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Exercise training restores the myogenic response in skeletal muscle resistance arteries and corrects peripheral edema in rats with heart failure

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[1]*Department of Physiology and Biophysics, Institute of Biomedical Science, University of São Paulo, São Paulo, Brazil;* [2]*Department of Pharmacology, Institute of Biomedical Science, University of São Paulo, São Paulo, Brazil;* [3]*Heart Institute, Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, University of São Paulo, São Paulo, Brazil;* [4]*School of Physical Education and Sport, University of São Paulo, São Paulo, Brazil; and* [5]*Department of Physiological Sciences, Federal University of Espírito Santo, Espírito Santo, Brazil*

Paula SM, Couto GK, Fontes MT, Costa SK, Negrão CE, Mill JG, Rossoni LV. Exercise training restores the myogenic response in skeletal muscle resistance arteries and corrects peripheral edema in rats with heart failure. *Am J Physiol Heart Circ Physiol* 317: H87–H96, 2019. First published May 3, 2019; doi:10.1152/ajpheart.00042.2019.—Impairment of the myogenic response can affect capillary hydrostatic pressure and contribute to peripheral edema and exercise intolerance, which are markers of heart failure (HF). The aim of this study was to assess the effects of exercise training (ET) on myogenic response in skeletal muscle resistance arteries and peripheral edema in HF rats, focusing on the potential signaling pathways involved in these adjustments. Male Wistar rats were submitted to either coronary artery occlusion or a sham-operated surgery. After 4 wk, an exercise test was performed, and the rats were divided into the following groups: untrained normal control (UNC) and untrained HF (UHF) and exercise- trained (on treadmill, 50–60% of maximal capacity) NC (TNC) and exercise-trained HF (THF). Caudal tibial artery (CTA) myogenic response was impaired in UHF compared with UNC, and ET restored this response in THF to NC levels and increased it in TNC. Rho kinase (ROCK) inhibitor abolished CTA myogenic response in the untrained and blunted it in exercise-trained groups. CTA-stored calcium (Ca^{2+}) mobilization was higher in exercise-trained rats compared with untrained rats. The paw volume was higher in UHF rats, and ET decreased this response compared with UNC. Myogenic constriction was positively correlated with maximal running distance and negatively correlated with paw volume. The results demonstrate, for the first time, that HF impairs the myogenic response in skeletal muscle arteries, which contributes to peripheral edema in this syndrome. ET restores the myogenic response in skeletal muscle arteries improving Ca^{2+} sensitization and handling. Additionally, this paradigm also improves peripheral edema and exercise intolerance.

NEW & NOTEWORTHY The novel and main finding of the present study is that moderate intensity exercise training restores the impaired myogenic response of skeletal muscle resistance arteries, exercise intolerance and peripheral edema in rats with heart failure. These results also show for the first time to our knowledge that exercise training improving calcium sensitization through the ROCK pathway and enhancing intracellular calcium handling could contribute to restoration of flow autoregulation to skeletal muscle in heart failure.

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heart failure; myogenic response; peripheral edema; physical training; ROCK

INTRODUCTION

The myogenic response is a central mechanism of blood flow autoregulation that contributes to the maintenance of regional flow despite blood pressure oscillations (10, 20, 37). It is pronounced in small arteries and arterioles in some vascular beds such as the skeletal muscle bed (12, 39) and plays a pivotal role in the maintenance of capillary hydrostatic pressure, establishment of basal vascular tonus, and regulation of peripheral vascular resistance (23, 31). Thus, myogenic autoregulation dysfunction can lead to changes in different organs, such as the formation of regional edema as a result of vasodilation triggered by calcium channel blockers (14).

Peripheral edema is a hallmark of heart failure (HF) (25). The pathogenesis of peripheral edema of cardiac origin follows a chain of events including increased circulating blood volume, increased venous pressure (43), and impairment of lymphatic flow (30, 44). However, the impact of myogenic response changes on the generation of cardiogenic edema remains unknown.

It is well known that aerobic exercise training is a helpful strategy to improve symptoms in patients with HF. Exercise training has been shown to restore endothelial function (7, 15, 26, 41), promote angiogenesis (28), and improve skeletal myopathy (2, 4, 8). The consequence of these changes is a remarkable increase in exercise tolerance (16, 35).

Some investigators demonstrated that the effects of exercise training also take place at the myogenic level. Exercise training has been shown to increase soleus muscle arteriole myogenic responses in young rats and to improve the reduced myogenic response in aging animals (12). However, the effects of exercise training on myogenic response in skeletal muscle resistance arteries and peripheral edema associated with HF remains unknown. Likewise, the involvement of Ca^{2+} sensitization and kinetics, which are pivotal mechanisms for myogenic response (10, 21, 48), are also unknown and a matter of interest.

Thus, the present study aimed to assess the impact of HF on the myogenic response in skeletal muscle arteries. We also

investigated whether aerobic exercise training changes peripheral edema and its signaling pathways, with a special interest with regards to Ca^{2+} handling and sensitization.

METHODS

Experimental model of HF. All experimental procedures were approved by the Animal Care and Use Committee of the Institute of Biomedical Sciences of the University of São Paulo (no. 118, sheet 24, book 3) and were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). Male Wistar rats (230–280 g) were anesthetized (ketamine-xylazine, 90 and 10 mg/kg ip, respectively; Sespo Indústria e Comércio, Paulínia, SP, Brazil) and subjected to myocardial infarction by permanent occlusion of the left coronary artery or to a sham operation, as previously described (6).

Four weeks after permanent occlusion of the left coronary arteries, cardiac function was determined by echocardiography and with brain natriuretic peptide (BNP) plasma concentrations. As previously reported (13), systolic function was assessed by left ventricle ejection fraction (LVEF). This parameter was used as the first criterion to include the animals in the present study. The rats showing a clear akinetic area in the left ventricular wall and with LVEF <35% were included in the present study (7). The BNP plasma concentrations were measured using an ELISA kit for BNP (cat. no. ab108815, BNP 32 Rat, Abcam, San Francisco, CA) according to the manufacturer's instructions. The BNP plasma concentration was used as the second criterion to include the animals in the present study. The rats that presented BNP plasma levels >0.3 ng/ml were included in the present study. In line with these parameters, 4 wk after myocardial infarction surgery, LVEF was reduced [sham operated: 72 ± 1.3% (n = 23) vs. HF: 29 ± 0.7% (n = 44); P < 0.05, unpaired t-test] and BNP plasma levels were increased [sham operated: 0.22 ± 0.03 ng/ml (n = 22) vs. HF: 0.47 ± 0.04 ng/ml (n = 60); P < 0.05, unpaired t-test] in HF rats compared with sham-operated rats, and there were no differences between the rats allocated to untrained and trained groups.

The selected animals were submitted to 1-wk treadmill adaptation (15–20 min at a speed of 0.3–0.6 km/h, 0% inclination, daily). After the adaptation period, the animals were submitted to the first maximum exercise test, starting at 0.3 km/h with increments of 0.3 km/h every 3 min until exhaustion, and the distance achieved was obtained (1). The animals were then subdivided into the following four groups: *1*) untrained sham operated or the normal control (UNC), *2*) untrained HF (UHF), *3*) exercise-trained NC (TNC), and *4*) exercise-trained HF (THF).

Exercise training protocol. The exercise-trained rats (TNC and THF groups) were submitted to an 8-wk training period on a treadmill (50 to 60% of the average maximal speed of the pretraining exercise test), 1 h per day, 5 days per week, with 0% inclination (7). At the end of the fourth week of training, the maximum exercise test was repeated to adjust the exercise intensity. A final exercise test was performed at the eighth training week to evaluate the increase in the aerobic capacity. The untrained groups were subjected to a short exercise period (once a week for 10 min at 0.3 km/h).

Hydropletismometry. The right hind paw volume was assessed by hydropletismometry (model 7120, Ugo Basile, Italy). This method is capable of making accurate volume measurements, as previously described (49). The animal right hind paw was immersed in an electrolytic solution up to the ankle level. Three repeated measurements were performed, and the average considered the final volume for each time point.

The measurements were performed before surgical procedures and at the end of the experiment (12 wk after surgery and 8 wk after exercise training, respectively) using the same animal's paw and environment, which was at the same temperature (25°C) and executed by the same operator.

Delta paw swelling of the right hind paw was calculated for each rat by subtracting the paw volume (ml) on the day before the myocardial infarction surgery from the last measured paw volume.

Hemodynamic and morphometric measurements. At the end of the 8 wk of exercise training or untrained period and after a resting period of 48 h, the arterial and left ventricle hemodynamics were obtained from anaesthetized animals as previously published by our group (6, 7).

The animals were then euthanized by exsanguination, and tissues such as the heart, lungs, tibia, and caudal tibial arteries were carefully dissected. The caudal tibial artery was chosen in the present study because it is a resistance artery (a third-order branch from the femoral artery) that irrigates several muscle groups in the pelvic limb of rodents (27). All these muscles are activated during aerobic exercise training.

The left and right ventricular hypertrophy indexes were obtained from the weights of the left ventricle (including the septum) and right ventricle, both normalized to the tibia length. The presence of pulmonary congestion was inferred by the lung weight normalized to the tibia length. The infarcted area was determined by planimetry (32), and the left ventricle scar tissue was expressed as a percentage of the total area of the left ventricle (including septum). Only animals that presented infarcted areas covering between 30 and 50% of the left ventricular surface were included in this study (6, 7). This was the third criterion used to include infarcted animals in the present study.

Myogenic reactivity analysis. The myogenic reactivity was evaluated using a pressure myograph (DMT, 115FP model, Aarhus, Denmark). The right and left caudal tibial arteries were dissected, freed of connective tissue, and placed in ice-cold Krebs-Henseleit solution (KHS) containing (in mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2 \cdot 2H_2O$, 1.2 KH_2PO_4, 1.2 $MgSO_4 \cdot 7H_2O$, 25 $NaHCO_3$, 11 glucose, and 0.01 EDTA. The artery was cut into 3- to 4-mm segments, cannulated with glass micropipettes on each side, and fixed with nylon thread inside the microvessel chamber containing oxygenated KHS (pH 7.4 and 37°C). The intraluminal pressure was then raised to 140 mmHg to adjust the longitudinal stretching and to confirm that the artery was free of leakage. The intraluminal pressure was then reduced to 70 mmHg and left to stabilize for 1 h. The ability of the vascular smooth muscle to contract was tested in the presence of 120 mM high-potassium solution. Arteries that did not exhibit a contractile response were discarded. After washing with KHS and 20 min of stabilization, the intraluminal pressure was reduced to 3 mmHg, and a pressure-diameter curve, in active condition, was performed by increasing the intraluminal pressure from 3 to 140 mmHg. Afterward, the intraluminal pressure was reduced to 70 mmHg, and the artery was washed with oxygenated Ca^{2+}-free KHS containing ethylene glycol tetraacetic acid (EGTA, 10 mM) and maintained for 20 min for complete relaxation. To confirm the depletion of the Ca^{2+} stocks, the artery was exposed again to high-potassium solution, and after another washing and 20 min of stabilization in Ca^{2+}-free KHS, the intraluminal pressure was reduced to 3 mmHg and a new pressure-diameter curve (3–140 mmHg) was performed in passive condition.

In an additional set of experiments, the participation of the Ca^{2+} sensitization pathway in the myogenic response was evaluated, in which 3 pressure-diameter response curves (3–140 mmHg) were performed. The first was performed in the control condition (presence of KHS), the second was performed after 30 min preincubation with the rho-kinase inhibitor Y-27632 (1 μM, Sigma-Aldrich, St. Louis, MO) (21, 45), and the third was performed in Ca^{2+}-free KHS containing 10 mM EGTA.

The myogenic constriction (MC) response was determined as the percentage of the constriction of the curve in active condition, the luminal diameter (LD) in the presence of Ca^{2+} (LD_{Ca}), compared with the curve in passive condition, in Ca^{2+} free KHS (LD_{0Ca}), at a given pressure: MC (%) = $100[(LD_{0Ca} - LD_{Ca})/LD_{0Ca}]$.

Vascular reactivity analysis. To investigate the role of Ca^{2+} kinetics in the segments of caudal tibial arteries, we used a wire

myograph (DMT, 610 M model). The arteries were dissected, freed of connective tissue, and cut into rings (~2 mm in length). Steel wires (2 at 40 μm in diameter) were introduced through the lumen of the artery mounted on a myograph chamber, and the vascular rings were stretched to a rest tension considered optimal to their LD (DMT Normalization Module; ADInstruments, Bella Vista, New South Wales, Australia), as previously published (6). After 30 min of stabilization (oxygenated KHS, pH 7.4 at 37°C), the vascular contractility was tested using a high-potassium solution (120 mM), and the contractile response was not different among the groups [UNC: 2.8 ± 0.3 ($n = 6$) vs. TNC: 3.0 ± 0.1 ($n = 9$) vs. UHF: 2.7 ± 0.1 ($n = 7$) vs. THF: 2.5 ± 0.1 mN/mm ($n = 9$); $P > 0.05$, two-way ANOVA]. Afterward, the arteries were washed with KHS, and after an additional 30-min stabilization, the endothelial integrity was evaluated by the ability of acetylcholine (10 μM) to induce relaxation when precontracted with a thromboxane receptor agonist (U46619, Sigma-Aldrich). All the experiments were performed in arteries with an intact endothelium.

To evaluate the role of intracellular Ca^{2+} on the contractile response, the arteries were washed in Ca^{2+}-free KHS containing EGTA (1 mM), and after 15 min of stabilization, the participation of intracellular Ca^{2+} was indirectly assessed by measuring the noradrenaline (10 μM)-induced phasic contraction (Fig. 3*A*). Following a period of phasic contractile response, $CaCl_2$ (2.5 mM) was added to the bath in the presence of noradrenaline (10 μM) (Fig. 3*A*). The tonic contractile response induced by $CaCl_2$ (2.5 mM), in the presence of noradrenaline, was considered as an index of Ca^{2+} inflow. The participation of Ca^{2+} (intracellular and influx) on noradrenaline-induced contraction was measured as a difference between the peak of phasic contraction or the plateau of $CaCl_2$-induced contraction and their respective basal tonus. The data were expressed as the active vascular tension (mN/mm).

Western blot analysis. A pool of 5 caudal tibial arteries were used for a single *n* value and homogenized in ice-cold radioimmunoprecipitation assay lysis buffer (Merck Millipore, Billerica, MA) containing protease inhibitors such as sodium orthovanadate (100 mM; Sigma-Aldrich), phenyl methane sulfonyl fluoride (10 mM; Amresco, Solon, OH), and protease inhibitor cocktail (1:5,000 dilution; Sigma-Aldrich) and phosphatase inhibitors such as sodium fluoride (100 mM, Synth, São Paulo, Brazil) and sodium pyrophosphate (10 mM; Synth). The homogenates were centrifuged (800 *g* for 15 min at 4°C), and the supernatant was isolated. The proteins were quantitated using a bicinchoninic acid protein assay kit (Pierce bicinchoninic acid protein assay kit, Thermo Scientific, Rockford, IL), extracted (20 μg), separated on a polyacrylamide gel (Mini-PROTEAN precast gels; Bio-Rad, Hercules, CA) by electrophoresis, and transferred to a PVDF membrane (Amersham-GE Healthcare, Little Chalfont, UK) for 12 h at 4°C using a Mini Trans-Blot Cell system (Bio-Rad) containing transfer buffer, consisting of 25 mM X Tris, 190 mM glycine, 20% methanol, and 0.05% SDS.

The membranes were then blocked with 5% bovine serum albumin (Inlab, São Paulo, Brazil) and incubated for 12 h at 4°C with primary anti-phospholamban (PLB) (1:1,000, cat. no. 05-205, Merck Millipore, Darmstadt), anti-sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2) (1:500, cat. no. sc-8095, Santa Cruz Biotechnology, Dallas, TX), anti-rho kinase I (ROCK I) (1:1,000, cat. no. ab45171, Abcam, Cambridge, UK), anti-rho kinase II (ROCK II) (1:1,000, cat. no. ab66320, Abcam, Cambridge, UK), anti-L-type voltage-gated Ca^{2+} channel ($Ca_V1.2$) (1:8,000, cat. no. ACC-003, Alomone, Jerusalem, IL), and anti-α-actin (1:10,000, cat. no. A 2547, Sigma-Aldrich) antibodies. The membranes were then washed and incubated with peroxidase-conjugated anti-mouse IgG antibody (Bio-Rad) for PLB and α-actin; anti-rabbit IgG antibody (Bio-Rad) for ROCK I, ROCK II, and $Ca_V1.2$; and anti-goat IgG antibody (Bio-Rad) for SERCA2. The membranes were washed and incubated with a horseradish peroxidase-luminol ECL (GE Healthcare, Buckinghamshire, UK) or ECL plus (Thermo Scientific) chemiluminescent system to detect the luminescent immunocomplexes, which were exposed to radiographic film (Kodak BioMax MR film, Sigma-Aldrich). Immunoblot signals were quantified using the ImageJ software (National Institutes of Health, Bethesda, MD). All densitometric raw data values were normalized to α-actin expression from the same sample resolved on the same gel/membrane, and the mean was expressed as a percentage of the UNC values.

Statistical analysis. Values are expressed as the means $\pm$ SE. The results were analyzed using the unpaired Student's *t*-test or paired and unpaired one- or two-way ANOVA when appropriate. When one- or two-way ANOVA showed a statistical significance, the Bonferroni post hoc test was applied (Graph Pad Prism Software, San Diego, CA). The results were considered statistically significant for *P* values <0.05.

RESULTS

At the end of the 8-wk exercise training protocol, the TNC rats showed a higher running distance compared with UNC rats (Table 1). The UHF rats showed a shorter running distance compared with UNC rats (Table 1), characterizing exercise intolerance. In contrast, the THF rats showed a greater running distance compared with UNC and UHF rats (Table 1).

The systolic blood pressure, left ventricle systolic pressure, and heart rate were reduced, whereas diastolic blood pressure and left ventricle end diastolic pressure were increased in UHF rats when compared with the UNC rats (Table 1). Additionally, the positive and negative first pressure derivative were significantly reduced in UHF rats compared with the UNC rats (Table 1). As previously shown by our group (7, 13), 8 wk of aerobic exercise training did not change these parameters in both TNC and THF rats (Table 1).

Table 1. *Running distance during the maximal exercise test and arterial and ventricular parameters from the UNC, TNC, UHF, and THF rats after 8 wk of sedentary lifestyle or exercise training on a treadmill*

	UNC	TNC	UHF	THF
Running distance, m	124 ± 7.9	$292 \pm 10.5*$	$91 \pm 4.9*$	$216 \pm 6.9*\#$
SBP, mmHg	128 ± 1.44	127 ± 1.34	$109 \pm 1.51*$	$106 \pm 1.33*$
DBP, mmHg	68 ± 1.89	68 ± 1.69	$75 \pm 2.01*$	$73 \pm 1.75*$
HR, beats/min	361 ± 4.99	350 ± 5.55	$340 \pm 4.99*$	$332 \pm 6.65*$
LVSP, mmHg	144 ± 1.53	141 ± 1.49	$123 \pm 2.14*$	$118 \pm 1.38*$
LVEDP, mmHg	5.0 ± 0.42	5.3 ± 0.46	$14.4 \pm 1.29*$	$13.0 \pm 0.99*$
+dP/d*t*, mmHg/s	$8,157 \pm 180$	$7,643 \pm 196$	$5,797 \pm 161*$	$5,448 \pm 117*$
−dP/d*t*, mmHg/s	$-4,691 \pm 109$	$-4,509 \pm 113$	$-4,115 \pm 115*$	$-3,911 \pm 91*$

Values are means $\pm$ SE. Statistical analysis was assessed by two-way ANOVA. +dP/d*t*, first time positive derivative; −dP/d*t*, first time negative derivative; DBP, diastolic blood pressure; HR, heart rate; LVSP, left ventricle systolic pressure; LVEDP, left ventricle end-diastolic pressure; SBP, systolic blood pressure; THF, exercise-trained heart failure; TNC, exercise-trained normal control; UHF, untrained heart failure; UNC, untrained normal control. *$P < 0.05$ vs. UNC. #$P < 0.05$ vs. UHF.

Body weight was significantly reduced in UHF, TNC, and THF rats compared with UNC rats (Table 2). A significant increase in the right ventricular hypertrophy index as well as in the lung weight-to-tibia length ratio were observed in the HF groups compared with NC rats (Table 2). These results indicate pulmonary congestion and right ventricle overload. Despite the presence of a large infarction area in the HF groups (Table 2), no change in the left ventricular hypertrophy index was observed among the groups (Table 2), suggesting ventricular remodeling in the remaining myocardium. Exercise training did not change these parameters (Table 2).

Impact of HF and exercise training on MC. The increment in the LD induced by the pressure-response curve in the active condition was different in caudal tibial arteries in UHF rats compared with UNC rats (Fig. 1, *A* and *C*). Indeed, the LD from 80 mmHg of intraluminal pressure was significantly lower in the active condition compared with the passive condition (Ca^{2+}-free medium) in arteries from UNC rats (Fig. 1*A*), which indicates the development of myogenic response in the caudal tibial arteries in UNC rats. However, the difference between the curves in the active and passive conditions was greatly attenuated in the arteries of UHF rats (Fig. 1*C*), which is suggestive of impaired myogenic response in skeletal muscle arteries from HF rats.

It was remarkable that exercise training enhanced myogenic response in caudal tibial arteries from NC rats (Fig. 1*B*) and HF rats (Fig. 1*D*). In NC rats, exercise training amplified the contractile response induced by intraluminal pressure (80 mmHg) under active conditions compared with UNC rats (Fig. 1*B*). In addition, in HF rats, exercise training restored the contractile response induced by intraluminal pressure (100 mmHg) under active conditions to NC levels (Fig. 1*D*). Additionally, as observed in Fig. 1*E*, these results suggest that exercise training was effective to enhance the myogenic response in NC rats and to reverse the impairment in myogenic response in skeletal muscle arteries of HF rats to a similar level as UNC arteries.

Effect of exercise training on ROCK and myogenic responses. In presence of the ROCK inhibitor Y-27632, the pressure-diameter curves in the active condition were similar to those observed in the passive condition in caudal tibial arteries from UNC rats (Fig. 2*A*), suggesting a pivotal role for the ROCK pathway in the myogenic response in caudal tibial arteries. In the caudal tibial arteries from UHF rats, the pressure-diameter curve in the presence of the ROCK inhibitor was similar to those observed under passive conditions (Fig. 2*C*). However, in TNC (Fig. 2*B*) and THF rat arteries (Fig. 2*D*), Y-27632

incubation induced an attenuation of the myogenic response observed under the active conditions, indicating that other pathways besides the ROCK pathway may be involved in the improvement of myogenic response induced by exercise training.

ROCK I expression was significantly enhanced in caudal tibial arteries from TNC and reduced in arteries from UHF and THF rats compared with UNC rats (Fig. 2*E*). In contrast, ROCK II expression was unaltered in caudal tibial arteries in TNC and UHF compared with UNC rats, though exercise training increased its expression in arteries from HF rats (Fig. 2*F*).

Effects of exercise training on intracellular Ca^{2+} handling. The phasic contraction induced by noradrenaline in Ca^{2+}-free KHS was similar in caudal tibial arteries from UHF and UNC rats (Fig. 3*B*). Exercise training increased the noradrenaline-induced contraction in arteries from NC and HF rats (Fig. 3*B*). In line with this result, the SERCA-2 and PLB expression ratio was significantly enhanced in caudal tibial arteries from the TNC and THF rats compared with their respective controls (Fig. 3*D*). No significant differences were observed in the noradrenaline-induced tonic contraction by Ca^{2+} influx (Fig. 3*C*) or in the $Ca_V1.2$ expression (Fig. 3*E*) among the groups.

Taken together these results suggest that the improvement in the myogenic response observed with exercise training in caudal tibial arteries from NC and HF rats may be, at least in part, associated with increased intracellular Ca^{2+} mobilization.

Impact of HF and exercise training on paw volume. Chronic HF rats presented a higher gain in paw volume than UNC rats (Fig. 4). Eight weeks of exercise training in HF rats reversed the paw volume toward normal levels (Fig. 4).

We observed an interesting negative correlation between the gain of paw volume and MC response in the caudal tibial arteries (Fig. 5*A*). Additionally, a positive correlation between running distance and the MC of the caudal tibial arteries was also observed (Fig. 5*B*). Taken together, these data strongly suggest that the increase of the gain of paw volume and exercise intolerance are related to changes in the myogenic response of skeletal muscle resistance arteries.

DISCUSSION

The novel and main finding of the present study is that moderate intensity exercise training restores the impaired myogenic response of skeletal muscle resistance arteries and peripheral edema in HF rats. These results also show for the first time that exercise training improving calcium sensitization

Table 2. *Morphometric parameters for the UNC, TNC, UHF, and THF rats after 8 wk of sedentary lifestyle or exercise training on a treadmill*

	UNC	TNC	UHF	THF
Infarct area, %LV			39.8 ± 0.66	39.4 ± 0.74
BW, g	476 ± 7.4	450 ± 7.9*	447 ± 6.8*	437 ± 5.9*
T, mm	41.5 ± 0.14	41.4 ± 0.17	41.4 ± 0.16	41.3 ± 0.15
RV/T, mg/mm	5.22 ± 0.12	5.26 ± 0.32	12.75 ± 0.37*	11.73 ± 0.41*
LV/T, mg/mm	19.14 ± 0.26	19.15 ± 0.27	18.15 ± 0.40	18.32 ± 0.34
L/T, mg/mm	50.04 ± 1.88	50.60 ± 2.97	87.89 ± 2.95*	82.85 ± 3.22*

Values are means ± SE. Statistical analysis was assessed by two-way ANOVA. BW, body weight; L/T, ratio of lung and tibia length; LV/T, ratio of left ventricle and tibia length; RV/T, ratio of right ventricle and tibia length; T, tibia length; THF, exercise-trained heart failure; TNC, exercise-trained normal control; UHF, untrained heart failure; UNC, untrained normal control. *$P < 0.05$ vs. UNC.
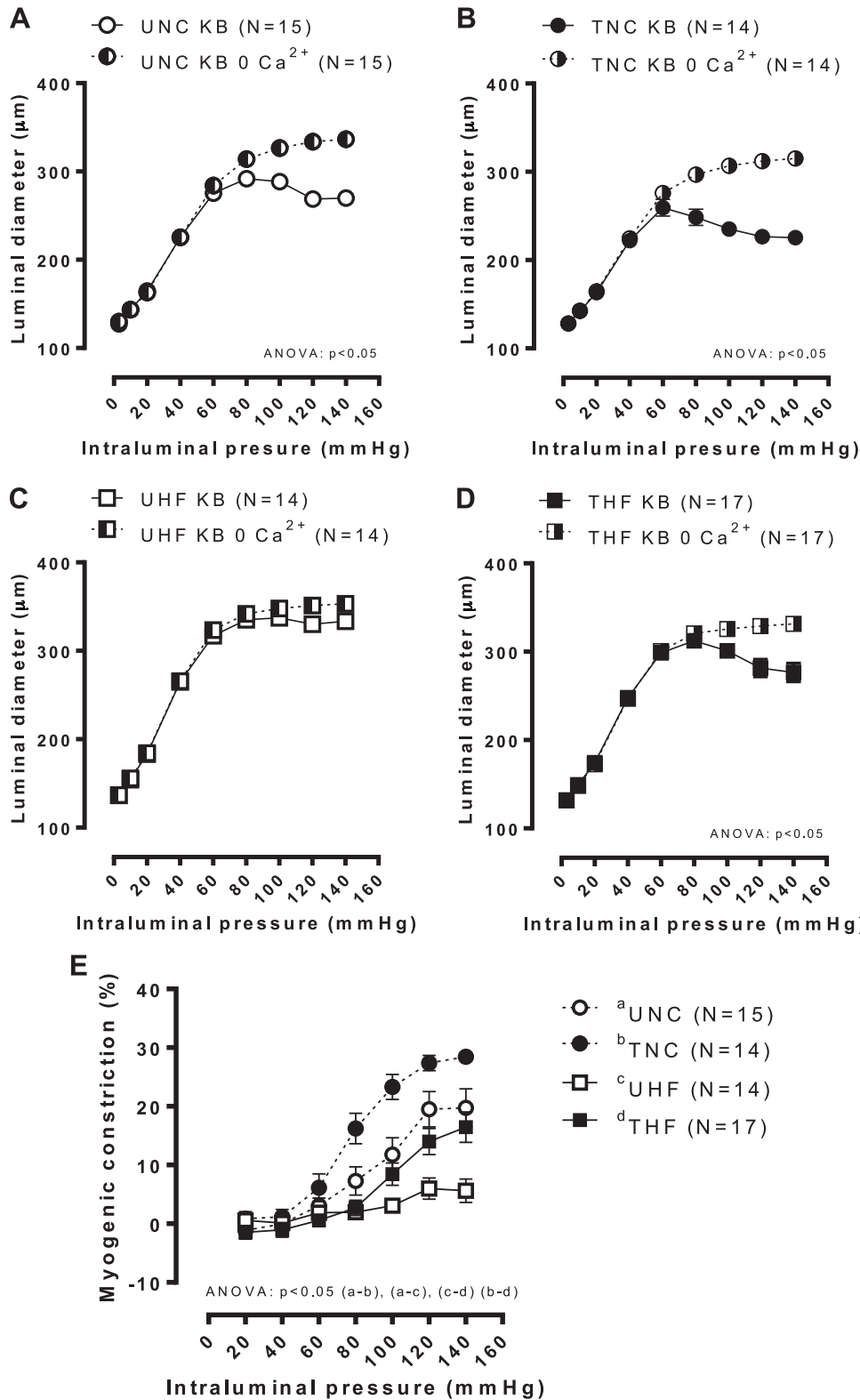
Fig. 1. Impairment in the myogenic constriction response in caudal tibial arteries from heart failure rats is restored by exercise training. Intraluminal pressure-luminal diameter curves performed in Krebs-Henseleit solution with Ca^{2+} (KB, active diameter) and Ca^{2+}-free Krebs-Henseleit solution (KB 0 Ca^{2+}, passive diameter) in tibial caudal arteries from untrained normal control (UNC) (A), exercise-trained normal control (TNC) (B), untrained heart failure (UHF) (C), and exercise-trained heart failure (THF) (D) rats. The myogenic constriction response to intraluminal pressure changes (E) in caudal tibial arteries from the groups. The number of animals used (n) in each of the experimental protocols is indicated in parentheses. The values correspond to the mean ± SE of the animals studied. The statistical analysis was assessed by two-way ANOVA.

through the ROCK pathway and enhancing intracellular calcium handling could contribute to restoration of flow autoregulation to skeletal muscle in HF.

Although previous observations (12, 39) and the present results have demonstrated that exercise training enhances myogenic function in arterioles from healthy rats, the present study extends this knowledge and adds that exercise training restores the impaired MC in caudal tibial arteries in HF.

Exercise intolerance in HF has been attributed not only to intrinsic abnormalities in skeletal myocytes (2, 4, 8, 9, 22) but also to perfusion (38, 47). The reduced myogenic response may play a role in decreased muscle blood flow, which in turn leads
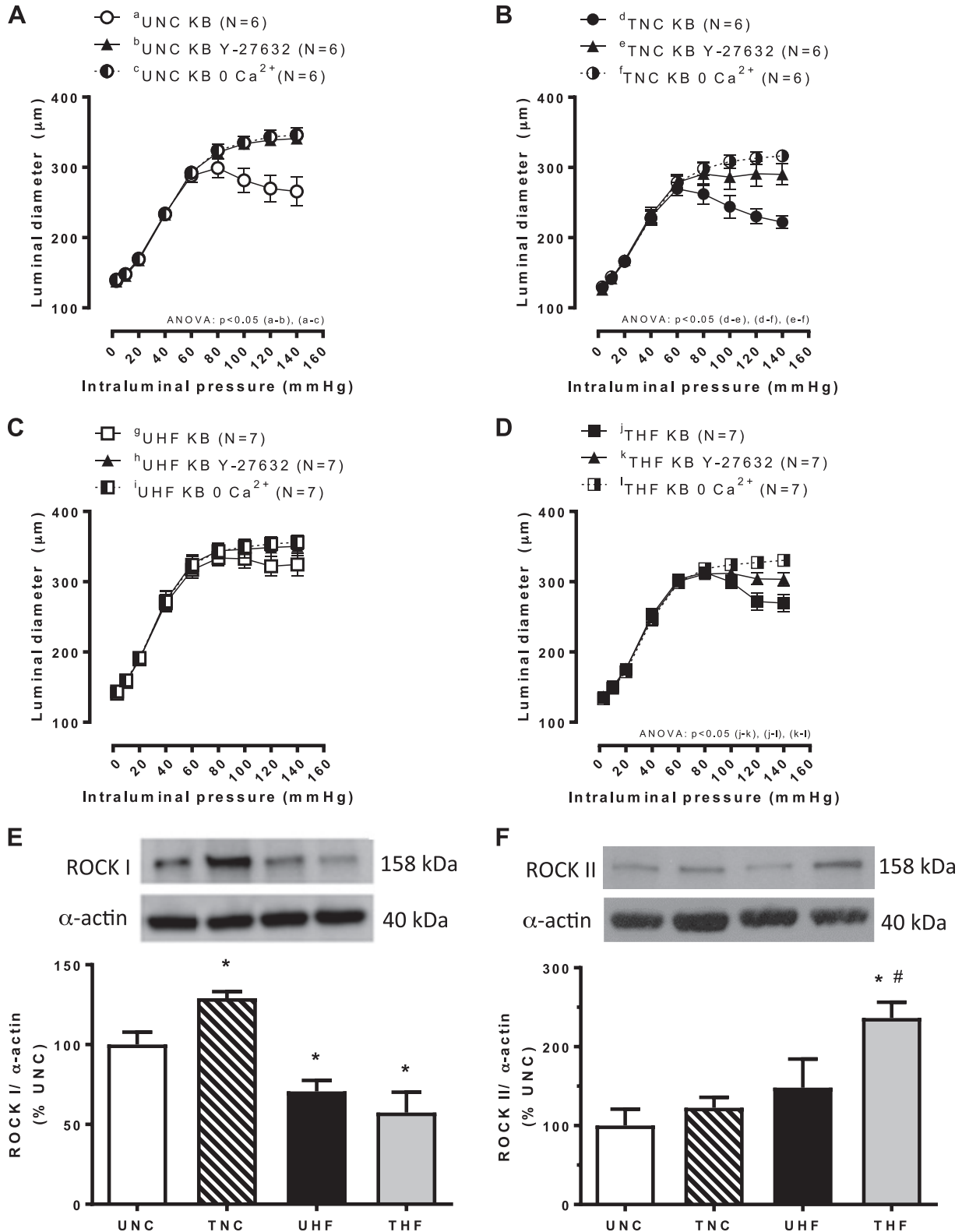
Fig. 2. The rho kinase (ROCK) pathway is involved in the improvement of the myogenic response in caudal tibial arteries from exercise-trained rats. Intraluminal pressure-luminal diameter curves performed in Krebs-Henseleit solution with Ca^{2+} in the absence (KB) and presence of ROCK inhibitor (KB Y-27632, 1 μM) and in Ca^{2+}-free Krebs-Henseleit solution (KB 0 Ca^{2+}) in tibial caudal arteries from untrained normal control (UNC) (*A*), exercise-trained normal control (TNC) (*B*), untrained heart failure (UHF) (*C*), and exercise-trained heart failure (THF) (*D*) rats. The number of animals used (*n*) in each of the experimental protocols is indicated in parentheses. The values correspond to the mean $\pm$ SE of the animals studied. The statistical analysis was assessed by two-way ANOVA. ROCK I (*E*) and ROCK II (*F*) expression in the caudal tibial arteries from the groups; the *top* of each graph represents typical Western blot autoradiographs. Protein expression was normalized to α-actin, and the results are expressed as the fold-change compared with UNC expression. The number of animals used was 5–8 per group, and a single *n* value corresponds to 5 pooled arteries. The values correspond to the mean $\pm$ SE of the animals studied. The statistical analysis was assessed by two-way ANOVA. *$P < 0.05$ vs. UNC and #$P < 0.05$ vs. UHF.
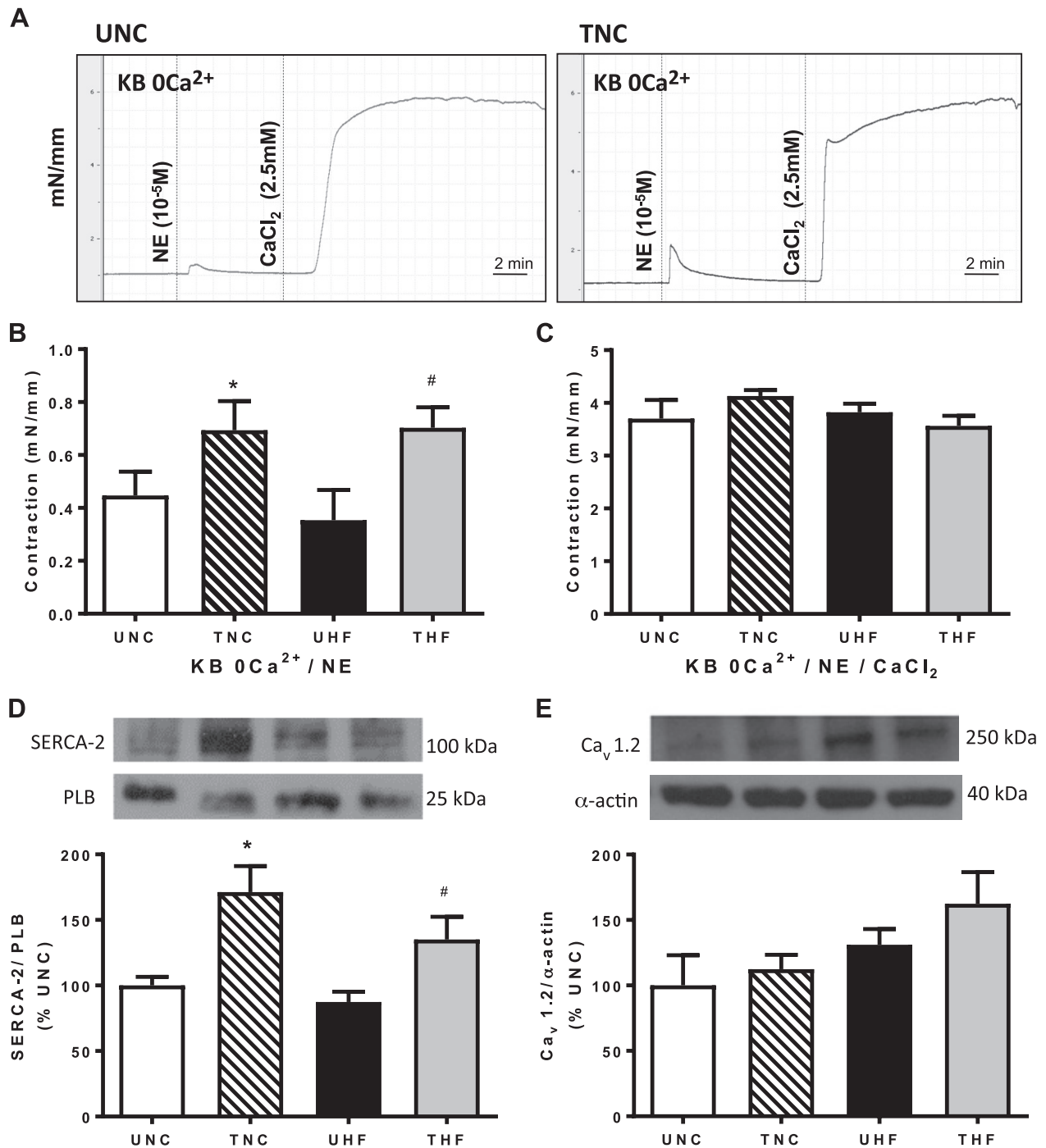
Fig. 3. Exercise training increases intracellular Ca^{2+} handling in caudal tibial arteries from normal control and heart failure rats. Typical traces represent the phasic contraction induced by noradrenaline (NE, 10 μM) in Ca^{2+}-free Krebs-Henseleit solution (KB 0 Ca^{2+}) and the tonic contraction induced by $CaCl_2$ (2.5 mM) in tibial caudal arteries from untrained normal control (UNC) and exercise-trained normal control (TNC) rats (*A*). NE-induced (10 μM) phasic contraction in KB 0 Ca^{2+} (*B*; $n = 6-8$ per group) and its tonic contraction after $CaCl_2$ (2.5 mM) was added to the bath (*C*, $n = 6-9$ per group) in tibial caudal arteries from UNC, TNC, untrained heart failure (UHF), and exercise-trained heart failure (THF) rats. The sarcoplasmic reticulum Ca^{2+} ATPase (SERCA-2) and phospholamban (PLB) ratio (*D*) and L-type voltage-gated Ca^{2+} channel ($Ca_V1.2$) expression normalized to α-actin (*E*) in caudal tibial arteries from the groups; *top* of each graph represents typical Western blot autoradiographs. The results are expressed as the fold change compared with UNC expression. The number of animals used was 4–9 per group, and a single *n* value corresponds to 5 pooled arteries. The values correspond to the mean ± SE of the animals studied. The statistical analysis was assessed by two-way ANOVA. *$P < 0.05$ vs. UNC and #$P < 0.05$ vs. UHF.

to exercise intolerance in HF. In fact, we observed that HF rats presented an impaired myogenic response in caudal tibial arteries and exercise intolerance. Of note, this arterial bed irrigates several muscular sites of the pelvic limb in rodents

(27), which are all active during exercise. The positive correlation between running distance and myogenic contraction strengthens this idea. Interestingly, an increased and significant paw volume was observed in HF rats compared with NC rats,
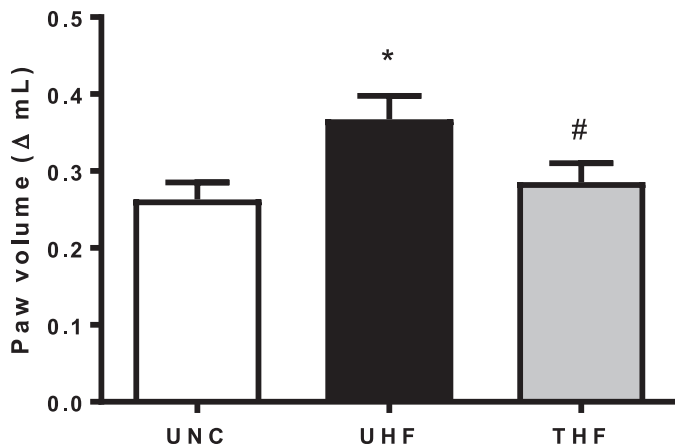
Fig. 4. The increase in paw volume induced by heart failure was restored by exercise training. Measurement of the right hind paw volume variation calculated as the difference between the volume before the myocardial infarction surgery and at end of the experiment (12 wk after surgery and 8 wk after exercise training) in the untrained normal control (UNC), untrained heart failure (UHF), and exercise-trained heart failure (THF) rats. The results are expressed as the mean ± SE ($n = 11$–13 per group). The statistical analysis was assessed by one-way ANOVA. *$P < 0.05$ vs. UNC and #$P < 0.05$ vs. UHF.

which strongly suggests peripheral edema. Peripheral edema resulting from the accumulation of fluid under the skin, often in the lower members, is a clinical marker in HF that is observed even in the early stages of this syndrome (25). The pathogenesis of peripheral edema of cardiac origin follows a chain of events including increased circulating blood volume, increased venous pressure (43), and impairment of lymphatic flow (30, 44). Since myogenic response is responsible for adequate adjusting of capillary resistance to maintain constant capillary pressure (19), an impairment of the myogenic response is a mechanism that may alter capillary pressure, autoregulation, and induce edema. The negative correlation between the paw volume and MC observed in our study corroborates this hypothesis. Thus, these data are consistent with the impairment in the myogenic response observed in the caudal tibial arteries of HF rats and may be one of the factors contributing to the increase in the paw volume found in this set of animals.

Another important finding of our study is that exercise training restores the MC in caudal tibial arteries of HF rats toward normal levels. This result points to the same conclusion

and is in line with a previous study in which exercise training improved myogenic response in the soleus muscle arteriole in elderly animals (12) and enhanced this response in arterioles from young rats (12, 39) and NC rats (present results). Altogether, these findings strongly demonstrate that 8 wk of aerobic exercise training is effective for restoring the myogenic response, the paw volume, and exercise intolerance observed in HF rats to NC levels.

The present study also shows that the essential role of the Ca^{2+} sensitization pathway through ROCK for the development and maintenance of the myogenic response in caudal tibial arteries is impaired in HF rats. The involvement of Ca^{2+} sensitization, because of myosin light-chain phosphatase inhibition by ROCK, and cytoskeletal reorganization, enhancing actin polymerization by ROCK and PKC, on the myogenic response of skeletal muscle resistance arteries is well known (33). Moreover, there are no data that associate myogenic responsiveness and ROCK in HF. Thus, our study shows that HF reduces ROCK I expression in skeletal muscle resistance arteries without changes in ROCK II or PKC (data not shown) expression. Classically, in vascular tissue, the ROCK isoforms increase the myosin light-chain phosphatase inhibition inducing contraction, with the myosin phosphatase target subunit 1 as a target (17). However, ROCK signaling induces different effects on the cellular function and cytoskeletal reorganization in the dependency of the tissue, subcellular distribution, activation and external influences (17, 42).

Furthermore, our study shows for the first time that exercise training modulates ROCK expression in caudal tibial arteries. Exercise training enhances ROCK I expression in caudal tibial arteries from NC rats, whereas it enhances ROCK II expression in caudal tibial arteries from HF rats. In line with this result, ROCK I and ROCK II expression were enhanced in skeletal muscle of NC rats by short-term exercise (34). Since the effect of the ROCK inhibitor Y-27632 on the myogenic response in arteries from exercise-trained NC and HF animals was limited when compared with NC rats, it is plausible to suggest that the ROCK pathway partially contributes to the amplification and restoration of myogenic control in exercise-trained NC and HF rats, respectively. These data suggest that calcium sensitization by the ROCK pathway is involved in the exercise training-induced improvement in the myogenic response. However, other pathways may also participate in this response.

Fig. 5. Impairment in the myogenic constriction response in caudal tibial arteries from heart failure rats is correlated with the gain in paw volume and exercise intolerance. Scatter plots represent the correlations between myogenic constriction in the caudal tibial arteries and the gain in paw volume (A) and the running distance at the last maximal exercise test on the treadmill (B). r = Pearson correlation coefficient.
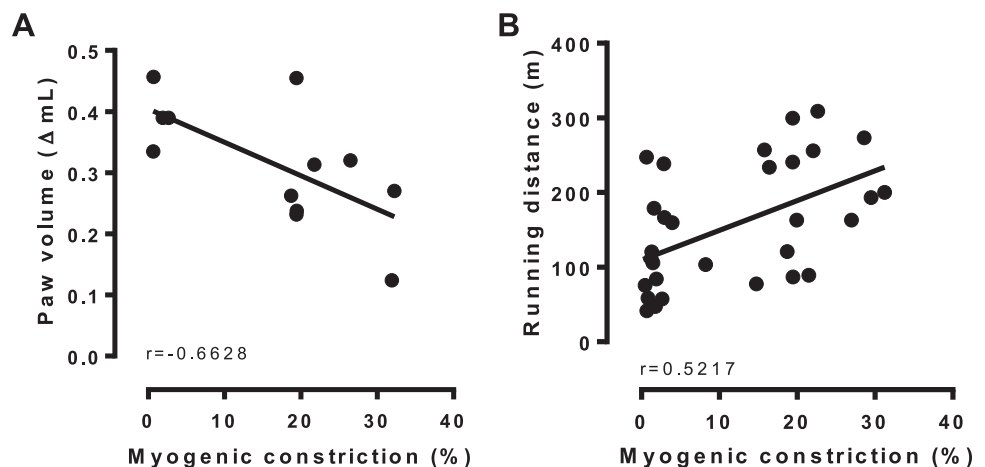
The role of Ca^{2+} handling in the myogenic response (10) and its dysregulation in cardiac muscle in HF animals has been well documented (11, 18, 40), but the way that Ca^{2+} handling pathways operate in myogenic response in the presence of HF is nevertheless obscure. In this respect, we hypothesize that Ca^{2+} handling pathways may be another mechanism by which exercise training contributes to improving the myogenic response in caudal tibial arteries from HF animals. In further experiments, we found an increase in Ca^{2+} mobilization in the sarcoplasmic reticulum in arteries from exercise-trained NC and HF rats, which was inferred by the increased contractile response to noradrenaline in the absence of extracellular Ca^{2+} compared with untrained rats. Corroborating this finding, the SERCA-2/PLB expression ratio was increased in caudal tibial arteries from exercise-trained animals. The present results are in agreement with studies that showed restoration of SERCA-2 expression in left ventricle samples (36) and skeletal muscles (5) from NC and HF mice submitted to exercise training.

$Ca_V1.2$ is a cellular mechanism that responds to membrane mechanical stretching. $Ca_V1.2$ is also involved in myogenic response with mandatory roles in the depolarization of vascular smooth muscle cells, leading to an increase in intracellular Ca^{2+} concentrations (24, 46). Increased voltage-gated Ca^{2+} channel current density has been described in coronary arteries from exercise-trained pigs (3). In the present study, the influx of extracellular Ca^{2+} assessed by the noradrenaline-induced contraction in response to extracellular Ca^{2+} as well as $Ca_V1.2$ protein expression was unchanged in caudal tibial arteries from all groups studied.

In addition to the benefits of exercise training for myogenic response, the present study also shows that this paradigm normalizes paw volume in HF rats. Since the capillary hydrostatic pressure is precisely regulated by myogenic response (29) and this response was restored in exercise-trained HF, it could be suggested that the reversal of the paw volume after exercise training is due to recovery of the functionality of the myogenic response of skeletal muscle resistance arteries. Moreover, the increased fluid mobilization in the interstitial compartments of active muscles, which is generated by the exercise session in association with the improvements in the flow autoregulation, may contribute to the proper restoration and transcapillary movement and fluid balance after exercise training.

In this study, we clearly showed for the first time that aerobic exercise training was able to restore the impairment in MC in arteries from HF rats. Adjustments in the MC induced by exercise training are associated with an improvement in the mobilization of Ca^{2+} by the sarcoplasmic reticulum, in addition to Ca^{2+} sensitization through the ROCK pathway. In association with the restoration of MC, exercise training also promoted the reversal of peripheral edema associated with HF. Thus, these beneficial insights on the myogenic response induced by exercise training may contribute to improving skeletal muscle perfusion and reducing classical symptoms of HF, such as exercise intolerance and peripheral edema, and can be a useful addition to therapeutic treatments.

AUTHOR CONTRIBUTIONS

S.M.P. and L.V.R. conceived and designed research; S.M.P., G.K.C., and M.T.F. performed experiments; S.M.P., G.K.C., M.T.F., and L.V.R. analyzed data; S.M.P., G.K.C., M.T.F., S.K.C., C.E.N., J.G.M., and L.V.R. interpreted results of experiments; S.M.P. prepared figures; S.M.P. drafted manuscript; S.M.P., S.K.C., C.E.N., J.G.M., and L.V.R. edited and revised manuscript; S.M.P., G.K.C., M.T.F., S.K.C., C.E.N., J.G.M., and L.V.R. approved final version of manuscript.

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