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Exercise Training Improves Vasoreactivity in the Knee Artery

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Abstract

Physical activity has been shown to enhance endothelial function of central and peripheral vascular beds. The primary purpose of the present study was to test the hypothesis that a short-term exercise training program would result in enhanced endothelium-dependent vasorelaxation of a major artery supplying blood flow to the knee joint, the middle genicular artery. Female Yucatan miniature swine were randomly assigned into exercise trained ($n = 7$) or sedentary ($n = 7$) groups. Exercise trained pigs underwent a daily exercise training program on treadmills for 7 days. In vitro assessment of vasorelaxation was determined in a dose response manner by administering increasing doses of 3 different dilators; adenosine diphosphate, bradykinin, and sodium nitroprusside. The role of nitric oxide synthase and cyclooxygenase pathways in vasomotor responses was evaluated with specific inhibitors using nitro-L-arginine methyl ester and indomethacin incubation, respectively. The results of this investigation indicate that adenosine and bradykinin-induced endothelium-dependent vasorelaxation were significantly enhanced in middle genicular artery from exercise trained pigs ($p < 0.05$). Endothelium-independent vasorelaxation was not altered with exercise training as determined by the response to sodium nitroprusside. The findings of the present investigation indicate that short-term exercise training enhances endothelial function of middle genicular artery through adaptations in the nitric oxide synthase and by non-nitric oxide synthase, non-cyclooxygenase pathways.

Keywords

physical activity; vasorelaxation; knee vasculature

Introduction

The endothelium, a dynamic inner layer covering the entire vascular system, plays an important role in maintaining healthy vascular function. This effect of the endothelium on vascular function is regulated largely through the endothelial release of protective bioactive substances; the most widely studied being nitric oxide (NO) [19]. Endothelial dysfunction can result from a number of causes but disturbed NO signaling causes endothelial dysfunction of a number of important processes including; diminished endothelium-dependent relaxation (EDR), increased endothelial permeability, and changes in endothelial cell phenotype [17].

Endothelial dysfunction contributes to the pathogenesis of different disease processes. For example, endothelial dysfunction is thought to be the first step in the chain of events that leads to the development of cardiovascular disease (CVD) [17,20]. Interestingly, endothelial dysfunction of atherosclerosis is not confined to the coronary arteries but rather appears to be a systemic disorder that affects peripheral vascular beds such as that supplying blood flow and nutrients to the joints with consequent disturbance of joint health. In that regard, Miller et al. [28] reported evidence for endothelial dysfunction in the vasculature of osteoarthritic rabbit knees. Furthermore, a recent review presents data supporting vascular disease as a causal factor in the initiation and/or progression of osteoarthritis, the most common disease of joints [7], and the leading cause of disability in the US [16]. Indeed, animal studies support the idea that the mechanisms that control knee blood flow are altered when the joint becomes inflamed [26,27] resulting in an increase joint diameter with a concomitant fall in basal blood flow. Chronic inflammation as seen in osteoarthritis might also alter normal vascular responses and promote endothelial dysfunction [7].

Among the numerous interventions that can enhance endothelial function, physical activity is recognized as critical for both primary and secondary intervention in humans [21,30] and animals [4,12]. There is cumulative evidence in the literature that reduced EDR is reversed and/or prevented by exercise [12,21]. Regular physical activity exerts both acute and chronic effects on the endothelium. For instance, exercise training has been shown to promote increases in the expression of NO synthase (NOS) and enhanced EDR in both arteries and resistance arterioles [24]. In addition, exercise has also been associated with anti-inflammatory and anti-atherogenic effects [14] mediated in part by the increase in NO bioavailability. Interestingly, there is also evidence that exercise and/or physical activity may play an important role in the preservation of joint integrity in humans [36,44] and animal [15,39] studies. In that regard, it has been reported that articular cartilage becomes attenuated with proteoglycan depletion in immobilized joints [34], whilst moderate exercise seems to enhance proteoglycan content and articular cartilage thickness [15,39,44]. There are also studies reporting that the development and/or progression of knee osteoarthritis are blunted by exercise [5,35]. Finally, among healthy community-based adults with no history of knee injury or disease, vigorous physical activity appears to promote a beneficial effect on tibial cartilage [36]. The positive effects of exercise on vasculature of articular cartilage can be, at least partly, attributed to diffusion of nutrients through the matrix from the synovial fluid [33] and/or instigation of mechanotransduction signals in chondrocytes [2]. Interestingly, vascular integrity might be another key component that influences articular cartilage health. Indeed, not only the synovial fluid (a dialysate of plasma with hyaluronic acid) but also the subchondral blood flow appears to be important in order to supply nutrition to the articular cartilage [7]. While articular cartilage degradation is considered the hallmark of osteoarthritis, the concept that the “joint is an organ” is quickly being recognized a fundamental to understand osteoarthritis. Periarticular tissues including subchondral bone, cruciate ligaments, and menisci are all vascularized tissues, and an adequate blood supply to these tissues should be essential in maintaining joint health [7]. Thus it appears that vascular contribution should be recognized as a key player in the initiation and progression of this condition. Further studies are needed in order to elucidate the involvement of vascular integrity in altering joint homeostasis.

Although many studies [4,12,21] have demonstrated that reduced EDR is reversed and/or prevented by exercise, we are unaware of any study that has tested the hypothesis that exercise can improve EDR of healthy knee vasculature. Further study is needed to elucidate a possible link between endothelial function and joint health. As a first step in that process the present study was primarily aimed to evaluate the effect of a short-term exercise training program on endothelial function of the middle genicular artery (MGA), a major blood supplier to the knee joint [40]. Previous training studies have reported enhanced dilation in

central [13,18,45] and peripheral vascular beds [23] after just one week of exercise, thus we hypothesized that short-term exercise training would also result in enhanced EDR in the MGA of exercise trained pigs.

Methods

Experimental animals

The experimental animals were adult female Yucatan miniature swine ($n = 14$), 30–33 months of age and weighed 33–58 kg. The pigs were housed in the animal care facility in the Department of Biomedical Sciences, University of Missouri, in rooms maintained at 20–23 °C with a 12-h: 12-h light-dark cycle. They were fed a normal diet (Purina Laboratory 5082 Mini-Pig Breeder Chow); calories provided by fat were 8 %. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Missouri. This study has also been performed in accordance with the ethical standards of the International Journal of Sports Medicine on research with experimental animals [8].

Training protocol

At the beginning of the study pigs were familiarized with running on a motorized treadmill and randomly assigned into exercise trained (Ex, $n = 7$) or cage confined/sedentary (Sed, $n = 7$) groups with ad libitum access to water. Sed pigs were restricted to their enclosures (2×4 -m pens) and did not exercise; whilst Ex pigs underwent a daily exercise training program on treadmills for 7 days. The exercise training program included a warm-up, endurance, and cool down stages, where the intensity and duration of exercise bouts were increased steadily. Similar exercise training protocols have been used previously in our laboratory [13,18]. Briefly, warm-up and cool down stages were run for 5 min each one at 2.0 mph during the 7 days. The endurance stage was run at 3.5 mph; on day 1 for 15 min, day 2 for 30 min, and from day 3 to 7 for a total time of 60 min. The relative intensity (i. e. percentage of maximal oxygen consumption) during the endurance stage of the training was roughly 25 ml/min/kg (an intensity that requires about 75 % maximal oxygen consumption), based on a previous study from our laboratory [25]. Heart weights and body weights were recorded at the end of the training or sedentary confinement program.

In vitro assessment of vessel reactivity

Vascular ring preparation—At the end of the 7 days exercise training, pigs were anesthetized with intramuscular ketamine (35 mg/kg)-xylazine (2.25 mg/kg), and intravenous thiopental (25 mg/kg) for deep anesthesia, and the heart was removed to achieve euthanasia. Immediately following sacrifice, the MGA was removed from the same site in the left knee of all pigs. The vessels were trimmed of connective tissue and fat in cold Krebs bicarbonate buffer solution, and cut into 5 vessel rings ~3–4 mm in length. Cut rings were photographed on an Olympus SZH video microscope which was connected to a Spot Insight camera (model 3.2.0., Diagnostic Instruments, Inc.). Then, the rings morphological characteristics (axial length, outer and inner diameters) were measured using Image J software (1.34n, NIH, USA).

Assessment of relaxation response of arterial rings—5 MGA rings were mounted on a myograph (Globaltown Microtech) by positioning 2 stainless steel wires in the lumen of the ring. Arterial rings were placed in a 20 mL bath of Krebs bicarbonate solution. The optimal point in the length-tension relationship (L_{max}) was established through incremental (10 % of passive outside diameter) increases in stretch in combination with potassium chloride (KCl: 50 mM) administration to the rings. The remainder of the study was performed at the experimentally determined L_{max} of each ring. Contractile responses to

KCl were performed prior to vasorelaxation studies. KCl (80 mM) was administered twice to all of the arterial rings until the increase in tension had reached a plateau (■10 min). Following the KCl studies, Krebs bicarbonate solution was replaced every 20 min until resting tension was achieved. Endothelium-dependent, dose-dependent vasorelaxation was assessed in all rings using cumulative addition of adenosine diphosphate (ADP; 10^{-9} – 10^{-4} M) and bradykinin (BK; 10^{-11} – 10^{-6} M); whilst the assessment of endothelium-independent vasorelaxation utilized increasing doses of sodium nitroprusside (SNP; 10^{-10} – 10^{-4} M). The 5 MGA rings were preconstricted with prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$; 30 μ M) and allowed to achieve a plateau in tension development before the addition of vasodilators. Ring 1 was left untreated; “intact” (in Krebs solution only). Ring 2 was pre-treated with nitro-L-arginine methyl ester (L-Name; 300 μ M) to inhibit the NOS pathway. The third ring was pretreated with indomethacin (Indo; 5 μ M) to inhibit cyclooxygenase (COX), the enzyme responsible for prostacyclin (PGI $_2$) and prostaglandin production. Ring 4 was pretreated with a combination of L-Name and Indo to assess the role of NOS and COX independent mechanisms of relaxation. Finally, on ring 5 the endothelium was removed; “denuded”, by gentle rubbing of the luminal surface with fine-tipped forceps to study the endothelium-independent mechanisms, explicitly smooth muscle relaxation. The order of agonists throughout the entire study was ADP, BK, and SNP. Following each agonist induced dose response; Krebs bicarbonate solution was replaced at 20 min intervals until resting tension of all arterial rings was reached (■60 min), before the next protocol was initiated.

Solutions and drugs

The Krebs bicarbonate buffer solution contained (in mM) 131.5 NaCl, 5.0 KCl, 1.2 NaH $_2$ PO $_4$, 1.2 MgCl $_2$, 2.5 CaCl $_2$, 11.2 glucose, 20.8 NaHCO $_3$, 0.003 propranolol, and 0.025 EDTA. Solutions were aerated with 95 % O $_2$ and 5 % CO $_2$ (pH 7.4) and maintained at 37 °C. All drugs and chemicals were purchased from Sigma Chemical (St Louis, MO, USA).

Statistical analysis

All values are means $\pm$ standard error (SE). Differences between groups regarding body weight, heart weight, heart weight-to-body weight ratio, ring characteristics, and half-maximal effective concentration (EC $_{50}$) values were determined via an unpaired 2-tailed Student's *t*-test where $p < 0.05$ was considered significant. All unpaired *t*-test data were analyzed in GraphPad Prism version 5.0a. EC $_{50}$ was defined as the vasodilator concentration that produced 50 % inhibition of a PGF $_{2\alpha}$ precontraction. The EC $_{50}$ values were determined for each animal using nonlinear regression analysis (GraphPad). Means of the EC $_{50}$ values are presented as the negative log of the molar concentration. The analysis of concentration-response curves was performed using the Mixed procedure in SAS version 9 (SAS Institute Inc. Cary, NC). The statistical model used was a 3-factor study with 1 between subjects factor (group; Ex or Sed), and 2 within subject factors (treatment and dose). The Mixed procedure also allows us to model for heterogeneous variances across doses. Pairwise comparisons were made between groups for fixed treatment and dose levels using Least Squares Means. Nonparametric statistical methods were used to perform a series of tests on dose-response data in the cases where examination of residual plots from a 3-factor analysis of variance model indicated that the assumption of normality of the error terms was suspect. Specifically, group differences at each dose-treatment combination were looked at with the Wilcoxon rank sum test. Treatment differences at each dose-group combination were also examined with the Wilcoxon signed-rank test on the differences. A false discovery rate adjustment was used for multiple tests in view of the large number of tests considered. Differences with false discovery rate-adjusted values of < 0.05 were considered significant.

Results

The exercise training program was successfully completed by all Ex pigs. The intensity and duration of exercise bouts were increased steadily every day so that by day 2 Ex pigs ran for 30 min, and from day 3 to 7 they were able to run for a total time of 60 min at 3.5 mph. Body weight of Sed pigs (47.14 ± 2.06 kg) was not statistically different than that of Ex pigs (39.37 ± 3.26 kg) ($p = 0.06$); furthermore, heart weights were not significantly different between the groups; Sed, 196 ± 6.00 g, and Ex, 205 ± 15.36 g; ($p = 0.55$). The exercise training protocol resulted in increased heart weight-to-body weight ratio; Sed, 4.17 ± 0.10 g/kg; and Ex, 5.22 ± 0.23 g/kg; ($p < 0.05$).

Vessel characteristics

Structural and functional characteristics of rings harvested from MGA are presented in Table 1. There were no significant differences in axial length, resting tension, compliance or 80 mM KCl and PGF_{2α} induced tension between Ex and Sed rings utilized for functional experiments. Interestingly, MGA rings from Ex pigs had significantly greater outer and inner diameters, and wall thickness than Sed pigs ($p < 0.05$).

Relaxation responses to ADP

ADP elicited a dose-dependent relaxation of MGA rings from Ex and Sed pigs (Fig. 1a). ADP induced a significantly enhanced relaxation in intact MGA rings from Ex pigs that was evident at dose 7 ($1e-6$) (Fig. 1a). ADP-induced relaxation was partially inhibited by L-Name in both groups; and ADP-induced relaxation remained greater in MGA rings of Ex pigs compared to Sed pigs (Fig. 1b). The addition of Indo, into the bath, abolished the differences between Ex and Sed MGAs (Fig. 1c). In the presence of L-Name and Indo, ADP-induced relaxation was present and it was similar between Ex and Sed MGAs (Fig. 1d) suggesting that pathways different from NOS and COX are responsible for the remaining relaxation observed. Finally, when the endothelium was removed from the MGA rings of Ex and Sed pigs, these vessels still exhibited some degree of ADP-induced relaxation indicating that ADP works on both endothelial and vascular smooth muscle cells of MGA rings (Fig. 3a). Also, because denuded MGA rings from Ex pigs no longer exhibited increased relaxation these results suggest that the effects of exercise training were focused to the endothelium.

The maximal relaxation and half-maximal effective concentration (EC₅₀) for ADP-induced relaxation are presented in Table 2. Briefly, in intact and L-Name treated MGA rings the maximal ADP-induced relaxation was lower in Sed than Ex pigs; however, these differences were not statistically significant (intact, $p = 0.27$; L-Name, $p = 0.25$). In the presence of Indo and L-Name+Indo, maximal ADP-induced relaxation was not different between MGA rings from Sed and Ex pigs. Although the sensitivity to ADP tended to be greater in intact and L-Name treated rings from Ex pigs, as indicated by the EC₅₀ values, these results did not reach significant difference (intact, $p = 0.09$; L-Name, $p = 0.08$). Finally, the EC₅₀ values were similar between Sed and Ex pigs when the rings were treated with Indo and L-Name+Indo.

Relaxation responses to BK

BK was used to assess endothelium-dependent relaxation. We observed that BK induced a concentration-dependent increase in relaxation in MGA rings from both Ex and Sed pigs (Fig. 2a). Interestingly, BK-induced relaxation was significantly greater in intact MGA rings from Ex pigs than Sed at dose 5 ($1e-9$) (Fig. 2a). Although BK-induced relaxation was partially inhibited by the addition of L-Name in both Ex and Sed MGAs; BK-induced relaxation remained significantly greater in MGA of Ex pigs at dose 5 ($1e-9$) (Fig. 2b). The addition of Indo abolished the differences between Ex and Sed BK-induced

relaxation (Fig. 2c). With the addition of both inhibitors, L-Name and Indo, BK-induced relaxation was still observed in both groups, being greater in Ex pigs, suggesting that the non-NOS, non-COX pathway was altered by exercise training. Finally, when the endothelium was removed, BK-induced relaxation was completely abolished in Ex and Sed MGAs, indicating that unlike ADP, BK-induced relaxation is endothelium dependent in MGA rings (Fig. 3b).

The maximal relaxation and EC_{50} for BK-induced relaxation are presented in Table 3. In intact and L-Name treated MGA rings the maximal BK-induced relaxation was lower in Sed than Ex pigs, similar to the results for ADP; however, these differences were not statistically significant (intact, $p = 0.13$; L-Name, $p = 0.43$). In the presence of Indo and L-Name+Indo, maximal BK-induced relaxation was not different between MGA rings from Sed and Ex pigs. The sensitivity to BK tended to be greater in intact and L-Name treated rings from Ex pigs, as indicated by the EC_{50} values; however, these results did not reach significant difference (intact, $p = 0.17$; L-Name, $p = 0.47$). Finally, the EC_{50} values were similar between Sed and Ex pigs when the rings were treated with Indo and L-Name+Indo.

Relaxation responses to SNP

Relaxation responses to SNP after $PGF_{2\alpha}$ -induced contraction are presented in Fig. 4. Across the entire range of SNP concentrations utilized, direct smooth-muscle relaxation induced by SNP was similar in MGA rings isolated from Ex and Sed pigs, we did not find significant differences.

Discussion

The main purpose of the present study was to test the hypothesis that a short-term exercise training program would enhance endothelial function of the MGA. The MGA, an artery that branches from the anterior aspect of the popliteal artery, has been confirmed as a major arterial supply vessel to the knee joint [40]. We report here that a short-term, 7-day exercise training program resulted in increased ADP and BK-induced endothelium dependent relaxation in MGA rings from Ex pigs. These results support our hypothesis that a short-term exercise training program would stimulate adaptations to MGA endothelium dependent relaxation similar to those reported in other vascular beds following exercise training [13,18,23,45]. To our knowledge this is the first study to document improvement of endothelium-dependent vasorelaxation responses in an artery providing blood flow to the knee joint, MGA, of healthy animals.

Training effectiveness

The present study demonstrates that endurance training for 7 days resulted in a significant increase in heart weight-to-body weight ratio of Ex pigs, an index of myocardial hypertrophy. These findings are comparable to results of other short-term exercise protocols in our laboratory [13,18].

Influence of exercise training on ADP-induced relaxation

The finding that exercise training promoted an enhanced ADP-induced EDR of the MGA rings from Ex pigs was consistent with our hypothesis. A previous study from our laboratory has reported that ADP-induced relaxation was also enhanced in coronary arteries of pigs following a short-term exercise program [18]. In addition, there is evidence that endurance exercise training improves ADP-induced EDR in femoral arteries of hypercholesterolemic pigs [46]. This is the first report in the literature of enhanced ADP-induced relaxation, in the MGA of healthy pigs following short-term exercise training. This finding is reflected in the fact that there were no differences between responses of Ex and Sed rings after denudation

(Fig. 3a). In addition, the results from the present investigation revealed that the ADP-induced relaxation of the MGA was partially inhibited (~20 %) by the addition of the NOS inhibitor L-Name; suggesting that the NOS pathway plays a role in the vasorelaxation responses of MGA rings. The fact that EDR was not entirely abolished by L-Name treatment indicates that the NOS pathway is not the single contributor to the response. A previous study reported that chronic NOS inhibition significantly diminished but did not abolish ADP-induced EDR in porcine coronary arteries [11]. Results indicate that ADP-induced relaxation is mediated by both endothelium-dependent and endothelium-independent processes (Fig. 1) but the effects of exercise training are entirely through endothelial adaptations (Fig. 3a).

ADP-induced EDR was still present in Ex and Sed rings in the presence of both inhibitors, L-Name and Indo. These results suggest that non-NOS non-COX pathways contribute to ADP-induced vasorelaxation in MGA rings. Comparable results have been reported in previous studies on peripheral arteries of swine [23,46]. Thus it can be speculated that the presence of endothelium-derived hyperpolarizing factor (EDHF) contributes to these responses. However, our results also indicate that ADP-induced relaxation is mediated in part by direct actions on vascular smooth muscle (Fig. 3a).

ADP-induced vasorelaxation was still present following removal of the endothelium from the MGA rings (Fig. 3a). These results indicate that ADP-induced vasorelaxation is at least in part mediated by receptors located in vascular smooth muscle of MGA rings. Indeed, ADP can induce vasodilation via nucleoside-selective P_{2Y1} receptors ("purinoceptors") located on vascular smooth muscle [6,38]. Ferrel et al. [6] reported that ADP-induced relaxation of denuded arteries is mediated by P_{2Y1} receptors located on the smooth muscle cells of rabbit knee joint blood vessels. In summary, the ADP-induced EDR in the MGA rings is significantly increased by short-term exercise training. This effect of training appears only partially NO-mediated because Ex MGAs exhibited greater relaxation in the presence of L-Name treatment (Fig. 1b). COX inhibition alone abolished the difference between the responses of Ex and Sed MGAs as did treatment with both L-Name and Indo suggesting that non-NOS, non-COX pathways are not involved in the training effect. Finally, because denuded MGAs from Ex pigs did not exhibit greater relaxation in response to ADP, the enhanced responses produced by Ex appear to be entirely mediated by changes in the endothelium.

Influence of exercise training on BK-induced relaxation

BK-induced EDR in MGA rings was significantly greater in Ex than in Sed pigs. These responses have not been previously documented in the knee vasculature; however, our laboratory has reported enhanced BK-induced vasorelaxation following a short-term exercise training program on coronary [18] and arteries perfusing skeletal muscle [23].

The present findings reveal that the BK-induced relaxation of the MGA was inhibited (~25 %) by the addition of the NOS inhibitor L-Name. While this indicates that the NOS pathway is a key contributor to BK-induced EDR in the MGA, the fact that Ex MGA exhibited greater relaxation than Sed during L-Name treatment suggests that the effects of training are not entirely mediated by increased NO release from NOS. These results are similar to previous studies addressing the role of NOS in BK-induced EDR in brachial and femoral arteries [31], as well as arteries that perfuse skeletal muscle [32]. Available human literature also suggests that the contribution of NO to EDR is relatively similar between limb vasculatures [37,43]. Results from Indo treatment indicate a slight reduction in BK-induced EDR but importantly, no differences between Ex and Sed rings (Fig. 2c). Thus, in the absence of COX activity, the effects of exercise training appear to be gone. In contrast, double blockade with L-Name and Indo (Fig. 2d) blunted relaxation of MGA from both

groups but Ex MGAs exhibited greater relaxation. Overall, these results suggest interactions among the 3 endothelial signaling pathways as reported previously [42] and do not allow us to conclude which of the endothelium-derived relaxing factors are most responsible for the enhanced EDR after exercise training.

The double treatment with L-Name and Indo results also indicate that when formation of endothelium-dependent NO and vasodilator prostaglandins were inhibited the remaining BK-induced vasorelaxation must result from a non-NOS, non-PGI₂, mechanism, probably EDHF. There is evidence that exercise training can increase sensitivity of coronary vascular smooth muscle to EDHF [29]. Furthermore, in human limbs, BK-induced EDR has been reported to be slightly reduced when NOS and COX are concurrently blocked, but the majority of the response has been attributed to EDHF or a NOS and COX-independent pathway [42]. The lack of BK-induced EDR when the endothelium was removed both confirms the endothelial-dependence of BK on MGA, and suggests that the MGA rings were properly denuded. The facts that Ex EDR exceeded Sed in untreated MGAs, in MGAs after L-Name treatment, and in MGAs after L-Name plus Indo suggest that the training effect on EDR is the result of increased PGI₂ and/or EDHF, not due to upregulation of the NOS pathway.

Influence of exercise training on SNP-induced relaxation

Our results demonstrate that SNP-induced vasorelaxation was similar in MGAs from Ex and Sed pigs. The lack of significant difference in the SNP-induced response in the MGA rings between Ex and Sed pigs indicates that this exercise training program did not cause modification in the smooth muscle responses to NO.

Evidence of structural remodeling in MGA of Ex pigs

Improved EDR has also been observed in central [18,45] and large peripheral conduit vessels after just 7 days of endurance training in pigs [23]. There is also evidence that short-term exercise training enhances eNOS protein expression and NO bioavailability in aortic tissues [4]. These findings suggest that the enhanced EDR with training may be the result of increased blood flow and shear stress acting directly on the endothelial layer and increasing NO production and/or bioavailability. These functional modifications represent the initial adaptation in response to training. On the other hand, extended training programs might induce structural remodeling of the vasculature (e. g. increase in lumen diameter) [1,10]. These structural modifications could result in alterations in the stimulus-response relationship as training becomes long-term. As a result there is a tendency for normalization of shear stress during prolonged exercise training (i. e. decrease in the shear stress gradient sensed by endothelial cells, or decreased response to the shear stress stimulus) [1]. Taking this information into consideration we are surprised to observe that MGA rings from Ex pigs had significantly greater outer and inner diameters, and wall thickness than those from Sed pigs of similar size. At this point we do not know the probable causes of these differences between the MGA from Ex and Sed pigs. We could consider that perhaps the smaller size of the MGA (diameter, ~1.6–2 mm) in combination with the anatomical location (popliteal fossa) and even the degree of shear stress could be also linked to the shorter time course of structural remodeling. In healthy young humans, peak and mean wall shear stress increased significantly along the femoral-popliteal axis following exercise [41]. Interestingly, the study by Schlager et al. [41] reported that the highest increase was observed in the popliteal artery. Unfortunately, we are unaware of any study that measured directly shear stress in the MGA, a branch of the popliteal artery. We could speculate that the MGA is also exposed to higher shear stress during exercise. If that will be the case, the increased shear stress would perhaps mediate early structural modifications in the MGA. Future studies are needed in order to confirm these unexpected findings reported here.

Conclusion

The purpose of this investigation was to test the hypothesis that a short-term exercise training program would promote increased endothelium-dependent relaxation of the MGA, an artery that provides blood flow to the knee joint. The most important finding is that the endothelium of arteries perfusing the knee joint adapts to exercise/physical activity. We report here that a short-term exercise training program results in a significant increase in both ADP and BK-induced EDR in the MGA rings. These EDR responses of the MGA following exercise training appear to be largely mediated by NOS, with non-NOS non-COX pathways also playing a role, most likely EDHF. It has been reported that patients with osteoarthritis exhibit a high prevalence of CVD [9,22]. A causal link between the progression of osteoarthritis and CVD has been also proposed [3]. Although coexistence of osteoarthritis and CVD might be merely an independent feature of advanced age and/or obesity, common major risk factors for both, knee osteoarthritis in women is more frequent in CVD cohorts independent of obesity [9,22]. Those studies supported the concept that joint health might be influenced by local and/or systemic vascular integrity. One of the possible mechanisms that might link these 2 disease conditions is endothelial function. Clearly the present results open the opportunity for future investigations willing to elucidate the physiological responses of the knee vasculature not only in health but also under pathologic conditions. In addition, these results imply a possible effect of vascular integrity on joint health and provide evidence for the potential beneficial effects of exercise as a therapeutic tool in order to restore and/or preserve the knee joint vasculature. If there is a pathogenic link between endothelial dysfunction and osteoarthritis and if this link can be understood, novel prevention and treatment strategies for these diseases could be determined, which would have tremendous benefit for millions of individuals in terms of morbidity and mortality.

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Fig. 1.

Concentration-response curves for adenosine (ADP)-induced relaxation of isolated medial genicular artery rings from exercise trained (Ex, n = 7) and sedentary (Sed, n = 7) pigs. Data were obtained for Intact vessels (panel **a**); in the presence of L-NAME (panel **b**); in the presence of Indomethacin (panel **c**); and in the presence of both, L-NAME+Indomethacin (panel **d**). Values are means $\pm$ SE. * $P < 0.05$ for Ex vs. Sed Intact.

Fig. 2.

Concentration-response curves for bradykinin (BK)-induced relaxation of isolated medial genicular artery rings from exercise trained (Ex, n = 7) and sedentary (Sed, n = 7) pigs. Data were obtained for Intact vessels (panel **a**); in the presence of L-NAME (panel **b**); in the presence of Indomethacin (panel **c**); and in the presence of both, L-NAME+Indomethacin (panel **d**). Values are means $\pm$ SE. * P < 0.05 for Ex vs. Sed Intact, and vs. Sed L-NAME.

Fig. 3. Concentration-response curves for adenosine (ADP) and bradykinin (BK)-induced relaxation of isolated medial genicular artery rings from exercise trained (Ex, n = 7) and sedentary (Sed, n = 7) pigs. Data were obtained for denuded vessels; ADP (panel **a**), and BK (panel **b**). Values are means $\pm$ SE. There were no significant differences between groups.

Fig. 4. Concentration-response curves for Sodium nitroprusside (SNP)-induced relaxation of isolated medial genicular artery rings from exercise trained (Ex, n = 7) and sedentary (Sed, n = 7) pigs. Values are means $\pm$ SE. There were no significant differences between groups.

Table 1

Vascular ring characteristics. Values are means $\pm$ SE. They were obtained from sedentary (Sed) and exercise trained (Ex) pigs. Potassium Chloride (KCl); prostaglandin F_{2 α} (PGF_{2 α}).

	Sed (n=7)	Ex (n=7)
axial length, mm	2.35 $\pm$ 0.15	2.64 $\pm$ 0.13
outer diameter, mm	1.68 $\pm$ 0.06	2.11 $\pm$ 0.05*
inner diameter, mm	0.68 $\pm$ 0.03	0.89 $\pm$ 0.06*
wall thickness, mm	1.01 $\pm$ 0.06	1.22 $\pm$ 0.05*
resting tension, g	4.35 $\pm$ 0.72	5.00 $\pm$ 0.87
80mM KCl tension, g	19.03 $\pm$ 4.13	24.19 $\pm$ 3.77
PGF _{2α} tension, g	18.58 $\pm$ 4.86	25.17 $\pm$ 4.67

* $P < 0.05$ for Sed vs. Ex comparison at the measured characteristic.

Table 2

Maximal relaxation and EC₅₀ values for ADP-induced relaxation. Values are means $\pm$ SE. They were obtained from sedentary (Sed) and exercise trained (Ex) pigs. Max. relaxation, maximal relaxation; half-maximal effective concentration (EC₅₀), concentration of vasorelaxing agent eliciting 50 % of maximal response.

Variable	Sed (n=7)	Ex (n=7)
Intact		
max. relaxation, %	73.19 $\pm$ 9.67	85.17 $\pm$ 3.88
EC ₅₀ , -log M	-4.92 $\pm$ 0.30	-5.62 $\pm$ 0.24
L-Name		
max. relaxation, %	49.03 $\pm$ 8.70	63.38 $\pm$ 7.89
EC ₅₀ , -log M	-4.13 $\pm$ 0.24	-4.83 $\pm$ 0.28
Indo		
max. relaxation, %	77.37 $\pm$ 3.93	72.19 $\pm$ 7.85
EC ₅₀ , -log M	-4.81 $\pm$ 0.21	-4.89 $\pm$ 0.24
L-Name+Indo		
max. relaxation, %	56.18 $\pm$ 5.45	62.12 $\pm$ 6.14
EC ₅₀ , -log M	-4.35 $\pm$ 0.15	-4.59 $\pm$ 0.16

Table 3

Maximal relaxation and EC₅₀ values for BK-induced relaxation. Values are means $\pm$ SE. They were obtained from sedentary (Sed) and exercise trained (Ex) pigs. Max. relaxation, maximal relaxation; half-maximal effective concentration (EC₅₀), concentration of vasorelaxing agent eliciting 50 % of maximal response.

Variable	Sed (n=7)	Ex (n=7)
Intact		
max. relaxation, %	61.30 $\pm$ 13.61	84.65 $\pm$ 6.12
EC ₅₀ , -log M	-7.76 $\pm$ 0.71	-8.90 $\pm$ 0.37
L-Name		
max. relaxation, %	44.01 $\pm$ 13.21	58.82 $\pm$ 12.72
EC ₅₀ , -log M	-6.81 $\pm$ 0.55	-7.44 $\pm$ 0.63
Indo		
max. relaxation, %	64.76 $\pm$ 9.65	62.24 $\pm$ 13.50
EC ₅₀ , -log M	-7.95 $\pm$ 0.51	-7.71 $\pm$ 0.71
L-Name+Indo		
max. relaxation, %	26.92 $\pm$ 4.85	45.63 $\pm$ 10.45
EC ₅₀ , -log M	-5.75 $\pm$ 0.18	-6.53 $\pm$ 0.48