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TRAINING-INDUCED VASCULAR ADAPTATIONS TO ISCHEMIC MUSCLE

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Peripheral arterial insufficiency is a progressive degenerative disease associated with an increased morbidity and mortality. It decreases exercise tolerance and often presents with symptoms of intermittent claudication. Enhanced physical activity is one of the most effective means of improving the life of affected patients. While this occurs for a variety of reasons, vascular remodeling can be an important means for improved oxygen exchange and blood flow delivery. Relevant exercise-induced signals stimulate angiogenesis, within the active muscle (*e.g.* hypoxia), and arteriogenesis (enlargement of pre-existing vessels via increased shear stress) to increase oxygen exchange and blood flow capacity, respectively. Evidence from pre-clinical studies shows that the increase in collateral blood flow observed with exercise progresses over time of training, is accompanied by significant enlargement of isolated collateral vessels, and enhances the responses observed with angiogenic growth factors (*e.g.* VEGF, FGF-2). Thus, enhanced physical activity can be an effective means of enlarging the structure and function of the collateral circuit. Interestingly, disrupting normal NO production (via L-NAME) eliminates this increase in collateral blood flow induced by training, but does not disturb the increase in muscle capillarity within the active muscle. Similarly, inhibiting VEGF receptor kinase activity eliminates the increase in collateral-dependent blood flow, and lessens, but does not eliminate, angiogenesis within the calf muscle. These findings illustrate distinctions between the processes influencing angiogenesis and arteriogenesis. Further, sympathetic modulation of the collateral circuit does not eliminate the increase in collateral circuit conductance induced by exercise training. These findings indicate that structural enlargement of the collateral vessels is essential to realize the increase in collateral-dependent blood flow capacity caused by exercise training. This raises the potential that meaningful vascular remodeling

can occur in patients with intermittent claudication who actively participate in exercise training.

Key words: *collateral blood flow, eNOS-NO, FGF, angiogenesis, arteriogenesis, peripheral arterial insufficiency*

INTRODUCTION

Peripheral arterial disease is an escalating, degenerative condition that increases morbidity and mortality. Quality of life is decreased by progressive reductions in blood flow to the legs, often leading to pain on exertion, and limited mobility. Even in the absence of leg pain on exertion, patients with peripheral arterial disease suffer poor functional performance and poorer quality of life (1). Advancing peripheral arterial insufficiency can lead to rest pain, increased risk of ulcers, inadequate blood flow to the distal tissues all too often resulting in amputation due to gangrene, an increased risk of premature death due to cardiovascular complications, and an overall depressed outlook on life (1, 2). There has been useful, but limited, success in managing these patients with pharmacological and surgical means. Treatments that modify blood rheology and clotting are beneficial. Surgical reconstruction is often successful, but is not universally applicable among patients. Enhanced physical activity has proven to be a very effective treatment available in managing these patients (2), even conjunction with surgical intervention (3). Participation in a daily exercise program leads to an improved walking tolerance, including a delayed onset of pain, longer walking distance, improved walking economy, a potentially greater activity effort (4), an enhanced outlook on life, reductions in disease progression, and a reduced mortality rate (5, 6). These benefits clearly provide a basis for recommending greater physical activity among patients with peripheral arterial disease (7).

While the value of enhance physical activity among patients with peripheral arterial insufficiency is almost universally observed, the reasons for the benefits are not always clear. An improved walking economy, an enhanced metabolic response, a greater accommodation to pain, and an enhanced blood flow to collateral-dependent muscle have been implicated. While large improvements in collateral blood flow are not typically observed in patients (3, 8 - 10), there is extensive evidence in preclinical models of peripheral arterial insufficiency indicating that meaningful vascular adaptations can be induced by exercise training (11-14). These adaptations can be separated into two types: those that modify microvascular (*i.e.* capillary) function within the muscle, and those that modify conduit vessel function, to increase blood flow circumventing the obstruction.

MICROVASCULAR ADAPTATIONS

Microvascular adaptations are expected to modify diffusive function within the active muscle, as nutrient exchange is dependent upon how well active muscle fibers are bathed by the surrounding capillaries. The greater the capillary abundance, the greater the expected capacity for diffusive exchange. Early work evaluating adaptations within muscles of claudicant patients demonstrated an enhanced capillary density and an increased mitochondrial enzyme activity (15-19) in the presence of the disease that is further enhanced with training (20, 21). Hypoxia within the collateral-dependent, ischemic muscle is the likely stimulus for the development of new capillaries, as a low pO_2 is a powerful stimulus for angiogenesis *via* the upregulation of vascular endothelial growth factor (VEGF) activated by the hypoxia response element (22, 23). This signaling cascade operates through the hypoxia-inducible factor HIF-1. Tissue pO_2 in the distal limb of claudicating patients can be rather low (24) and well within the range necessary to upregulate VEGF (25). There is a point, however, where disease progression is so severe that pathological changes within the distal tissue preempt these adaptations. Training enhances and/or retains muscle capillarity, even if surgical intervention is successful to lessen or eliminate tissue hypoxia (21). In normal athletes that adapt to training there are characteristic increases in capillarity of the active muscle. Findings in humans (26) and animals (27-29) demonstrate that an enhanced capillarity is coincident with a greater ability to extract oxygen. In effect, the muscle exhibits a greater diffusion capacity, even when blood flow is not increased (28, 30). An increase in maximal oxygen consumption of as much as 30% translates into a marked improvement in muscle performance (30). This adaptive increase in capillarity could have contributed to the increase in maximal oxygen extraction observed in claudicating patients who were exercise trained (31, 32), although a redistribution of the limited blood flow within the limb, to better bathe the active muscle fibers (33), could have also been important. This benefit of being more physically active could be central, because it can occur independent of any increase in total blood flow to the collateral dependent muscle. It should be recognized, however, that the magnitude of this benefit is limited, since it 'only' optimizes oxygen extraction from the limited flow that is available. Nonetheless, this improvement in oxygen extraction could be the difference in contributing to improvement in the activities of daily life. This could measurably improve the quality of life of affected patients.

CONDUIT VESSEL ADAPTATIONS

Enlargement of pre-existing arterioles to form a functioning collateral circuit has been termed arteriogenesis (34-37). The primary stimulus expected to bring about enlargement of these vessels is a result of increased blood flow associated

with redistribution of flow caused by the reduction in downstream luminal pressure. The attendant increase in shear stress imparts a powerful stimulus for vessel enlargement (38), by a process that involves vessel wall remodeling and NO signaling (39-41). The critical role of shear stress in vascular enlargement makes this a self-limiting process, since diameter directly affects shear stress. The initial increase in shear stress imparts a signal to increase vessel diameter, which upon enlargement will reduce shear stress for the given flow. As the vessel continues to increase in diameter, shear stress will decrease and return to 'normal' values at which point there is no longer any stimulus for further vessel enlargement (40). On the other hand, hypoxia is an unlikely signal to stimulate arteriogenesis, since the collateral vessels that enlarge are typically well upstream from the collateral-dependent ischemic tissue (14, 42). These vessels are bathed by high pO_2 arterial blood characteristic of that exiting the lungs. Thus, it is difficult to imagine how the endothelium, which is critical for vascular remodeling (43), is subjected to a low pO_2 . This illustrates one of the differences in signaling stimuli that distinguishes arteriogenesis from angiogenesis. However, similar to the events stimulating angiogenesis, there are clear implications that growth factors, that exhibit angiogenic activity (*e.g.* VEGF, FGF-2, PDGF, *etc.*), contribute to vessel enlargement. In addition, there are a host of other cytokines that are implicated in arteriogenesis, since an inflammatory response appears to be a vital part of the tissue response to vascular occlusion (44).

GROWTH FACTOR STIMULATED ARTERIOGENESIS

Although arteriogenesis is a complicated process that requires an integration of distinct aspects (*e.g.* cell proliferation, migration, extracellular remodeling), simple administration of the well-established angiogenic cytokines, VEGF and FGF-2, is sufficient to induce significant vascular remodeling following vascular occlusion (22, 45, 46). VEGF and FGF-2 increase collateral-dependent blood flow within approximately two weeks of administration (14, 39), likely due to an improved responsiveness of the arteriogenic signaling pathways to the prevailing stimuli that promote vascular enlargement (*e.g.* increased shear stress). Interestingly, the remodeling of the vessels due to VEGF and FGF-2 is specific to those vessels that form the collateral circuit within the limb that experienced vascular occlusion (47, 48). Vessels of the contralateral limb, that did not receive vascular occlusion, exhibited no response to these cytokines, even though they were subjected to the same circulating concentrations of VEGF and FGF-2. Thus, there is clearly some distinct feature caused by vascular occlusion that renders those affected vessels responsive to angiogenic growth factors. Fortunately, this phenomenon represents a potential advantage in patient management, by establishing specificity should therapeutic growth factor treatment become available. Further, it is clear that VEGF and FGF-2 treatment combined with

enhanced shear stress, caused by daily exercise, leads to a greater increase in collateral-dependent blood flow than with growth factor delivery alone (14, 39). This amplification is likely due to the vessels being more responsive to increases in shear stress due to an enhanced sensitivity of the vascular remodeling process, induced by these growth factors,

EXERCISE TRAINING INDUCED ARTERIOGENESIS

Even in the absence of exogenous administration of angiogenic growth factors, participation in a daily exercise program significantly increases collateral-dependent blood flow (11-13). As illustrated in *Fig. 1*, there is an increase in collateral blood flow, evident from the onset of training that reaches an asymptote after approximately three weeks (11). The magnitude of training adaptations is typically related to the intensity and duration of training (49). Therefore, it is possible that the increase in collateral blood flow could become even greater, if the training program had progressed longer and become progressively more intense, as the collateral blood flow and exercise capacity increased over time. It is important to recognize that, contrary to measuring an index of limb blood flow in an anesthetized rested animal (50), blood flows measured in this training study represent maximal collateral-dependent blood flows observed physiologically. In order to know the extent of vascular remodeling induced by a treatment, it is essential to measure the maximal conductance of the collateral circuit. In order to appropriately measure the conductance capacity of the collateral circuit, it is necessary to minimize the

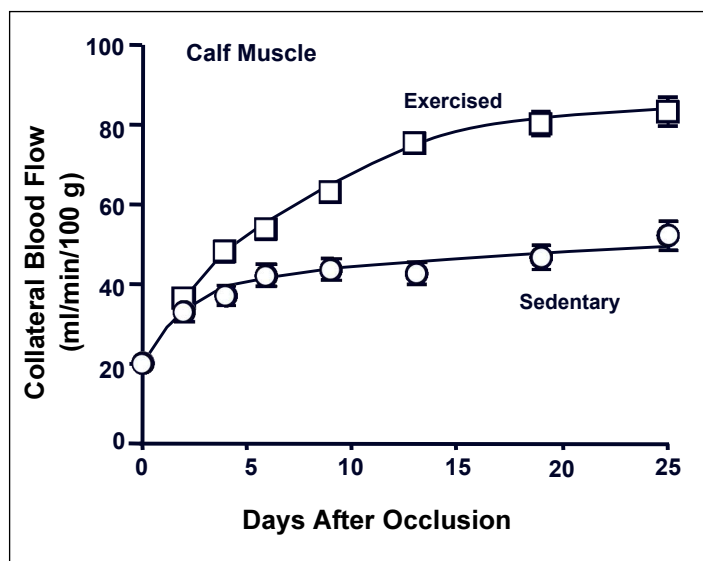


Fig. 1. Time-course of the increased capacity of collateral blood induced by exercise training. Adapted from Prior *et al.* (11) with permission.

resistance of the distal vascular circuit that is in series with the collateral circuit resistance. When this goal is achieved, blood flow to the distal limb (*e.g.* calf muscle) is essentially determined by the upstream resistance of the collateral circuit. Since muscle contractions represent the most powerful stimulus for vasodilatation (51, 57), we employed treadmill exercise to minimize the vascular resistance distal to the collateral circuit. Other work has demonstrated that, during treadmill exercise, the upstream resistance of the collateral circuit constitutes 75-85% of the resistance of the total circuit (52, 53) and effectively determines blood flow to the calf muscle. The collateral blood flows to the calf muscles, illustrated in *Fig. 1*, are initially 2-3 times the resting blood flow needs of the muscle (54), and progress over time to 5-6 times and ~10 times resting, for the sedentary and trained animals, respectively. This flow capacity still remains well below the ~30-fold increase in muscle blood flow that can occur in the absence of vascular occlusion (51, 54). The changes in actual blood flows to individual muscle fiber sections within the calf muscles will correspond to the differences in vascular capacities inherent to the muscle fiber type (11).

It is apparent from *Fig. 1* that the relatively low collateral-dependent blood flow, observed initially upon occlusion of the femoral artery, rapidly changes over time even in the sedentary group. This increase in blood flow over the first week post-occlusion is probably related to modest remodeling, inherent to the vasculature, which occurs upon occlusion of the primary supply vessel. A potential contributor to this response is the change in collateral vessel compliance that occurs within the first three days, post-occlusion. The increase in elasticity of remodeled vessels is likely due to the decreased intraluminal pressure, approximately 35% of normal, experienced by these vessels *in vivo* (52, 53). As illustrated in *Fig. 2*, there is a

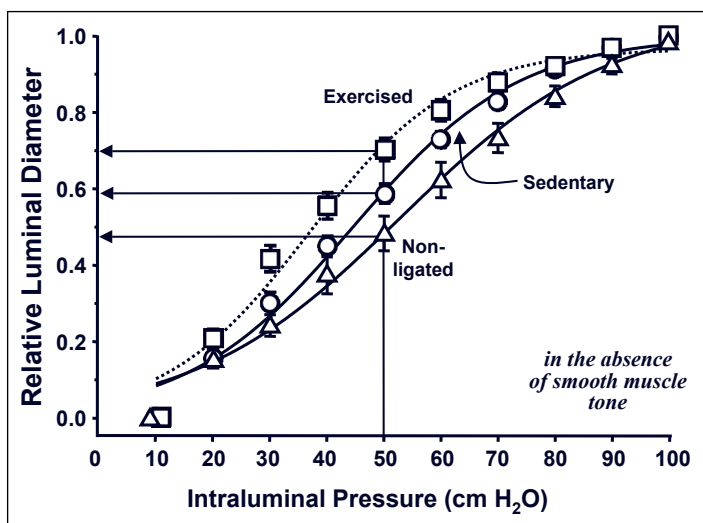


Fig. 2. Time-course of the change in collateral compliance induced by exercise training. Adapted from Prior *et al.* (11) with permission.

larger passive diameter at the lower pressures (e.g. 50 cm H₂O) that could alter radial wall tension to result in a larger luminal diameter *in vivo*, even with no change in maximal vessel diameter. The larger diameter would help reduce resistance of the collateral circuit and contribute to the rapid-onset of improvement in collateral dependent blood flow shown for both the sedentary and trained animals (Fig. 1). In addition, maximal diameter of the isolated collateral vessel increases, progressively over time in the trained animals and after approximately a two-week delay in the sedentary animals. These larger maximal diameters would further enhance the potential for improved collateral-dependent blood flow. However, it becomes clear in comparing the time-course of response in blood flows (Fig. 1), to that of vessel diameter changes (Fig. 3), that there is no direct cause-effect correspondence. Indeed, the increase in blood flow progresses ahead of the time-course in vessel enlargement. This apparent inconsistency is especially significant recognizing that resistance, and therefore blood flow capacity, changes as a fourth power of the radius. The discordance could be explained if the particular collateral vessel that was sampled does not represent the whole of the collateral circuit resistance change. Indeed, the collateral circuit is comprised of an expansive network of small arterioles that are in the process of enlarging (11, 30). A further complication could be in the vascular control of the collateral circuit. The collateral circuit is subjected to sympathetic vasoconstriction that could render the effective resistance well above that possible by the caliber of the enlarged plumbing (52), resulting in no simple relationship between vessel size determined *in vitro* and functional vessel size *in vivo*. Subsequent work, however, has demonstrated that trained animals exhibit a significantly greater collateral-dependent blood flow that sedentary controls, even in the presence of sympathetic inhibition (55). Thus, the

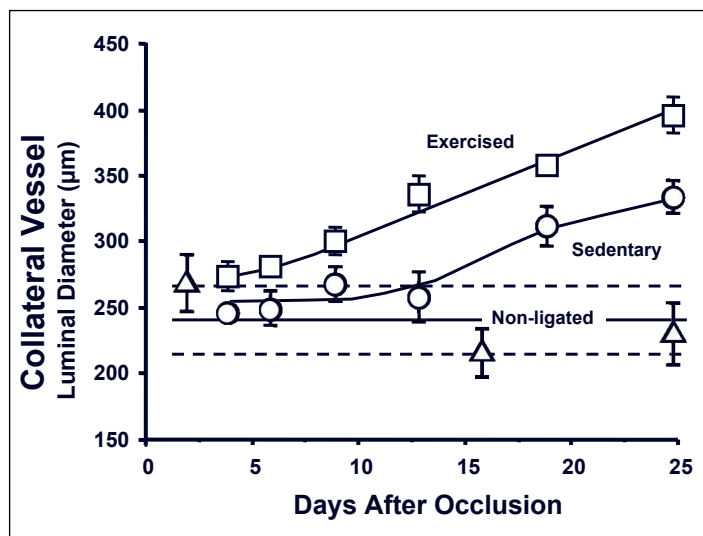


Fig. 3. Time-course of the increase in collateral vessel diameter induced by exercise training. The solid horizontal line, bordered by broken lines give the mean and 95% confidence interval for vessels from normal non-occluded animals. Adapted from Prior *et al.* (11) with permission.

structural increase in vessel caliber appears to be an essential element supporting the increase in collateral-dependent blood flow induced by training.

SIGNALS STIMULATING ARTERIOGENESIS

While the stimuli of an increased shear stress, within vessels experiencing a reduced luminal pressure, may be essential factors leading to vessel enlargement, there is neither a comprehensive understanding of all of the signals and processes involved, nor how they are orchestrated to yield the final enlarged collateral circuit. A number of factors are upregulated within collateral vessels upon occlusion of a primary supply artery. These factors could give insights into some of the processes involved. For example, just as in ischemic muscle that is active during daily training (56), eNOS is up regulated in collateral vessels (11). This increase in endothelial nitric oxide synthase (eNOS) mRNA expression is typical of vessels used to support exercise, leads to an increase in eNOS protein, and is thought to be an important adaptation in local vascular control within active muscle (57). However, in the case of vascular remodeling, it is likely more critical, since NO signaling is essential for vascular enlargement (39, 40, 58-60). For example, Tronc and coworkers (40) reported that inhibiting normal nitric oxide (NO) production eliminated carotid artery enlargement following surgical introduction of an arterial-venous anastomosis to enhance blood flow. Normal NO production is also important in collateral vessel development, as chronic administration of L-NAME to the animals completely preempted the increase in collateral-dependent blood flow capacity typically induced by VEGF and FGF-2

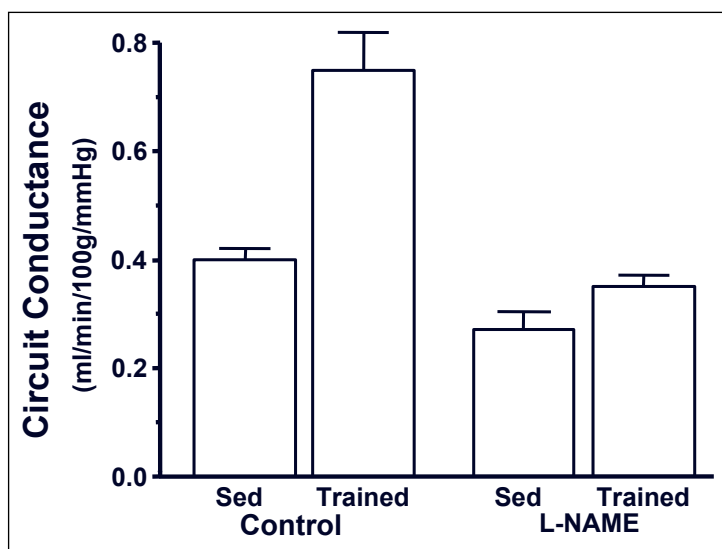


Fig. 4. Inhibition of the training-induced increase in collateral blood flow capacity caused by disruption of normal nitric oxide production with L-NAME. Adapted from Lloyd *et al.* (61) with permission.

(39). Collateral circuit conductance was not greater than that observed in control animals; in fact, it was slightly less, likely owing to the acute effects of obstructing NO signaling in local vascular dilatation (39). Further, the typical enlargement of collateral vessel diameter induced by VEGF administration ($357 \pm 5.6 \mu\text{m}$) is preempted by chronic eNOS inhibition, as the collateral vessel of the occluded hind limb of animals given VEGF and L-Name ($300 \pm 13.1 \mu\text{m}$) was not different from the same vessel in the contralateral non-occluded hind limb ($277 \pm 13.3 \mu\text{m}$; $n = 8$; $P = \text{NS}$; Li Z, HT Yang, and RL Terjung, unpublished observations). Similarly, inhibition of normal NO production eliminated the increase in collateral blood flow typically induced by exercise training (*Fig. 4*, (61)). A recent study, using eNOS null mice that received surgical ligation of the femoral artery of one limb, appears to contradict these findings (62). Magnetic resonance imaging and Hb-oxygen saturation evidence demonstrated a good recovery over time in the ischemic limb, fairly similar to that observed in wild type animals. Unfortunately, these observations are based upon the relative response of the ischemic limb, compared to the response of the non-occluded contralateral limb of the quiescent anesthetized animal (62). As such, comparisons are made to the normal limb during exceptionally low flow conditions apparent at 'rest'. Since the capacity for blood flow to normal limb muscles of rodents is more than 30-fold 'rest' (51, 54), it is apparent that recovery of flow to such a low-flow standard cannot provide insights into the changes in the capacity of the vascular circuit. Therefore, it is probable that significant deficits in flow capacity of the collateral circuit of the eNOS null mice remained. Thus, recognition that vessel enlargement induced by increased shear stress is dependent upon NO signaling remains. On the other hand, angiogenesis within the active ischemic muscle of the trained animals was just as extensive even with NO inhibition (61), similar to that observed in ischemic muscle of eNOS^{-/-} mice (62). Thus, distinctly different stimuli can be operating within conduit vessels, important for arteriogenesis, compared to microvessels, which are susceptible to tissue hypoxia.

Upregulation of VEGF mRNA and that of its receptor VEGFR2, occurs initially after the onset of vascular occlusion, but abates over a 1-2 wk time period (11). Interestingly, VEGFR1 mRNA, which is characteristically upregulated by hypoxia and whose soluble form has been proposed as a marker for critical limb ischemia (63), is upregulated by training over the initial 2-3 wk period, but only on day 2 post-occlusion in the absence of exercise. This upregulation of the VEGF signaling system could be important to the enhancement of arteriogenesis induced by exercise training (11, 13, 64). VEGF receptor signaling is further implicated in the remodeling of the collateral circuit, since selective VEGF receptor inhibition eliminated the increase in collateral blood flow typically induced by training (64). Interestingly, angiogenesis in active muscle was only partially inhibited. As with the different responses with NO inhibition, this discrepancy again illustrates that signals produced by exercise (*e.g.* shear stress, radial wall tension, longitudinal tension, hypoxia) can utilize distinct pathways to effect angiogenesis and

arteriogenesis. Further, it is apparent that a multitude of other signaling events, yet to be determined or understood, can occur in collateral vessels in response to vascular occlusion and the imposition of daily exercise. For example, placental growth factor, an angiogenic growth factor in the VEGF family, is markedly upregulated by occlusion and exercise (11). In addition, there is extensive evidence for the infiltration of monocytes, associated with an inflammatory process, into the perivascular region of the collateral vessels following vascular occlusion. This raises the potential for extensive local cytokine production that could help orchestrate vascular remodeling of the collateral circuit (35, 44, 65, 66).

FUNCTIONAL SIGNIFICANCE OF TRAINING ADAPTATIONS IN PATIENTS WITH INTERMITTENT CLAUDICATION

While there is compelling evidence from pre-clinical models of peripheral arterial insufficiency that collateral blood flow can be increased by exercise training, this adaptation does not appear to be wide-spread among patients with intermittent claudication who participate in an exercise program (2). Several factors may contribute to this apparent contradiction. First, peripheral vascular disease is a complex degenerative disease that often presents with multiple levels of obstruction. This presents exceptional demands for development of an effective collateral circuit, since it must accommodate successive obstructions with increasingly poorer perfusion pressure at each level. Rather, the most easily realized collateral circuit would be to circumvent a single proximal lesion. Second, it is possible that greater changes in collateral blood flow have been induced in patients by exercise, but not appropriately measured. While it is exceedingly difficult to assess collateral blood flow in patients, inadequacy in measurement techniques would not preempt the appearance of exceptionally large improvements in performance. While significant improvements in exercise tolerance are routinely observed, the benefits do not seem to match the increases in blood flow that occur in those patients who undergo successful surgical intervention (3). Third, it may be that the exercise stimulus was insufficient to bring about substantial vascular remodeling. This could occur due to rather brief exercise bouts each day, infrequent daily activity, and/or an abbreviated total time of training. Fourth, it may be that the large collateral vessels, which are necessary to be effective in patients, cannot be formed. Recall that collateral vessels enlarge from small arterioles, a process that appears to be relative easy in small rodents where modest enlargement can be easily achieved. On the other hand, significant enlargement of conduit vessels contributing to collateral blood flow can occur in a large animal (38, 67). Therefore, given a sufficient stimulus for collateral vessel development, it is likely that exceptionally large collateral conduit can be developed. However, this may be effectively preempted if the vascular endothelium is not 'healthy' in its NO-dependent signaling. Unfortunately, endothelial dysfunction is common among patients with

chronic cardiovascular disease (68, 69). This could dull and possibly even prevent meaningful vascular remodeling induced by the increased shear stress caused by physical activity. Fortunately, one of the fundamental vascular adaptations induced by exercise training is an improvement in endothelial function and, specifically, an increase in NO responsiveness (70, 71). Thus, participation in an exercise program appears to benefit patients in multiple ways. Further research work is expected to help clarify the means by which exercise programs can be so beneficial for patients with peripheral vascular disease.

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