INTRODUCTION

Physical training is an effective therapeutic means to ameliorate symptoms in patients with peripheral arterial insufficiency (1). Training can improve a number of contributing factors, including metabolic (2, 3), vascular control (4), and collateral circulatory functions (5). The latter is potentially most significant as flow limits to the ischemic muscle would be lessened. The developed collateral vessels can effectively support prolonged physical exercise, albeit at a reduced intensity (6). In an animal model of peripheral arterial insufficiency, established with bilateral femoral artery occlusion, collateral dependent blood flow to the calf muscles increases with relatively short-term (2-4 weeks) (7). However, the recovery of flow capacity remains relatively limited. Administration of exogenous angiogenic growth factors (FGF-2, VEGF) in combination with physical training can further increase collateral blood flow (8-11); yet, the absolute flows do not approach the normal maximal flow capacity. This is due to the high, dominant resistance of the up-stream collateral circuit (e.g., ~85% of total limb resistance; 12, 13), as the maximal conductance of the down stream vascular circuit within the muscle remains high as seen in normal animals (14). Thus, while exercise can be an effective means to prompt vascular remodeling (7, 15), recovery of flow capacity to the occluded distal muscles has not been observed. An increase in vessel wall shear stress is thought to be a major determinant inducing vascular remodeling. Vessels adapt to the high shear by outward remodeling via arteriogenesis such that the increased shear stress returns to normal (16, 17). In conditions of peripheral arterial insufficiency, the increase in blood flow through pre-existing collateral vessels, caused by distal muscle hyperemia during exercise (18), has been implicated as a stimulus for collateral vessel enlargement (19). Yet, the limited collateral flow recovery below the normal value with training implies an insufficient training stimulus, or reduction of endothelium sensitivity to shear stress. Increasing the flow stimulus by increasing exercise intensity is inherently difficult, since exercise capacity is markedly reduced in peripheral arterial insufficiency. Thus, it is unclear whether an exercise program utilizing an extended daily training duration may drive collateral dependent blood flow close to the normal hind limb values.

Recognizing the importance of shear stress in vascular remodeling, Eitenmuler and co-workers (20) conducted an intriguing experiment where a femoral artery-to-femoral vein...
anastomosis was surgically introduced distal to the site of femoral artery occlusion. This model established a chronically increased flow shear stress in the collateral circuit, since a significant portion of the collateral flow drained into the low-resistance femoral vein. Thus, the reduced distal resistance prompted an unsatiated stimulus for blood flow through the collateral vessels. They found that conductance of the collateral circuit was markedly increased, well beyond that previously observed experimentally. Further, Eitenmuler et al.'s study confirms the findings by others that high blood flow conditions, which are expected to increase shear stress, is a primary stimulus for arterial remodeling (16, 21-23). These adaptations were mediated by the endothelial isoform of nitric oxide synthase-nitric oxide (eNOS-NO) system, since the NOS inhibitor L-NAME abolished the outward remodeling of the vessels (16, 20). Interestingly, vascular endothelial growth factor (VEGF), a potent angiogenic growth factor, facilitates NO release from the endothelium (24). Indeed, administration of exogenous VEGF to animals with femoral artery occlusion enlarges collateral vessels and increases collateral-dependent blood flow to the distal hind limb (9, 25). We hypothesized that A-V shunt treatment, in combination with exogenous VEGF administration, would maximize the collateral vascular remodeling in our rat model with femoral artery occlusion.

In the present study, we compared effects of two types of stimuli to induce collateral flow expansion; a prolonged exercise training program (extended exercise twice per day until fatigue, up to 15 weeks), and a femoral artery to femoral vein (end-to-side) shunt, distal to the site of femoral occlusion to exaggerate the distal flow demand through the collateral circuit. This permitted us to evaluate whether a greater volume of exercise training would materially increase collateral blood flow, over that observed previously with shorter-duration training (2, 7), and compare this with, what should be an exaggerated tissue response induced by an optimal flow stimulus.

MATERIAL AND METHODS

Experimental design

This study was performed in two separate experiments. The first experiment was a prolonged exercise training experiment by using adult female (275-300 g) Sprague-Dawley rats. The adult females, chosen as their body mass has less variation over time, were divided into four groups: 1) non-occluded control group (n=6); 2) acute occlusion group (n=6), that received acute bilateral femoral artery occlusion for 6 hours; 3) bilateral occlusion group that was trained for 15 weeks (n=13); and 4) bilateral occlusion group that was limited to cage activity for 15 weeks (Sed, n=9). Collateral dependent blood flow to the distal limb muscles was determined during treadmill running to reduce distal limb resistance (12, 13).

The second experiment was an A-V shunt study using adult male (400-425 g) Sprague-Dawley rats that received bilateral occlusion on the femoral arteries, and an A-V anastomosis was created on one side distal to the occlusion (Fig. 1). The placement of the A-V anastomosis established a low-resistance venous circuit for blood, derived distally via the collateral circuit, to return to the heart without perfusing the distal muscle. Preliminary experiments indicated that approximately 35-45% of collateral blood flow returned to the heart via the shunt. The femoral-occluded contralateral side served as a control. Rats were either assigned to vehicle control group (n=9) or VEGF treated group (n=4). After 2 weeks of treatment, rats were anesthetized, and surgically prepared for blood flow determination using an isolated hindquarter perfusion procedure, as done before (2, 26).

Collateral dependent blood flow to both hind limbs was measured under constant aortic perfusion pressure (~65 mmHg) when the A-V shunt patent and when closed. This use of larger animals and an isolated perfused hindquarter system permitted facilitated the A-V surgical procedure and the acute closure of the A-V shunt, necessary to appropriately measure collateral blood flow to the distal muscles.

Animal care

Rats (Harlan, Indianapolis, IN) were housed two per cage in a temperature controlled (20±1°C) room with a 12:12 h light:dark cycle. Rats were fed a standard rat chow diet and tap water ad libitum. Upon arrival, all female rats were familiarized with the motor-driven treadmill as done by others (27); by running 5-6 min/d for 4-5 days. This brief exposure to the treadmill ensures running performance during the training and blood flow determination without imparting training adaptations (2, 7).

Care and treatment of the animals and all experimental procedures were carried out in accordance with the National Institutes of Health guidelines and approved by the Animal Care and Use Committee of the University of Missouri.

Femoral artery occlusion

Details of the artery occlusion procedure have been described previously (28). Briefly, all animals were anesthetized with a mixture of ketamine (100 mg/kg) and acepromizide (0.5 mg/kg) i.p. Femoral arteries were exposed and isolated immediately distal to the inguinal ligament. A ligature (3-0 silk) was placed tightly around the artery. This procedure ensures
uniform closure of the femoral artery that reduces blood flow reserve to ~10-20% of normal capacity and retains sufficient resting blood flow (12, 13, 18).

**Femoral artery to vein anastomosis**

Following bilateral occlusion of the femoral arteries an artery to vein anastomosis was introduced distal to the occlusion on the left limb. In brief, a skin incision ~2 cm in length was made on the left medial thigh area and both femoral artery and vein were dissected. The femoral artery was tied with 3-0 surgical silk and severed at ~5 mm distal to the inguinal ligament. The distal end of the femoral artery was trimmed to a bevel shape to maximize the size of anastomosis. The distal end of the femoral artery was then anastomosed to the side of the femoral vein under 60X magnification with 10-0 ophthalmologic suture. This resulted a ~2 x 5 mm opening of the anastomosis (Fig. 1). Patency of the anastomosis was confirmed by color change of the femoral vein at the proximal side of the A-V shunt.

**Exogenous vascular endothelial growth factor165 infusion**

Human recombinant VEGF<sub>165</sub> (Genentech Inc, San Francisco, CA) was delivered by mini-osmotic pumps (model 2002, Alzet) at a dose (10 µg/kg/d, for 14 days via the left jugular vein) show to increase collateral blood flow. The control group received vehicle solution infused via the mini-osmotic pumps as described in our previous reports (8-11). The animals were sacrificed at day 16 following depletion of VEGF from the osmotic pump.

**Exercise training**

Rats in the training group began walking on a motor-driven treadmill 24 hours after bilateral femoral artery occlusion. Animals were trained twice per day, 5 days per week at a constant treadmill speed (20 m/min, 15% grade) until fatigue as characterized by uneven gait and transitional hopping. Time to fatigue was recorded for each animal.

**Blood flow determination during running**

Surgical preparations for blood flow determination while running on the treadmill have been described previously (28). Briefly, under (ketamine (100 mg/kg) and acepromizide (0.5 mg/kg) i.p.) anesthesia, catheters (PE-50 tubing) were inserted into the carotid artery for microsphere infusion, and the caudal artery for microsphere infusion. A well-mixed suspension of microspheres was infused into the arch of the aorta over ~20 s through the carotid catheter, followed by a saline flush. At the same time, a reference blood sample was withdrawn from the caudal artery catheter at a rate of 500 µ/min beginning 10 s before microsphere infusion. After the second microsphere injection, rats were anesthetized with sodium pentobarbital (60 mg/kg intra-arterially). Tissue samples comprising the entire hind limb, and the middle third of each kidney were counted to within a 1% error (Wallac Wizard 1480 Auto gamma Counter, Turku Finland) and corrected for "spillover" between isotope counting windows. Excellent mixing of the microspheres in cardiac output was verified by matching blood flows in the left and right sections of the kidneys (left vs. right: 1.02±0.02, n=68 observations) and in non-ischemic (abdominal, psoas) muscles. Muscle blood flow (ml·min<sup>-1</sup>·100 g<sup>-1</sup>) was calculated as:

\[
\text{Blood flow} = \frac{(0.50 \text{ ml·min}^{-1} \times \text{CPM RBS-1}) \times \left(\frac{\text{CPM tissue}}{\text{tissue mass}}\right) \times 100}{\text{g}}
\]

where, CPM is counts per minute and RBS is reference blood sample.

**Blood flow measurement with hindquarter perfusion system**

The detail perfusion preparation procedure was reported previously (2, 26, 29). Briefly, 16 days following A-V shunt surgery and VEGF<sub>165</sub> delivery, under sodium pentobarbital (60 mg/kg, i.p.) anesthesia, a PE-50 catheter was placed into the left carotid artery for injection of heparin (2000 IU). Through an abdominal incision, the abdominal aorta and vena cava were exposed for teflon catheter insertion (IV catheter; Becton-Dickinson, Rutherford, NJ). The animals were sacrificed and flow rate and perfusion pressure were gradually increased until the net aortic pressure reached ~65 mmHg. A bolus of 85Sr labeled microspheres was delivered via an inline arterial port followed by a second infusion of 141Ce labeled microspheres after closing the femoral artery to vein shunt. Limb tissues were dissected for gamma counting. Blood flow (ml/min) to each individual muscle was calculated from the flow rate time the fraction of total cpm within the given muscle section (tissue cpm/total cpm in tissues), and expressed as ml·min<sup>-1</sup>·100 g<sup>-1</sup> by correcting to tissue weight. For perfusion media preparation, see details in (29).

**Statistics**

Values are presented as means ±S.E. Data were analyzed by analysis of variance, with repeated measures as appropriate. Specific mean differences were evaluated using Tukey's post hoc analysis. Significant differences were accepted when p<0.05.

**RESULTS**

**Series I. Treadmill running**

**General response to training**

Daily exercise duration in the trained group increased markedly over the course of study, beginning at 5 to 10 min/d, and reaching a maximum of ~360 min/day by 15 weeks of training (Fig. 2). An increased citrate synthase activity in the deep portion of the gastrocnemius muscle (trained: 53.6±6.7 vs. sed: 41.3±5.5 umol·min<sup>-1</sup>·g<sup>-1</sup>, p<0.05) indicated training efficacy.

Body weights of groups were between 265 and 300 g (average=283±5.0). Tissue weights of the limb muscle were not materially different among the groups. Similar blood pressures and heart rates were observed across treatment groups at each of the running speeds used for blood flow determination (data not shown).

**Exercise training increased blood flow to occluded hind limb**

Prolonged treadmill running for 15 weeks significantly increased blood flow to the total (p<0.001), the proximal (p<0.005), and the distal (p<0.001) hind limb segments (Table 1). Collateral-dependent blood flow to the calf muscles was
markedly improved with training, increasing more than 100% more than the sed animals following occlusion of the femoral artery (cf., Table 1). The absence of an increase in calf muscle blood flow at the higher 25 m/min running speed in the occluded animals indicates that the upstream resistance (i.e., collateral circuit) was limiting and determined absolute blood flow.

Blood flow distribution within quadriceps and calf muscles varied between the individual fiber types because of inherent differences in vascular capacity. Further, blood flows to the corresponding fiber type regions of the proximal limb are greater than those of the lower limb, since the thigh muscles are not completely collateral-dependent with vascular supply from the internal iliac artery. As expected, within the muscle of the lower limb, the high oxidative red portion of the gastrocnemius and the soleus muscles maintained the highest blood flow, followed by the mixed fiber type regions of the hind limb (plantaris and mixed gastrocnemius muscles) and then the white portion of the gastrocnemius muscle (Table 2).

Blood flows to non-ischemic muscles (abdominal, psoas, and diaphragm muscles) were not different among groups (data

Table 1. Series I. Hind limb blood flow (ml·min⁻¹·100g⁻¹):

<table>
<thead>
<tr>
<th>Femoral artery occlusion</th>
<th>AOV</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-occluded</td>
<td>Acute</td>
<td>Sedentary</td>
</tr>
<tr>
<td>Hind limb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>82 ± 4.7</td>
<td>37 ± 1.5 ‡</td>
</tr>
<tr>
<td>Proximal</td>
<td>77 ± 6.9</td>
<td>51 ± 2.8 *</td>
</tr>
<tr>
<td>Distal</td>
<td>91 ± 3.8</td>
<td>15 ± 1.8 ‡</td>
</tr>
<tr>
<td>Calf muscle.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 m/min</td>
<td>141 ± 3.1</td>
<td>17 ± 2.2 ‡</td>
</tr>
<tr>
<td>25 m/min</td>
<td>132 ± 11.5</td>
<td>21 ± 3.0 ‡</td>
</tr>
</tbody>
</table>

Data expressed as mean±S.E.; N=number of observations. Acute=femoral artery occlusion 6 hr, Sedentary and Trained group occluded for 15 wks. AOV=Analysis of variance. Significantly different from: *Non-Occluded; ¶both Acute and Sed Occluded group; ‡different from all other groups by Tukey’s test (p<0.05).

Table 2. Series I. Hind limb muscle fiber type section blood flow (ml·min⁻¹·100g⁻¹):

<table>
<thead>
<tr>
<th>Femoral artery occlusion</th>
<th>AOV</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-occluded</td>
<td>Acute</td>
<td>Sedentary</td>
</tr>
<tr>
<td>Proximal Hind limb tissue:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White quadriceps</td>
<td>21 ± 3.2</td>
<td>26 ± 3.5</td>
</tr>
<tr>
<td>Red quadriceps</td>
<td>362 ± 25.2</td>
<td>297 ± 20.6 *</td>
</tr>
<tr>
<td>Mixed quadriceps</td>
<td>96 ± 8.2</td>
<td>71 ± 4.3 *</td>
</tr>
<tr>
<td>Distal Hind limb tissue:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White gastrocnemius</td>
<td>25 ± 3.8</td>
<td>13 ± 1.6 *</td>
</tr>
<tr>
<td>Red gastrocnemius</td>
<td>319 ± 27.9</td>
<td>37 ± 6.2 ‡</td>
</tr>
<tr>
<td>Mixed gastrocnemius</td>
<td>116 ± 8.6</td>
<td>17 ± 2.2 ‡</td>
</tr>
<tr>
<td>Soleus</td>
<td>282 ± 27.0</td>
<td>35 ± 5.6 *</td>
</tr>
<tr>
<td>Plantaris</td>
<td>138 ± 9.2</td>
<td>16 ± 1.6 ‡</td>
</tr>
</tbody>
</table>

Data expressed as mean±S.E.; N=number of observations. Acute=femoral artery occlusion 6 hr, Sedentary and Trained group occluded for 15 wks. Significantly different from: *Non-Occluded; ¶both Non-occluded and Acute occluded groups; ‡different from all other groups by Tukey’s test (p<0.05).
Renal blood flows were 340 to 370 ml·min⁻¹·100g⁻¹, and did not differ among groups. As illustrated in Fig. 3, administration of VEGF occluded groups (p<0.01). *significantly different from no VEGF occluded groups (p<0.01). ‡significantly differ from other occluded groups (p<0.01). Shadow bar: flow measured before closing A-V shunt.

Fig. 3. Blood flow to calf muscles during treadmill running (Series I), and during hindlimb perfusion (Series II). ANOVA showed significant treatment effect (p<0.001). Shadow bar: flow measured before closing A-V shunt.  §significantly different from other occluded groups (p<0.01). *significantly different from no VEGF occluded groups (p<0.01).

not shown). Renal blood flows were 340 to 370 ml·min⁻¹·100g⁻¹ for the low speed and 200 to 230 ml·min⁻¹·100g⁻¹ for the high speed in each of the non-trained groups. This reduction in renal blood flow with increased intensity of running is typical of that observed and probably represents an increase in sympathetic vasoconstriction outflow. The same reduction in renal blood flow was observed in the trained group; however, absolute blood flows were greater (506±27 and 286±33 ml·min⁻¹·100g⁻¹, at the low and high speeds, respectively), likely owing to a reduced sympathetic outflow typical of a training response.

Series II. Hind limb perfusion

General response to arterial-venous shunt

Animals in Series II weighted approximately 395±10.5 g and did not differ among groups. As illustrated in Fig. 3 introduction of the shunt increased collateral-dependent blood flow to the calf muscles modestly. Similarly, administration of VEGF demonstrated its arteriogenic efficacy by increasing collateral blood flow (cf., Fig. 3). Calf blood flows in the shunt groups show in shaded bars were determined prior to closure of the shunt, thereby permitting a significant 'steal' of collateral blood flow (~35%) in the venous return without delivery through the calf muscles. Interestingly, the combination of increased blood flow through the collateral circuit, caused by the a-v shunt, in the presence of VEGF administration markedly increased collateral-dependent blood flow to the calf muscles (cf., Fig. 3). While the size of this group was rather small (n=4), there was sufficient statistical power to demonstrate a meaningful effect of VEGF (p<0.01).

The combined treatments of VEGF and expected high shear induced an exceptionally high blood flow, potentially equivalent to that supplied to normal muscle in the absence of femoral artery occlusion. This is not evident from the blood flows in Fig. 3, since these flows were determined in different experiments and at different perfusion pressures. When the blood flows are corrected for perfusion pressures and expressed as vascular conductance a more valid comparison can be made. Calf muscle conductance of ~1 ml·min⁻¹·100g⁻¹·mmHg⁻¹, determined while running at submaximal 25 m/min compares favorably with a conductance approaching ~2 ml·min⁻¹·100g⁻¹·mmHg⁻¹ at higher treadmill speeds (40-60 m/min) (14, 30). Calf muscle conductance of the VEGF + shunt animals approached this high value that is close to maximum for normal animals in the absence of vascular occlusion Fig. 4. Thus, the combination of an angiogenic growth factor and putative enhanced shear recovered flow capacity of the collateral-dependent muscle to near normal.

DISCUSSION

Physical exercise exerts a powerful angiogenic stimulus for vascular growth. While this is most apparent by an enhanced capillarity in the active muscle of trained individuals (3, 31, 32), aerobic type exercise has been shown effective at expanding the collateral circuit surrounding an obstruction of the femoral artery in preclinical studies (7, 19). Significant but limited increases in collateral-dependent blood flow (~30-60%) have been observed with relatively short-term training programs of treadmill running of a few weeks (7, 26, 30, 33). The purpose of the present study was to evaluate whether a prolonged training program of extended daily running would induce a recovery of flow capacity to the collateral-dependent muscles of the lower limb. This extended training program induced a marked increase in collateral blood flow of ~100% above that observed in animals kept sedentary. However, the vascular capacity remained well below that observed in animals in the absence of occlusion of the femoral artery. We then attempted to determine the extent to which the collateral circuit could adapt to recover blood flow capacity back to normal. We employed a surgical procedure by which a high blood flow would be required through the collateral circuit 24 h/day. This was expected to increase shear stress in the collateral vessels, a factor known to impart massive vessel enlargement (16). In addition, we included delivery of a powerful angiogenic growth factor, VEGF, in an attempt to optimize collateral vessel growth. In these experiments vascular conductance of the collateral circuit reached near-normal values of ~2 ml·min⁻¹·100g⁻¹·mmHg⁻¹. Thus, vascular adaptations within the collateral circuit are capable of massive growth, when given a sufficient stimulus for development. While exercise in the form of treadmill running remains a powerful stimulus for increasing collateral blood flow to the ischemic distal muscle, the adaptation appears to be self-limiting in that flow capacity did not return to normal. Nonetheless, the induced increase in the flow capacity to the active muscles with training is significant and results in a marked increase in exercise capacity.

Daily physical training expands collateral dependent blood flow

As in the past (7, 30), we observed a significant increase in collateral-dependent blood flow to the calf muscles; however, in contrast with this previous work the increase was measurably greater owing to the greater volume of exercise training. Rats with bilateral femoral artery occlusion experienced exercise-induced muscle hyperemia for both a longer run duration per day...
normal animals running at the same speed (~75% of that observed in Non-occluded calf muscle of greater volume of treadmill running, calf muscle blood flow was for collateral vessel enlargement. However, despite this much speed, but for a much longer duration imparts a greater stimulus for collateral arteries (19). The up-regulated expression of eNOS in vessels has been shown to be mediated by NO production in the trained vessels is thought to be mediated by the elevated shear stress induced by exercise (40) or by reduced reactive oxygen species that inhibit NO production or bioavailability in the vascular system (41). This raises the potential that increased function of eNOS-NO in the vascular system, introduced by physical training, plays a role in both structural and functional adaptations of the collateral arteries. Indeed, collateral vessel remodeling, induced either by exercise training (33) or by exogenous angiogenic growth factors (9), is impaired with L-NAME (NOS inhibitor) administration. While NO likely plays a critical role in the vascular remodeling observed in this study, there also appears to be an interaction among NO and angiogenic growth factors that could be important. VEGF has been shown to increase both VEGFR-2 (42) and eNOS expression (43). We have previously reported that, in vivo, VEGF receptor antagonists impaired the outward remodeling of collateral arteries and the concomitant increased collateral blood flow induced by exercise training and VEGF infusion (44). Similarly, inhibition of VEGFR-2 kinase decreased flow-mediated NO-dependent arterial dilation (45, 46). In an in vitro study, Jin et al. reported that laminar flow (shear stress=12 dyne/cm²) across endothelial...
cells could activate vascular endothelial growth factor receptor 2 (VEGFR-2) in a ligand-independent manner and induce eNOS activation (45). Similarly, Chien and colleagues (47, 48) demonstrated that shear stress activation of integrins caused the ligand-independent autophosphorylation of VEGFR-2. Phosphorylation of VEGFR-2 is expected to activate the PI3K/Akt pathway leading to eNOS mediated NO production (49, 50, 51). Interestingly, NO has been shown to be a downstream regulator for VEGF action (52), and a competent eNOS-NO system is required for exogenous VEGF-induced expansion of collateral blood flow (9). Thus, greater VEGF-2 expression could putatively result in greater NO release via both ligand dependent and independent mechanisms. Furthermore, shear stress may also trigger expression of other angiogenic growth factors (FGF-2, PDGF-B) (53) that also have potency in collateral vascular remodeling (8-11, 54). However, the exact roles of FGF-2, PDGF-B and other possible cytokines in prompting and optimizing collateral vasculature remodeling are unclear at present. In addition, it is likely that when the caliber of the vessels enlarged with the A-V shunt+VEGF administration, the downstream pressure increased. This has been shown by other treatments to increase collateral blood flow (12, 13). As a result, the elevated pressure would greatly increase the absolute blood flow returning centrally through the shunt. This, in turn, would further increase down stream flow demands, thereby enhancing flow through the collateral circuit. The increase flow through the collateral circuit would be expected to increase shear stress, thereby sustaining a signal for continued collateral vessels enlargement. This positive feed-back process would continue until the shunt resistance becomes a significant outflow resistance leading to a tempering of collateral flow demand and a tempering of the stimulus for continued vessel enlargement. This could account for why the shunt flow fraction is rather large in the A-V shunt plus VEGF group (approaching ~40%), compared to the A-V shunt minus VEGF group where the magnitude of the absolute flows were very different (cf., Fig. 4).

One of the limitations in the present study is that we did not assess the adaptations of small and pre-capillary arterioles which normally contribute the most resistance in the vascular circuit. Since endurance training increases the density of arterioles in both white and red sections of gastrocnemius muscle of non-occluded rat (55), it may be presumed that our 15 wk training program could contribute to the increase in blood flow to the calf muscles. However, our previous work shows that, with femoral artery occlusion, the collateral circuit resistance is 75-85% of the total resistance in the vascular circuit of the distal limb muscles (12, 13) when the conductance of the actual muscles is near maximal (~2 nl/min.mmHg−1), compared to the V-A shunt minus VEGF group where the magnitude of the absolute flows were very different. Thus, in the presence of femoral artery occlusion, blood flow to the distal hind limb is essentially dependent on the resistance of the collateral circuit. However, it is clear that the conductance of the distal muscles can increase with training (57) and markedly so with alpha-adrenergic and neuroepitope Y receptor blockade (56, 57). Any such increase in distal muscle conductance would exert a modest influence on total blood flow, however, since the change is only operating on 15-25% of the circuit’s total resistance. Thus, we believe that the large changes in collateral dependent blood flow to the calf muscle reflects marked enlargement of the collateral vascular circuit. This makes even the likely arteriolar adaptation in the trained animals as relatively unimportant and mathematically incapable of producing the large flow increases observed in the present study.

In summary, we studied the functional recovery of collateral circulation in the hind limbs of a preclinical model of peripheral arterial insufficiency established with bilateral occlusion of femoral arteries. An intervention of physiological relevance, that of exercise training, significantly increased collateral blood flow, but did not return vascular capacity to normal or that observed with an experimental intervention that caused sustained collateral flow demand plus VEGF treatment. While exercise training has the potential for meaningfully improving collateral blood flow, and thereby mobility and exercise capacity, the stimulus for adaptation is not maximal. Nonetheless, physical activity represents a powerful means of imparting physiologically relevant and tissue specific vascular remodeling that can be potentially meaningful for patients with peripheral arterial insufficiency.

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