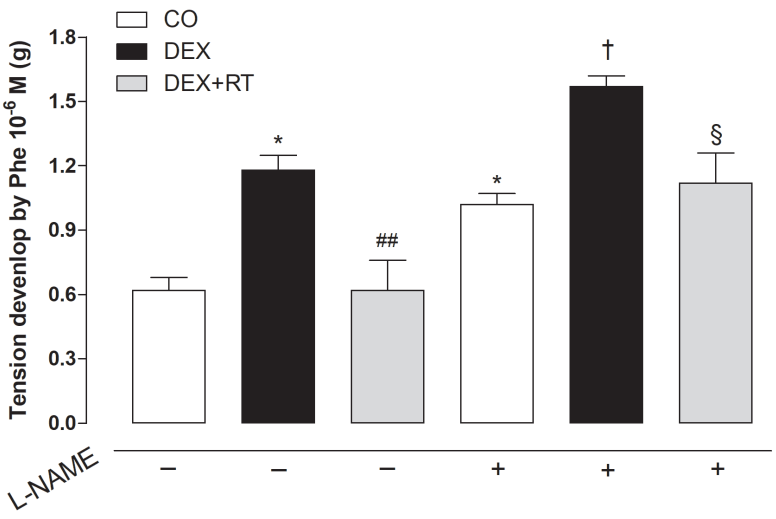

Figure 5. The developed tension elicited by Phe (10^{-6} M) was evaluated in mesenteric artery of control (CO), dexamethasone-treated (DEX) and dexamethasone + resistance training (DEX+RT) groups, in the absence or presence of L-NAME (100 µM). The results are expressed as the mean ± SEM for 8–10 experiments in each group. *p < 0.05 vs CO; #p< 0.05 without L-NAME; ##p< 0.01 vs DEX without L-NAME; †p < 0.05, vs. CO with L-NAME; §p< 0.05 vs DEX with L-NAME.

the PI3K, and reducing the response of insulin signaling (Kuo et al. 2015). Thus, we evaluated insulin-induced vascular effects in the presence of LY294002, and found that vasoconstriction was present in the DEX group, but was avoided with RT.

RT can increase the metabolic demands of contracting muscle, promoting vasodilation and increasing blood flow in active muscle tissue. As a result, it is believed that shear stress is elevated in the vasculatures supplying blood to the active cardiac and skeletal muscle (Padilla et al. 2011), and, consequently, activates the PI3K signaling pathway (Fontes et al. 2014). This increases the degree of phosphorylation and activity of Akt protein (Wang et al. 2010, Dai et al. 2020), which promotes an increase in the phosphorylation of serine residues in position 1177 of the eNOS, and increases the bioavailability of NO and vasodilation (Barbosa et al. 2013, Fontes et al. 2014). Besides, during RT sessions, repeated episodes of high shear stress occur, acting as a primary physiological signal, stimulating endothelial adaptations in the area of muscle tissue, a greater relative increase in fiber activity, and possibly stimulating significantly higher expression of phosphorylated eNOS through activated Akt (Padilla et al. 2011, Muniyappa & Sowers 2013, Li et al. 2015). Thus, the beneficial effects of this type of exercise may involve enhanced insulin signaling, increased eNOS activity, leading to the rebalancing of the vasoconstrictor and vasodilator actions of insulin (Muniyappa & Sowers 2013).

The vascular bed studied here regulates about 20% of total blood flow in the body and changes in its vascular perfusion can represent significant alterations in total vascular peripheral resistance (Blanco-Rivero et al. 2013). During exercise, mesenteric arteries suffer a decrease in blood flow, an intensity-dependent phenomenon known as reactive hyperemia (Joyner & Casey 2015). In our study, RT was able to prevent damage to the PI3K pathway caused by glucocorticoids. This may have been the result of increased shear stress, which increases PI3K activity and stimulates phosphorylation and activation of Akt, directly phosphorylating eNOS at Ser1177. This results in increased eNOS activity and subsequent NO production (Muniyappa & Sowers 2013).

NO plays a key role in the control of vascular tone by acting as the main inducer of relaxation in vascular beds (Muniyappa & Sowers 2013). NO

participation in insulin-mediated vasodilation was not only attenuated, but the concentration–response curve reversed after eNOS inhibition in the DEX group, due to a reduction in NO bioavailability mediated by a decrease in eNOS expression. Studies have demonstrated that GC treatment can significantly down-regulate eNOS expression (Schäfer et al. 2005, Blecharz-Lang & Burek 2017), uncoupling eNOS through the inhibition of essential cofactors, and reducing NO production (Verhoeven et al. 2016).

However, the contractile effect was prevented in the DEX+RT group. Some studies show that exercise training is able improve endothelial function through up-regulating eNOS expression and increasing phosphorylation, and consequently increasing NO bioavailability. Moreover, some factors that can be involved in eNOS activation and subsequent NO synthesis, such as hypoxia and shear stress, are present during exercise and post-exercise. Furthermore, the reversion of the concentration response curve could be due activation of vasoconstrictor mechanisms. The literature shows that insulin pathway disturbance can induce vasoconstriction by an endothelium-dependent mechanism through the activation of the MAPK/ET-1 pathway (Cardillo et al. 2000, Jiang et al. 2003).

To evaluate this hypothesis BQ123+L-NAME were used simultaneously. In this condition, insulin-induced vasoconstriction was inhibited, suggesting that this effect appears be due to a predominance of MAPK/ET-1 about the PI3K/eNOS pathway in the DEX group, which can lead to a reduction in NO bioavailability and increased ET-1 activation. In contrast, in the DEX+RT group no change was observed in the presence of BQ123; this response can be explained by the blood flow redistribution (reactive hyperemia) that occurs during and immediately after RT to the required tissues, increased shear stress, and consequently the activation of the PI3K/eNOS signaling pathway. Also, the activation of the ET-A receptor appears to have no negative effect on vascular reactivity for insulin. Some authors have demonstrated that in normal conditions, the effect of ET-A activation is compensated for by an increase in NO bioavailability caused by the activation of the PI3K/eNOS pathway (Arce-Esquivel et al. 2013, Muniyappa & Sowers 2013). Therefore, RT may help to maintain vascular tone, in addition to promoting adjustments to the PI3K/eNOS and MAPK/ET-1 pathways, through flow redistribution in the exercised muscle restoring the balance between the pathways (Mikus et al. 2012).

CONCLUSION

In conclusion, changes to the vascular endothelium in the presence of high doses of glucocorticoids damage endothelial homeostasis, which can be a factor and/or critical indicator of a risk factor associated with cardiovascular diseases. However, in the present study, it was demonstrated that RT, even in the presence of high doses of glucocorticoids, was able to prevent damage to the vasodilator PI3K/eNOS pathway, attenuate the vasoconstrictor response through the MAPK/ET-1 pathway, and reduce contractile responsiveness to phenylephrine.

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