Research Article

Moderate-Intensity Exercise Training Reduces Vasorelaxation of Mesenteric Arteries: Role of BK_{Ca} Channels and Nitric Oxide

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Short Title: **Moderate intensity exercise training and vasorelaxation**

Summary

Exercise training (ET) is well established to induce vascular adaptations on the metabolically active muscles. These adaptations include increased function of vascular potassium channels and enhanced endothelium-dependent relaxations. However, the available data on the effect of ET on vasculatures that normally constrict during exercise, such as mesenteric arteries (MA), are scarce and not conclusive. Therefore, this study hypothesized that 10 weeks of moderate-intensity ET would result in adaptations towards more vasoconstriction or/and less vasodilatation of MA.

Young Fischer 344 rats were randomly assigned to a sedentary group (SED; n=24) or exercise training group (EXE; n=28). The EXE rats underwent a progressive treadmill ET program for 10 weeks. Isometric tensions of small (SED; 252.9±29.5 μm, EXE; 248.6±34.4 μm) and large (SED; 397.7±85.3 μm, EXE; 414.0±86.95 μm) MA were recorded in response to cumulative phenylephrine concentrations (PE; 0-30 μM) in the presence and absence of the BK_{Ca} channel blocker, Iberiotoxin (100 nM). In another set of experiments, tensions in response to cumulative concentration-response curves of acetylcholine (ACh) or sodium nitroprusside (SNP) were obtained, and pEC_{50s} were compared. Immunoblotting was performed to measure protein expression levels of the BK_{Ca} channel and eNOS.

ET did not alter the basal tension of small and large MA but significantly increased their responses to PE, and reduced the effect of BK_{Ca} channels in opposing the contractile responses to PE without changes in the protein expression level of BK_{Ca} subunits. ET also elicited a size-dependent functional adaptations that involved reduced endothelium-independent and endothelium-dependent relaxations. In large MA the sensitivity to SNP was decreased more than in small MA suggesting impaired Nitric oxide (NO)-dependent mechanisms within the vascular smooth muscle cells of ET group. Whereas the shift in pEC_{50} of ACh-induced relaxation of small MA would suggest more effect on the production of NO within the endothelium, which is not changed in large MA of ET group. However, the eNOS expression was not significantly changed between the ET and SED groups.

In conclusion, our results indicate an increase in contraction and reduced relaxation of MA after 10 weeks of ET, an adaptation that may help shunt blood flow to metabolically active tissues during acute exercise.

Keywords: Exercise training, Mesenteric arteries, large conductance calcium-activated potassium channel, vascular smooth muscle cells, nitric oxide.

Introduction

Exercise training (ET), unequivocally, was shown to induce functional and structural alterations in blood vessels of a number of body organs (Perrino *et al*. 2011). These changes varied depending on the type, intensity and duration of ET. In addition to the dependence on the pattern of ET, these adaptations are also reported to be heterogeneous along the arterial tree of the same vasculature such as in conduit and resistance arteries (Thijssen *et al.* 2010). Among the important adaptations to ET is the remodelling of K^+ channels in the vascular smooth muscle cells (VSMCs). Several studies focused specifically on change in the function, expression, and/or electrical currents of the large-conductance calcium-activated potassium channels (BK_{Ca}) (Shi *et al.* 2013), since BK_{Ca} channels play an important role in opposing VSMCs contraction and, therefore favour vasodilatation (Latorre *et al*. 2017). The impact of these channels becomes magnified due to its high conductance, which makes the opening or closing of few channels sufficient to alter the membrane potential and to induce arterial relaxation or constriction respectively (Dopico *et al*. 2018).

The contraction of VSMCs depends mainly on the influx of Ca^{2+} through L-type voltage-gated calcium channels (LTCC) (Ets *et al*. 2016). Gating of these channels is regulated by the membrane potential, which is mostly under the influence of K^+ channels. The opening of K^+ channels hyperpolarizes the membrane, leading to the closure of LTCC and vasorelaxation. In contrast, their closure depolarizes the membrane to a threshold potential for the opening of LTCC, leading to Ca^+ influx and vasoconstriction (Sobey 2001). Therefore, adaptations that tend to increase or decrease the expression of K^+ channels in VSMCs result in enhanced vasorelaxation or contraction, respectively (Zhang *et al*. 2017).

Vascular smooth muscle tone is also modulated by factors released from the endothelium. Nitric oxide (NO) has been recognized for decades to be the most important endothelium-dependent vasorelaxant factor. Its vasorelaxant effect was reported to depend on the size of the blood vessels to be more important in large arteries compared to small ones which are involved in regulating vascular resistance and blood pressure (Hilgers *et al*. 2006, Rajendran *et al*. 2013).

The physiological significance of upregulating the vasodilatory tools such as; BK_{Ca} channels as well as the endothelial nitric oxide synthase (eNOS) in response to ET is well documented in blood vessels such as coronary and skeletal muscle that normally vasodilate during acute exercise (Duncker & Bache 2008, Cocks *et al*. 2013). However, it is not yet clear what is the adaptation of arteries such as; mesenteric arteries (MA), which generally constrict in response to acute exercise when exposed to a moderate intensity ET.

In this study, we hypothesized that MA would downregulate its vasodilatory tools, specifically BK_{Ca} and/or eNOS when exposed to repeated bouts of a moderate-intensity ET. This adaptation may lead to enhance its contractility and/or reduce its vasodilatory ability in order to better shunt blood to more active organs during acute exercise. Since small arteries and arterioles contribute to total peripheral resistance and blood pressure (Pries *et al*. 2015), and they are the main vessels responsible for shunting blood, this study used both small and large MA in a comparable fashion. Therefore, we investigated the adaptations to a moderate-intensity ET in small and large MA at the level of whole vessel vasoreactivity and the involvement of BK_{Ca} channels and NO, in addition to changes in BK_{Ca} and eNOS protein expression.

Materials and Methods

Chemicals

All chemicals were obtained from Sigma Chemicals (Steinheim, Germany) unless otherwise stated. The physiological saline solution (PSS) contained (mM): 119 NaCl, 4.7 KCl, 1.18 KH_2PO_4, 1.17 $MgSO_4$, 25 $NaHCO_3$, 5.5 glucose and 1.6 $CaCl_2$, pH 7.4. Iberiotoxin (IbTx) was obtained from TOCRIS (Abingdon, United Kingdom), and was prepared in 100 μM stock solution in PSS, and stored in aliquots at -20 ˚C.

Experimental animals

All procedures were performed after the approval of the Animal Ethics Committee according to Sultan Qaboos University Research Ethics Policy in accordace with the Guide for the Care and Use of Laboratory Animals (1985), NIH, Bethesda under the project (SQU/AEC/2017-18/04).

Fifty-two male Fischer 344 rats, aged 2–3 months were housed in Sultan Qaboos University Small Animal House facility in a temperature-controlled room (22 ±2 ˚C) with a 12h light/12h dark cycle, and received food and water *ad libitum*. Two groups of rats were used comparatively; a sedentary group (SED, n=24) and an exercise-training group (EXE, n=28). Since rats are nocturnal animals, their normal light/dark cycle was inverted two weeks before the start of exercise training so that the training sessions take place during the active period of the rats. In this study, it was between 9 am-12 pm.

Exercise training protocol

Exercise-training (ET) protocol consisted of a moderate-intensity aerobic training as described by Albarwani *et al*., (2010). Rats walked on a rodent motor-driven treadmill (IITC Life Science, California, USA) for the duration of 10 weeks (5 days/week, speed of 15 m/min). Exercise time was increased gradually from 20 min/day with 0% slope (week 1-3) to 40 min/day with 0% slope (week 4), to the final level of 60 min/day, with a slope of 5% from week 5 to week 10.

Measurement of body weight, heart rate, and blood pressure

The body weight of rats of both groups was measured immediately at the end of ET period. Twenty-four hours after the last training session, heart rate and systolic blood pressure were measured using tail-cuff blood pressure monitor (BP-2000-R-2 series II, Visitech Systems, NC, USA).

Exercise training efficacy assessment

Rats were sacrificed by intra-peritoneal injection of an overdose of a mixture of ketamine (140 mg/Kg) and xylazine (40 mg/Kg) within 24–48h after the last exercise session. The left soleus muscle was collected randomly from six animals of each SED and EXE group and immediately stored at -80 $^{\circ}$C until citrate synthase activity (CSA) was measured. CSA was evaluated according to the manufacturer protocol using the Citrate Synthase Activity Colorimetric Assay Kit (BioVision, USA). CSA was expressed as mU/mg of protein /min.

Vascular reactivity assessment

First-order (large) and second-order (small) MA were isolated and mounted onto a wire myograph chamber (620M, Danish Myo Technology, Aarhus, Denmark) containing PSS for tension recording. After mounting, the arterial segment was stretched progressively to an internal circumference equivalent to 90% of the circumference that the vessels would have reached if exposed to 100 mmHg transmural pressures.

At the beginning of each experiment, the basal tension of arteries was measured after an equilibration period of 30 mins. Then, the viability of arteries and the integrity of the endothelium were tested by first contracting the vessels with phenylephrine (PE; 4 μM) followed by relaxing them with acetylcholine (ACh; 1 μM). The endothelium was considered intact if the artery relaxed by ≥70% in response to 1 μM ACh.

To examine the contribution of BK_{Ca} channels to the basal tone, PE-induced contractions were obtained in a cumulative concentration response manner of 0.01 μM, 0.05 μM, 0.1 μM, 0.5 μM, 1 μM, 3 μM, 5 μM, 10 μM, 30 μM first in the absence of IbTx then repeated after incubating the vessels with a potent blocker of BK_{Ca} channels, IbTx; 100 nM for 20 mins. In a different set of experiments, endothelium-dependent and endothelium-independent relaxations were assessed on arteries precontracted with 4 μM PE and then relaxed with a cumulative concentration of ACh; 0.01 nM, 0.1 nM, 1 nM, 10 nM, 100 nM, 1 μM, 10 μM or with a NO donor, sodium nitroprusside (SNP), also in a cumulative concentration of 0.01 nM, 0.1 nM, 1 nM, 10 nM, 100 nM, 1 μM, 10 μM.

Western immunoblotting

Western immunoblotting was performed as described earlier by Albarwani *et al*. (2010). Protein samples were prepared by homogenizing arteries that were pooled from three to four rats for each group in 50 µl lysis buffer (2 mM EDTA, 2 mM EGTA, 250 mM Sucrose, 50 mM MOPS, protease inhibitor; pH:7.4). For each blot one sample of 30 µg from each of the four groups of arteries was loaded in one lane and separated by 7.5% SDS-PAGE. Membranes were blocked with 10% skimmed milk (BIO-RAD Laboratories, USA) for 1 h at room temperature and then incubated overnight at 8 ˚C with monoclonal anti-BK_{Ca}-α (1:500, BD Bioscience, USA) or anti-BKCa-β (1:500, Abcam, USA), or monoclonal anti-eNOS (1:1,000, BD Bioscience, USA). The binding of antibodies was detected with horseradish peroxidase-conjugated secondary antibodies (dilution 1:5,000, Santa Cruz Biotechnology, USA). Immunoreactive bands corresponding to the molecular weight were detected by enhanced chemiluminescence with Supersignal West Dura Substrate (ThermoScientific, USA). β-actin antibody (1:1000, Santa Cruz Biotechnology, USA) was used as an internal standard to the normalize loading of protein.

Data analysis and statistics

LabChart (ADInstruments) software was used to calculate the actual diameter and the active tension of the arterial wall. The maximal tension (100%) was calculated from the difference in tension between the tension maximally induced by PE and the basal tension. Relaxations in response to ACh and SNP are expressed as the % relaxation from contractions induced by 4 µM PE. The concentrations of PE, ACh, and SNP that produced half-maximal responses (EC_{50}) were calculated using GraphPad Prism Software (San Diego, CA, USA). The EC_{50} values were expressed as the negative logarithm of the molar concentration (pEC_{50}).

Proteins were quantified using densitometry analysis normalized for loading differences to β-actin signal. Four blots were run for each protein and, each run used protein isolated from three to four rats. All values were expressed as means ± SEM, (n) represents the number of vessels used except for blood pressure and weight it represents the number of animals.

Data from each arterial size (small or large) were analyzed independently of each other. Effect of ET on PE-concentration response curves was analyzed using two-way ANOVA for comparing SED vs EXE and the effect of IbTx on each group. Paired t-test was used for comparing the effect of ACh or SNP between SED and EXE for each arterial size. Differences were considered statistically significant at $P<0.05$.

Results

Effect of exercise training on body weight, heart rate, blood pressure, and citrate synthase activity

Table 1 shows that 10 weeks of moderate intensity ET had no significant effect on the body weight, heart rate, systolic blood pressure, the CSA and vessel diameters.

Effect of exercise training on basal tone and phenylephrine-induced contractions

The basal tension (BT, mN/mm) of small MA isolated from the SED group (SED-S) was 0.77±0.15 (n=33), and from the EXE group (EXE-S) was 0.74±0.11 (n=40). The BT of large MA isolated from the SED group (SED-L) was 1.24±0.20 (n=16), and from the EXE group (EXE-L) was 0.97±0.06 (n=39). ET did not significantly alter the BT of small (P=0.83) nor large (P=0.11) MA.

In the small MA isolated from the EXE group, the pEC_{50} of PE-concentration response curves were significantly lower (P=0.03) than of those MA isolated from SED group. The pEC_{50} were; 5.72±0.03 (n=9) and 5.54±0.02 (n=15) for EXE-S and SED-S respectively (Fig. 1A). In contrary to the small MA, the responses of the large MA to PE were significantly higher in EXE group compared to SED group. The pEC_{50} of EXE-L and the SED-L were 5.56±0.01 (n=9) and 5.83±0.03 (n=17) respectively (Fig 1B) (P<0.0001). The maximal contractile responses to PE were not significantly different between SD and EXE groups (Table 2).

Effect of exercise training on contribution of BK_{Ca} channels to basal tone and vascular reactivity

Incubating small and large MA isolated from both SED and EXE rats with 100 nM IbTx resulted in no significant change (P = 0.26-0.93) in the basal tension of the small (SED-S; 0.56±0.11 mN/mm, SED-S-IbTx; 0.98±0.27 mN/mm, n=7, and EXE-S; 0.71±0.07 mN/mm, EXE-S-IbTx; 0.96±0.10 mN/mm, n=16), or large MA (SED-L; 0.82±0.16 mN/mm, SED-L-IbTx; 0.79±0.17 mN/mm, n=4, and EXE-L; 0.96±0.06 mN/mm, EXE-L-IbTx; 1.05±0.13 mN/mm, n=16). However, IbTx significantly shifted PE-concentration response curves to the left in SED-S, SED-L and to a less extent in EXE-S, but not in EXE-L (Fig. 2A-D). In the presence of IbTx, the pEC_{50} of SED-S increased from 5.72±0.03 to 6.11±0.06 (n=9, P<0.0001), of SED-L from 5.56±0.01 to 6.05±0.03 (n=8, P<0.0001) and in EXE-S from 5.54±0.02 to 5.71±0.05 (n=15, P= 0.01). In EXE-L, the pEC_{50} was 5.83±0.03 in the absence and 5.80±0.03 in the presence of Ibtx (n=17, P=0.86). The pEC_{50} values are also provided in Table 2. Concentration-response curves plotted using absolute tensions (mN/mm) of the same data are shown in inserts of each respective figure (Fig. 2A-D).

Effect of exercise training on endothelium-dependent and independent relaxations

Relaxations of arteries were studied by constructing normalized endothelium-dependent and endothelium-independent relaxation response curves using ACh and SNP, respectively. ET shifted the

ACh concentration-dependent relaxation curves to the right, but the shift was significant only in small MA. The pEC_{50} was 8.01±0.11 (n=17) for SED-S and 7.55±0.09 for EXE-S (n=23) (P=0.02). In large MA, the pEC_{50} was 7.86±0.10 (n=18) SED-L and 7.66±0.09, (n=26) for EXE-L (P=0.17) (Fig. 3A, 3B).

In contrast, ET shifted the SNP concentration- relaxation curves to the right but the shift was significant only in the large MA. The pEC_{50} was 7.43±0.39 for SED-S (n=17) and 7.03±0.06 for EXE-S (n=23) (P=0.47). For the large MA, pEC_{50} was 7.73±0.17 for SED-L (n=18) and 6.87±0.11 (n=26) for EXE-L (P=0.01) (Fig. 4A, 4B). The pEC_{50} values for ACh and SNP are given in Table 3.

Western immunobloting

To associate functional changes observed in vasoreactivity of EXE rats, the protein expression level of α-subunits and β-subunits of BK_{Ca} channels and eNOS were detected using their specific antibodies. The corresponding immunoreactive bands of the pore-forming α-subunit and the auxiliary β-subunit of BK_{Ca} (n=4 runs, 3 to 4 rats/run), when normalized to its β -actin signal of the same membranes resulted in no significant difference between SED and EXE for α- subunits of small (P=0.27) and large MA (P=0.48) also of β -subunits of small (P=0.39) and of large MA (P=0.86) (Fig. 5A, and Fig.5B).
Similarly, the expression level of eNOS protein normalized to the β-actin signal in different membranes indicated no significant difference in the level of expression of eNOS in both small MA (SED-S vs. EXE-S, P=0.20) and large MA (SED-L *vs* EXE-L, P=0.49) (Fig. 5C).

Discussion

Studies on humans and animals have demonstrated that the blood flow to almost all splanchnic vascular trees is reduced during acute exercise and shunted to metabolically active tissue (Padilla *et al*. 2011). We, therefore, hypothesized that moderate-intensity ET of 10 weeks duration would enforce the efficiency of MA in shunting blood flow from splanchnic circulation to muscle tissue (Laughlin *et al*. 2008, Nagashima *et al*. 2012) by reducing its vasodilatory and/or enhancing its vasoconstrictive capacity. More specifically, we hypothesized that BK_{Ca} channels would play an important role in the resulted adaptation. Because of the functional heterogeneity that exists in the same arterial tree based on the size of the arteries, this study was conducted using small and large MA.

The results obtained from this study demonstrated four main observations that are arterial size dependent. First, ET increased the response of large MA to PE. Second, the contribution of BK_{Ca} channels' in opposing PE contractile responses was reduced in small MA and completely abolished in large MA after ET. Third, ET reduced endothelium-dependent vasodilatation in small MA and endothelium-independent vasodilatation in large MA. Fourth, the immunoblotting showed that the

above-mentioned functional changes were not associated with significant changes in protein expression levels of eNOS or the BK_{Ca} pore-forming and regulatory subunits.

Efficacy of exercise training

Our results showed no significant increases in CSA in the soleus muscle after 10 weeks of a moderate intensity ET. The reason for this discrepancy is not clear at this time. However, few studies also reported unchanged CSA; in mice after 8 weeks of wheel-running (Momken *et al*. 2004) and in men after 6 weeks of aerobic endurance training (Heilbronn *et al*. 2007). Leek *et al*. (2001) raised concerns about using CSA as a biomarker for mitochondria density after ET without optimizing tissue sampling points. He attempted to provide explanation to the variability in CSA levels after ET, such as the timing of muscular tissue sampling, whether immediately or after 24 hours after exercise being important in results obtained. In our study, muscle samples were obtained within 24-48 hours after the final bout of exercise.

Effect of exercise training on the vasoreactivity of mesenteric arteries

Examining the vascular responsiveness to PE, demonstrated that 10 weeks of a moderate intensity ET had altered the responses of small MA and large MA differently by increasing the responsiveness of the large MA but decreasing that of the small MA. Our results on the large MA are in agreement with those of Lash *et al.* (1993), who reported increased PE-induced responses of Sprague-Dawley rats' intestinal vessels after 11 weeks of treadmill aerobic ET. However, Jansakul and Hirunpan (1999), using in vitro perfused mesenteric arterial beds, showed a lower vascular response to PE in the superior MA of young WKY rats after 33 days of swimming ET. Likewise, Chies *et al.* (2004) also reported a decreased PE-induced vasoconstriction through a non-endothelial nitric oxide related mechanism in the Wistar rats' superior mesenteric arteries after five weeks of forced swimming. It is not clear if the type of exercise; treadmill vs. swimming, or the arterial size has contributed to the observed contradicting responses with some of the above studies.

Effect of exercise training on vasodilatory response and eNOS expression

In this study, the endothelial-dependent vasodilatation was tested by relaxing precontracted MA with ACh in a concentration-response manner and comparing the pEC_{50} values of arteries from different groups. Our results showed that, ET reduced the endothelium-dependent relaxations in small and large MA. However, statistical significance was only observed in small MA. ACh binds to muscarinic receptors on the vascular endothelium and stimulates an influx of Ca^{2+} which in turn activates several mediators that ultimately lead to vasodilatation. The most studied mediator is NO which once synthesized by eNOS diffuses to the adjacent VSMCs, and causes vasorelaxation (Sandoo *et al*. 2010).

The importance of each endothelium-dependent vasodilator is believed to be vasculature and arterial size-dependent (Rajendran *et al*. 2013). For example, NO is more potent to vasodilate large arteries while, small arteries and arterioles rely mainly on endothelium-dependent hyperpolarizing factors (EDHF) (Hilgers *et al*. 2006). Differential ACh-dependent relaxations in response to ET according to vessel sizes have been reported earlier in coronary arteries (Duncker & Bache 2008), in MA, and aorta (Chen *et al*. 2001, Hilgers *et al*. 2006).

Inconsistent with the above, the current study showed that there was no significant change in the expression of eNOS protein in small and large MA from SED and EXE rats. It is therefore, may be speculated that the attenuation of ACh-induced relaxations observed in EXE groups was brought about by endothelium-dependent mediators other than those related to changes in expression of eNOS. Some studies have reported that in mesenteric resistance arteries of spontaneously hypertensive rats, the ACh-induced relaxation brought about by NO can be strongly compensated by other endothelium-vasodilators such as; the EDHF which induces VSMCs hyperpolarization and relaxation mainly by activating K^+ channels (Albarwani *et al*. 2015), indicating the overwhelming effect of EDHF mechanisms in this vasculature. Conversely, Chen *et al*. (2001) reported that ET enhanced NO production in Wistar rats' MA through BK_{Ca} channels activation after 8 weeks of treadmill ET. The cause of this discrepancy is not clear at this point but may be due to the difference in ET protocols and/or in animal species used.

The present study also examined the endothelium-independent relaxations and similarly found that they were reduced after ET as indicated by the decreased pEC_{50} when vessels were treated with the NO donor, SNP. This effect was observed in both small and large MA but was only significant only in large MA. NO activates the soluble enzyme guanylate cyclase and causes the production of cGMP, reduction in intracellular Ca^{2+}, and consequently vasorelaxation (Green *et al*. 2004). Hence, the reduced potency of SNP to elicit relaxation in the vascular smooth muscles of MA in the EXE group could be due to changes in any of the molecules involved in the above-mentioned pathway.

Effect of exercise training on BK_{Ca} channels activity and expression of its subunits

Several studies have shown that the expression profile/activity of arterial BK_{Ca} channels is altered in many pathological and physiological conditions such as hypertension (Yang *et al*. 2013), aging (Albarwani *et al*. 2010), and exercise (Shi *et al*. 2013, Zhang *et al*. 2017). Hence, these channels serve as an excellent target to examine for any alteration in vasoreactivity that may occur due to ET.

Our results showed no differences in BT of small and large MA after blocking BK_{Ca} channels, indicating an insignificant contribution of BK_{Ca} channels in maintaining the BT of MA. These results are contrary to what was reported earlier in coronary arteries of F344 rats (Albarwani *et al*. 2010), and of Zucker

rats (Climent *et al*. 2017), and femoral arteries of Wistar rats (Al-Brakati *et al*. 2015), where BK_{Ca} channels blockade caused a significant increase in the BT.

On the other hand, the contribution of BK_{Ca} channels in limiting PE-induced contractions was significantly lower in small MA isolated from the EXE group compared to the SED group and was abolished in the large MA of EXE group as indicated by the shift of PE-concentration response curves to the left when arteries were incubated with IbTx. This response may have resulted from ET-induced down-regulation of the BK_{Ca} channels, altered intracellular mediators that affect channel gating (Hou *et al*. 2009) or channel modulation by the endothelium-derived vasoactive substances such as, NO, Prostaglandins, EDHFs (Tanaka *et al*. 2004).

To find out whether these ET-induced functional changes are associated with molecular changes in BK_{Ca} channel subunits, we measured the expression levels of the pore-forming (α) and the regulatory (β) subunits of BK_{Ca} channels in small and large MA after ET. In contrary to what has been reported earlier by Shi *et al*. (2013) that ET increased contribution of BK_{Ca} channels to the regulation of mesenteric arterial tone by upregulating its β-subunit, we found no difference in the protein expression level of both subunits after 10 weeks of a moderate intensity ET. It should be noted that Western blotting in the current study measured overall subunit protein level not only the membranous protein, and hence we cannot negate that there could be differences in channel densities inserted in the membrane of VSMCs that have caused the observed functional changes.

Conclusion

The results obtained support our hypothesis that a moderate intensity ET of 10 weeks duration is sufficient to elicit arterial size-dependent functional adaptations favouring increased contractility and reduced vasorelaxation in the MA. The increased sensitivity of the large MA to PE and the abolished contribution of BK_{Ca} channels in opposing these contractions favour increased contractility. The reduced sensitivity of large MA to SNP suggests impaired NO-dependent mechanisms within the VSMC of ET group. Whereas the ACh-induced relaxation of small MA would suggest more an effect on the production of NO within the endothelium, which was not changed in large MA of ET group. These alterations were not associated with significant changes in the protein expression level of BK_{Ca} channel subunits or eNOS. Taken together, these results indicate a tendency to an increased vascular tone of MA after ten weeks of a moderate intensity ET. The physiological significance of these adaptations may be to effectively shunt more blood to more metabolically active tissues during acute exercises, such as; the heart and the exercising skeletal muscle.

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Competing interests
The authors declare that they have no competing interests.

Contributions:
Farid Al-Dhuhli conducted the experiments, analysed the results, and wrote the manuscript; Sultan Al-Siyabi assisted in running the experiments, analysing the results, and editing the manuscript; Hamed Al-Maamari conducted part of the experiments; Said Al-Farsi conducted part of the experiments; Sulayma Albarwani* contributed to overall study design and discussion, and edited the manuscript.

All authors read and reviewed the manuscript.

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	Body weight (gram)	Systolic blood pressure (mmHg)	Heart rate (beat/min)	Citrate synthase activity (mU/mg/min)	Vessel diameter (μm)
SED	317.4±6.2 n=24	161.8 ± 11.6 n=7	383.5±11.7 n=7	332.8±18.8 n=6	S: 252.9±29.5, n=17 L: 397.7±85.3, n=18
EXE	318.7±3.7 n=27	167.3±5.3 n=7	411.0±6.0 n=7	308.7±32.4 n=6	S: 248.6±34.4, n=23 L: 414.0±86.95, n=26
P-value	P = 0.55	P = 0.70	P = 0.85	P = 0.54	S: P = 0.68 L: P = 0.54

Table 1. Body weight, heart rate, systolic blood pressure, citrate synthase activity, and diameters of vessels used in the study for sedentary (SED) and exercised (EXE) rats. Each value represents mean ± S.E.M.

Figure 1

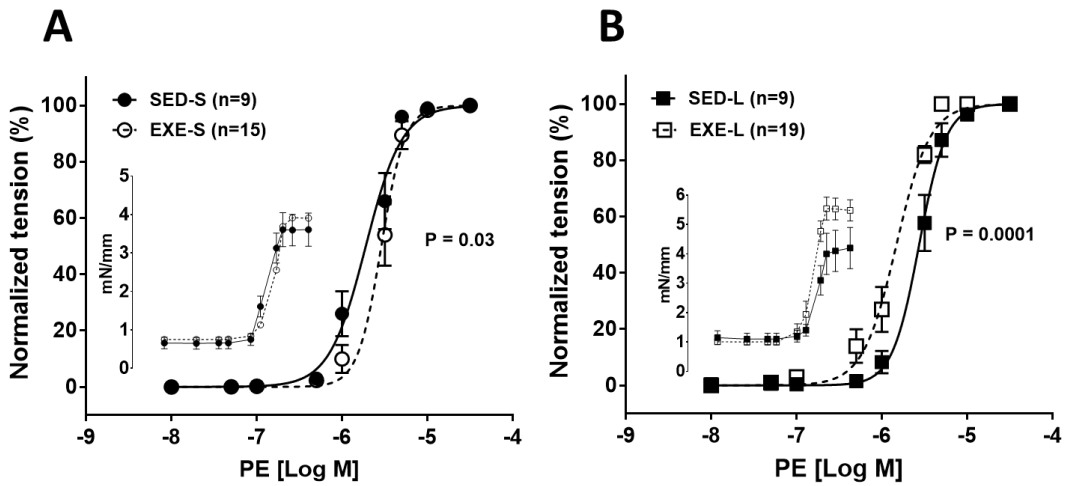

Figure 1. Effect of exercise training on phenylephrine-concentration response curves
Phenylephrine (PE) normalized concentration-response curves of small (A) and large (B) mesenteric arteries isolated from SED and EXE rats. Insert of each respective figure shows concentration-response curve of the same data plotted using absolute tensions (mN/mm). SED-S: sedentary small, SED-L: sedentary large, EXE-S: exercise small, EXE-L: exercise large. Each value represents mean ± S.E.M.

Vessel size	Rat group	Emax* mN/mm	-ve IbTx pEC_{50} ± S.E.M.	+ve IbTx pEC_{50} ± S.E.M.	(P-value)
Small arteries	SED-S (n=9)	3.60±0.43	5.72±0.03	6.11±0.06	P < 0.0001
	EXE-S (n=15)	3.92±0.30	5.54±0.02	5.71±0.05	P = 0.01
(P-value)		*P = 0.54	*P = 0.03	*P < 0.0001	
Large arteries	SED-L (n=8)	4.22±0.74	5.56±0.01	6.05±0.03	P < 0.0001
	EXE-L (n=17)	5.54±0.39	5.83±0.03	5.80±0.03	P = 0.86
(P-value)		*P = 0.10	*P < 0.0001	*P < 0.0001	

Table 2. pEC_{50} of phenylephrine concentration response curves of mesenteric arteries isolated from sedentary (SED) and exercised rats (EXE) in the absence (-ve) and presence (+ve) of iberiotoxin (IbTx). *P: the vertical significance level between SED and EXE of the same vessel size.

Figure 2

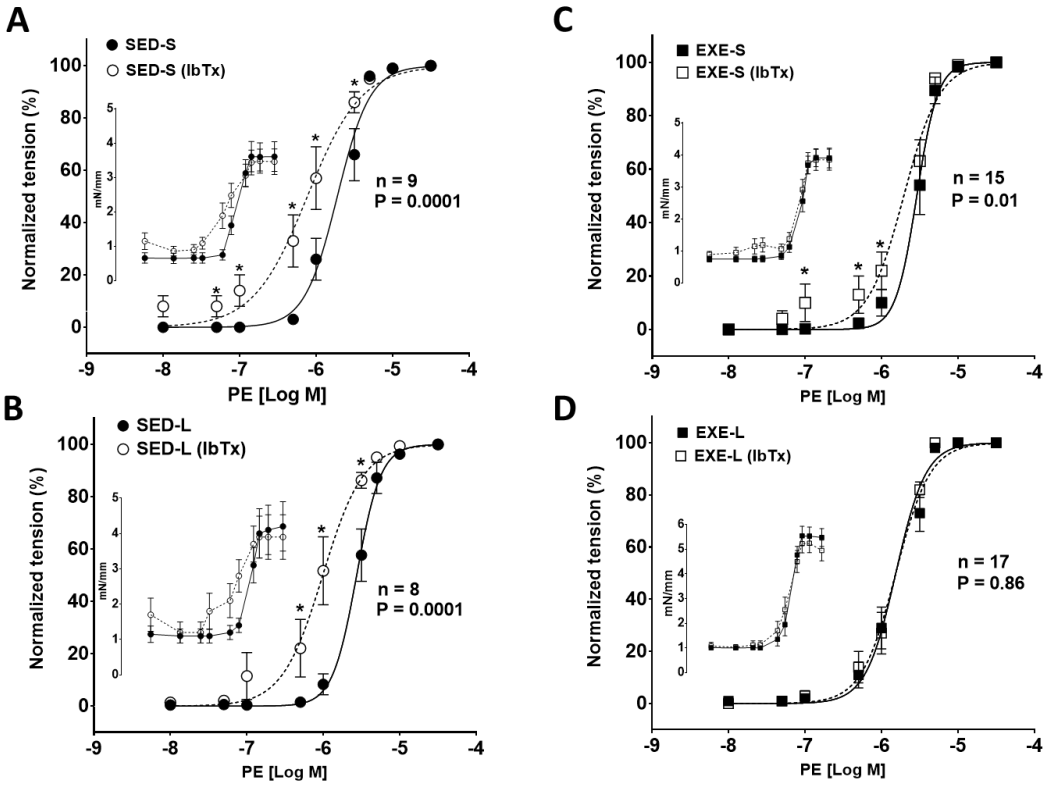

Figure 2. Effect of Iberiotoxin on phenylephrine concentration-response curves
Phenylephrine (PE) cumulative concentration-response curves constructed from normalized contractions of mesenteric arteries in the presence and absence of BK_{Ca} channel blocker, Iberiotoxin (IbTx, 100 nM) in small (A) and large (B) arteries isolated from sedentary rats and in small (C) and large (D) arteries isolated from exercised rats. Insert of each respective figure shows similar concentration-response curve plotted using absolute tensions (mN/mm). SED-S: sedentary small, SED-L: sedentary large, EXE-S: exercise small, and EXE-L: exercise large,. Each value represents mean ± S.E.M and (*): P<0.05.

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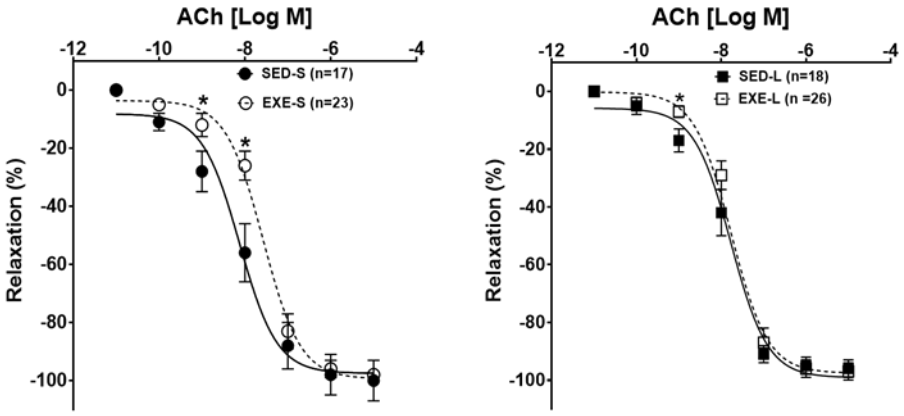
Figure 3



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563 **Figure 3. Effects of exercise training on acetylcholine concentration-dependent relaxations.**
564 Acetylcholine (Ach) cumulative concentration-response curves constructed from normalized
565 relaxations of small and large mesenteric arteries from sedentary and exercise groups. SED-S: sedentary
566 small, SED-L: sedentary large, EXE-S: exercise small, and EXE-L: exercise large. (*): P<0.05. Each
567 value represents mean ± S.E.M.
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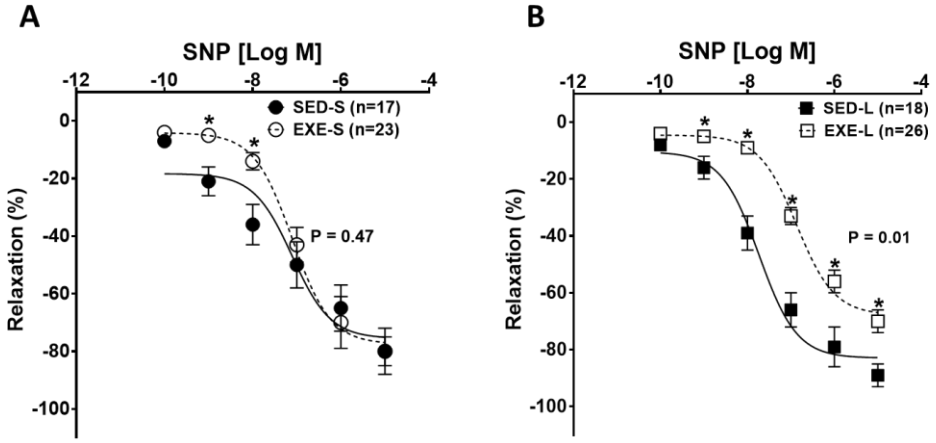
Figure 4



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574 **Figure 4. Effect of exercise training on sodium nitroprusside concentration-dependent**
575 **relaxation.**

Sodium nitroprusside (SNP) cumulative concentration-response curves constructed from normalized relaxations of small and large mesenteric arteries from sedentary and exercise groups. Insert of each respective figure shows similar concentration-response relaxations plotted using absolute tensions (mN/mm). SNP: sodium nitroprusside, SED-S: sedentary small, SED-L: sedentary large, EXE-S: exercise small, EXE-L: exercise large, and r^2: R-value. (*): $P<0.05$. Each value represents mean ± S.E.M.

Vasodilator	Vessel size	SED pEC_{50} ± S.E.M.	EXE pEC_{50} ± S.E.M.	(P-value)
ACh	small	8.01±0.11 (n=17)	7.55±0.09 (n=23)	P = 0.02
	large	7.86±0.10 (n=18)	7.66±0.09 (n =26)	P = 0.17
SNP	small	7.43±0.39 (n=17)	7.03±0.06 (n=23)	P = 0.47
	large	7.73±0.17 (n=18)	6.87±0.11 (n=26)	P = 0.01

Table 3. pEC_{50} of acetylcholine (ACh) and sodium nitroprusside (SNP) cumulative concentration-response curves of small and large mesenteric arteries isolated from sedentary (SED) and exercise (EXE) rats. Each value represents mean ± S.E.M.

Figure 5

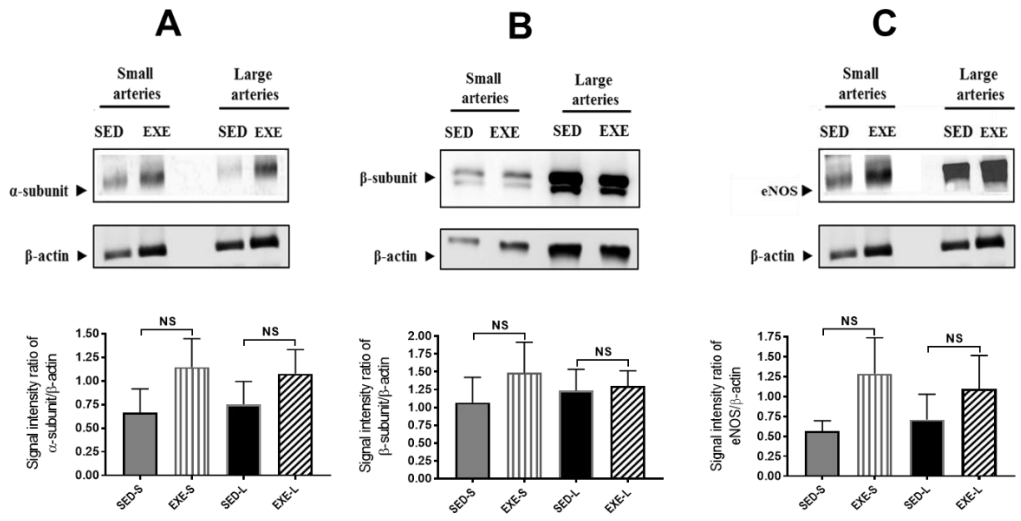

Figure 5. Representative Western immunoblots and expression levels of the α- and β-subunits of the BK_{Ca} channel and of eNOS in small and large mesenteric arteries.
Representative Immunoblots (upper panel) of BK_{Ca} channel α-subunit (A) and β-subunit (B) proteins and of eNOS (C) each with its corresponding β-actin signals for arteries from small and large mesenteric arteries isolated from sedentary and exercise rats. Corresponding bars (lower panel) represent means ± S.E.M of pooled data from 4 blots, each using arteries isolated from 3 to 4 rats per run and normalized

to β-actin protein signal. SED-S: sedentary small, EXE-S: exercise small, SED-L: sedentary large and EXE-L: exercise large. NS: not significant. Each value represents mean ± S.E.M.