Peripheral Adaptations in Trained Aged Rats With Femoral Artery Stenosis

H.T. Yang, Robert W. Ogilvie, Ronald L. Terjung

Abstract The development and functional significance of exercise-induced peripheral adaptations were evaluated in aged animals with peripheral arterial insufficiency. Fisher 344 male rats (21 months old) were subjected to bilateral stenosis of the femoral arteries sufficient to limit active hyperemia but not to impair resting blood flow. Beginning the third day after stenosis, animals were (1) exercised by walking (n=12) on a treadmill at 20 m/min at 15% inclination, twice a day, 5 days per week, or (2) limited to cage activity (n=10). Exercise tolerance improved from ~5 to ~35 minutes (P<.001) over the 8 weeks of the training program but increased only marginally to ~8 minutes for the sedentary group. An isolated hind limb preparation perfused at equivalent blood flows (=1 ml · min⁻¹ · g⁻¹ with an arterial blood oxygen content of ~20 vol%) was used to assess the functional and metabolic impact of muscle-specific adaptations during sequential contraction periods at 4, 8, 15, 30, 45, 60, 75, and 90 tetani per minute. An initially similar force development of ~10 N/g was better maintained (P<.001) by the trained group. The peak oxygen consumption attained by the trained group of 5.68±0.34 μmol · min⁻¹ · g⁻¹ was greater (P<.01) than that of the sedentary group (4.34±0.29 μmol · min⁻¹ · g⁻¹). This was due to a greater oxygen extraction, since oxygen delivery was the same (~10 μmol · min⁻¹ · g⁻¹) to muscles of both groups. The improved muscle performance and greater oxygen extraction of trained muscle is attributed to an enriched capillary network (5.70±0.10 versus 4.68±0.11 capillary contacts per fiber, P<.001) and a 25% to 100% greater mitochondrial capacity (citrate synthase activity, P<.001) of the fibers. Thus, aged animals with peripheral arterial insufficiency are capable of developing training-induced adaptations that serve to improve muscle performance, even if blood flow is not improved. Similar activity-related adaptations in elderly patients with intermittent claudication could contribute to an improved exercise tolerance. (Circ Res. 1994;74:235-243.)

Key Words • oxygen consumption • blood flow • capillarity • fiber type • intermittent claudication

Exercise-induced peripheral adaptations develop in healthy young individuals and probably contribute to their improved performance and metabolic response during exercise. These muscle-specific adaptations involve a greater mitochondrial capacity for aerobic ATP provision and an enriched capillary network that should enhance blood-tissue exchange properties. Extensive evidence indicates that the exercise-induced peripheral adaptation of an increased mitochondrial content develops in healthy elderly individuals and animals. In contrast to evidence with younger individuals, there is little known about peripheral vascular and microvascular adaptations to training in the elderly. An increase in muscle capillarity has been observed with exercise training in the slow-twitch soleus muscle of healthy aged rats but not in the diaphragm or, more importantly, in fast-twitch muscle, which constitutes most of the rat’s musculature. Thus, the general applicability of the capillarity change in the soleus muscle has not been established. Unlike their findings in young adults, Denis et al found no evidence for angiogenesis in older humans after training, even though the cycle exercise program was sufficient to produce an increase in maximal oxygen consumption. They concluded that training adaptations in muscle are different between young and older individuals. Thus, it is unclear whether microvascular adaptations to exercise occur in the elderly.

The development of exercise-induced peripheral adaptations may be most significant in elderly patients with peripheral arterial insufficiency, where limb blood flow is limited by vascular obstruction. Numerous studies have demonstrated that enhanced physical activity improves muscle function (exercise tolerance) in patients with intermittent claudication. An increase in collateral-dependent blood flow has contributed to the improved exercise tolerance of some patients with intermittent claudication and of animals with peripheral arterial insufficiency. However, most clinical studies demonstrate an improvement in exercise tolerance without any accompanying increase in limb blood flow. The basis for this improvement is thought to be peripheral, within the limb or muscle, and not to involve changes in collateral blood flow to the limb. One possible explanation is a redistribution of the limited flow within the limb, to better perfuse the contracting fibers specifically involved in the activity. On the other hand, even if blood flow is not improved, training can enhance muscle function and lead to a greater peak oxygen consumption of the active muscles of young adult animals. This apparent increase in the oxygen exchange capacity of the muscle is likely due to the change in mitochondrial content and distribution within the fiber and microvascular adaptations that enhance the capillary network surrounding the fibers. Similar muscle adaptations in elderly individuals with intermittent claudication could contribute to their improved exercise tolerance. Although an increase in mitochon-

Received March 3, 1993; accepted September 28, 1993.

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bral content may be expected in elderly individuals with peripheral arterial insufficiency who are physically active, the development of microvascular adaptations has not been established. Thus, it is our hypothesis that exercise-induced microvascular changes develop within the muscles of aged animals with peripheral arterial insufficiency and serve to improve muscle performance, even when blood flow (oxygen delivery) is not improved. We evaluated this hypothesis using an animal model of peripheral arterial insufficiency established through surgical bilateral stenosis of the femoral arteries that was sufficient to limit active hyperemia without impairing resting blood flow. This is similar to the clinical situation of intermittent claudication caused by a discrete proximal obstruction without the more advanced complications associated with ischemia at rest, ulceration, and/or gangrene. Our results indicate that biochemical and microvascular adaptations improve muscle function, even when blood flow is maintained equivalent between muscles of trained and sedentary animals.

Materials and Methods

Animal Care

Male 20-month-old Fisher 344 rats, weighing 438±3.9 g, were obtained from Charles Rivers (National Institute on Aging colony). Rats were housed in an animal quarter at 22°C with a 12-hour light-dark cycle. Rats were fed with Purina chow and tap water ad libitum. On arrival, all rats were exercised on a motor-driven treadmill at a speed of 20 m/min and at 15% grade for 5 minutes, twice a day for 10 days, to familiarize them with treadmill walking. Animals were randomly assigned to a control or experimental group. The care and treatment of the animals in the present study were approved by the State University of New York Health Science Center at Syracuse institutional committee for the humane use of animals.

Surgical Procedures

The animal model of peripheral arterial insufficiency is established by surgical bilateral stenosis of the femoral arteries at a proximal site just outside the inguinal ligament. Although this abrupt introduction of stenosis is not typical of the progressive vascular obstruction that is common in patients, it does provide a constant and reproducible treatment useful in experimental evaluation of adaptive responses to peripheral insufficiency. We have shown that this procedure allows normal resting muscle flow, while limiting blood flow to the distal limb musculature during exercise to ≈25% to 50% of normal, depending on the muscle fiber type section. Verification that the stenosis remained its original caliber was obtained at autopsy.

The procedures for femoral artery stenosis have been described in detail previously. Briefly, under ketamine hydrochloride/acepromazine maleate (100 mg/0.5 mg per kilogram body weight) anesthesia, the femoral arteries were isolated just distal to the inguinal ligament. A ligature (0.1 surgical silk) was placed tightly around the femoral artery and a stainless-steel wire (0.014-in diameter). The wire was then carefully removed, and arterial driving pressure restored the patency of the femoral artery. Topical antibiotic powder (Neurod, Upjohn) was placed on the wound before closure with skin clips.

Physical Training

Beginning 72 hours after surgery, animals in the trained group (n=12) were exercised by walking twice a day (at least 4 hours apart between exercise sessions), 5 days per week, on a treadmill of 15% grade set at 20 m/min. Initially, the rats walked for 5 to 10 minutes; walking duration progressively increased for each session as exercise tolerance improved. Rats were carefully monitored and taken off the treadmill with signs of fatigue, characterized by a change in gait followed by exaggerated hops. Daily run time gradually increased so that, at the end of 8 weeks, rats walked 35 to 40 minutes per session or ≈75 minutes per day. Sedentary animals (n=10) were limited to cage activity, except for evaluation of exercise tolerance at 6 and 8 weeks of training.

Hind Limb Preparation

After 8 weeks of exercise training, sedentary and trained rats were randomly selected, anesthetized with sodium pentobarbital (60 mg/kg), and prepared for hind limb perfusion, as previously described in detail. The Achilles tendon of the left gastrocnemius-plantaris-soleus muscle group was prepared for connection to a lever system (series 300 B, Cambridge Technology, Inc, Watertown, Mass) for measurement of isometric force development. The sciatic nerve was isolated, tied, and cut for placement across a platinum bipolar electrode for stimulation. The femoral artery was carefully dissected, distal to the site of stenosis, to permit insertion of the arterial catheter and perfusion of the limb without the marked pressure drop across the stenosis site.

Perfusion Procedure

When surgery was complete, the animal was transferred to a temperature-controlled (37°C) perfusion apparatus that has been previously described. After an arterial injection of heparin (2000 U), the left femoral artery was cannulated with a 20-gauge Teflon catheter (IV catheter, Becton Dickinson, Rutherford, N.J). Flow was initiated within 20 seconds, the animal was killed with 0.5 mL sodium pentobarbital injected into the carotid artery, and the venous catheter (18-gauge Teflon IV catheter, Becton Dickinson) was inserted into the femoral vein, via the common iliac vein, to establish a closed vascular circuit. After an initial washout period, flow of ≈250-mL perfusion medium was recirculated. Flow rate was measured by timed collections of the venous effluent. A pressure transducer was located at the height of the hind limb for determination of perfusion pressure at the head of the inflow catheter. Perfusion pressure in the femoral artery was obtained by subtracting the pressure drop across the inflow catheter that was determined previously for the corresponding flow and hematocrit.

Perfusion Medium

The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer containing washed bovine erythrocytes, 4 g/100 mL bovine serum albumin, 100 μU/mL bovine insulin, amino acids typical for rat blood, and 5 mmol/L glucose (maintained constant with periodic additions of glucose), with a 39% to 41% hematocrit (see Table 1).

Muscle Stimulation

Tetanic contractions of only the distal hind limb muscles were elicited by supramaximal square-wave pulses (0.1-millisecond duration, ≈8 V) delivered in 100-millisecond trains at 100 Hz (model S48 stimulator, Grass Instruments, Quincy, Mass), using successive contraction frequencies of 4, 8, 15, 30, 45, 60, 75, and 90 tetani per minute (TPM), at 10-minute stimulation for each train frequency. This train-stimulation protocol produced maximal isometric tetanic tension over a broad range of energy demands. Steady-state measurements of tension development and oxygen consumption were generally evident between the 5th and 10th minute of each train frequency, as found previously. Tension output of the gastrocnemius-plantaris-soleus muscle group was continuously recorded using a Gould five-channel polygraph (Gould Inc, Cleveland, Ohio). Average tension for each contraction frequency was calculated from the tension measured at the 5th and 10th minute of contractions.
Oxygen Consumption

Oxygen consumption was calculated as a product of the flow rate and the arteriovenous oxygen content difference. The oxygen content was determined by a LEXOCON (Lexington Instruments, Waltham, Mass). The resting oxygen consumption was determined from the mean of three observations over a 30-minute rest period. During contractions, oxygen consumption was determined from duplicates obtained at the 5th and 10th minute of each contraction frequency. Peak oxygen consumption was determined as the highest oxygen consumption value during the entire contraction sequence.

Oxygen Cost of Tetanic Contractions

The energy cost of brief isometric contractions is directly proportional to the tension developed by the muscle. Therefore, the oxygen cost per contraction at initial force development was obtained from the slope (micromoles per gram per contraction at initial tension) of the relationship between oxygen consumption, corrected for loss in tension development with fatigue, and contraction frequency. The oxygen cost per contraction can be taken as the entire energy cost per contraction, since we have demonstrated previously that the fraction of energy derived from glycolysis is small (<6%) during this contraction protocol.

Blood Flow Distribution

The distribution of blood flow in the hind limb was determined with radiolabeled 48Sr microspheres after the 10 minutes of stimulation at 90 TPM, as described previously. The fraction of radioactivity found in a tissue, relative to the total activity infused, is determined by its relative blood flow. Absolute blood flow was calculated as follows: Tissue blood flow (mL/min) = [(CPM tissue/CPM injected) x perfusion flow rate, where CPM = counts per minute in tissue and CPM injected is total counts per minute injected. Muscle blood flow is expressed per gram of tissue weight.

Biochemical and Histochemical Analyses

Muscle sections from the contralateral superficial white (predominantly type IIb) and deep red (predominantly type Ila) quadriceps, superficial white medial (predominantly type Iib), and deep lateral red gastrocnemius (predominantly type Ila) and soleus (predominantly type I) muscles were dissected immediately after the animal was killed. All muscle samples were frozen and stored at ~80°C until analyzed for citrate synthase activity, as described by Sercr. The entire contralateral plantaris muscle (mixed fiber type) was affixed to a piece of index card at approximately resting length and frozen in isopentane cooled in liquid nitrogen. Capillarity was determined from frozen sections obtained at the midpoint of the plantaris first by reaction for alkaline phosphatase according to Seligman et al., then by the periodic acid Schiff reaction for glycogen and glycoprotein after removal of glycogen with diastase, and subsequently by metanil yellow counterstain. This sequence of reactions and stains renders the capillaries blue-black, the fibers yellow, and the external lamina just outside the sarcolemma magenta. Muscle sections from control and trained animals were paired on the same slide. Myocyte and capillary number, fiber area, and number of capillaries surrounding fibers (capillary-to-fiber contacts) were determined from 20 nonoverlapping fields (0.06 mm2 each) from ×400 projections of each muscle. The capillaries around fibers were tabulated for at least 100 fibers for each section.

Fiber type (type I, type Ila, and type IIB) distributions were determined from myofibrillar ATP-stained midbelly sections of the plantaris muscle. Sections were stained with toluidine blue after preincubation at pH 4.5 for 5 to 7 minutes at room temperature and subsequently incubated with ATP at pH 9.4 for 30 minutes at room temperature. Muscle fiber oxidative enzyme distribution was determined with NADH tetrazolium reductase. Fiber number and area were obtained by point counting.

Materials

Radioactive 48Sr-labeled microspheres (14.2±0.92 μm) with a specific activity of 9.6 mCi/g were obtained from 3M, St Paul, Minn, in a suspension of 10% dextran containing 0.05% Tween 80 surfactant. Bovine serum albumin (fraction V) and the reagents used for biochemical analysis were obtained from Sigma Chemical Co, St Louis, Mo. Fresh bovine erythrocytes were prepared by extensive washing (>20 vol) of acid-citrate-dextrose titrated blood collected at a local meat packer.

Results

Training Response

Exercise tolerance improved markedly with daily treadmill walking. After the first week of exercise, walking duration increased from =6 to 35 to 40 minutes per session over the 2-month training program (Fig 1). Actual daily exercise time increased to ≈75 minutes a day, since there were two exercise sessions. Exercise tolerance of the sedentary rats limited to cage activity increased marginally and remained at ≈10 minutes per session 6 and 8 weeks after femoral stenosis. Typical of endurance-type exercise programs, the trained male rats weighed less and exhibited a slightly smaller muscle mass, as compared with the sedentary animals (see Table 1). Muscle-specific adaptations also resulted from the treadmill walking program. The activity of citrate synthase, a mitochondrial marker enzyme, increased ≈100% in the trained superficial white quadriceps, ≈50% in the deep red quadriceps, ≈100% in the superficial white gastrocnemius, and ≈25% in the deep red gastrocnemius, when compared with the sedentary rats (see Table 2).

Perfusion Conditions and Oxygen Delivery

Muscle blood flows were high (≈1 mL·min⁻¹·g⁻¹) and similar between the sedentary and trained animals (Table
TABLE 1. Body and Muscle Weight, Perfusion Pressure, Blood Flow and O2 Delivery to Contracting Muscle, and Force Development

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight, g</th>
<th>Muscle Weight, g</th>
<th>Hind Limb Perfusion Pressure, mm Hg</th>
<th>Blood Flow, mL/min</th>
<th>O2 Delivery, μmol/min·g-1</th>
<th>Initial Force, N/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary (n=10)</td>
<td>433±12</td>
<td>4.17±0.18</td>
<td>8.4±0.3</td>
<td>75±5.9</td>
<td>91±6.5</td>
<td>1.08±0.15</td>
</tr>
<tr>
<td>Trained (n=12)</td>
<td>384±5*</td>
<td>3.68±0.09*</td>
<td>6.5±0.1*</td>
<td>64±3.8</td>
<td>79±8.7</td>
<td>1.08±0.08</td>
</tr>
</tbody>
</table>

Hct indicates hematocrit. Values are mean±SEM.
P<.05 vs corresponding sedentary value.

1). Similar flows to the contracting musculature of the distal limb were achieved by providing a slightly higher inflow to the entire hind limb of the sedentary animal (Table 3). This was necessary, since muscle mass was slightly greater and training has been determined to enhance the fraction of total flow delivered to the distal tissue.16,19 Further, similar arterial blood oxygen contents of ≈20 vol% established similar high oxygen deliveries to the contracting muscles (=10 μmol·min-1·g-1) of each group (Table 1). Finally, blood flow distributions among specific fast-twitch white, fast-twitch red, and slow-twitch red fiber sections of the contracting musculature were not different between groups (data not shown). Thus, the essential criterion of the same oxygen delivery was met in order to evaluate the influence of training-induced muscle adaptations on muscle performance and oxygen exchange.

Muscle Performance

Initial force development of ≈10 N/g was similar between groups (Table 1). However, the ability to maintain this force development was markedly better in the trained group (Fig 2). The trained animals demonstrated greater force development at the higher contraction demands of 30, 45, 60, 75, and 90 TPM (P<.001).

Oxygen Consumption

Resting oxygen consumption was not different between the trained (0.31±0.02 μmol·min-1·g-1) and sedentary (0.36±0.03 μmol·min-1·g-1) animals and was similar to values obtained previously for young adult rats.20,24 Oxygen consumption of the contracting muscle in the sedentary group increased as the frequency of contractions increased, to a peak of 4.34±0.29 μmol·min-1·g-1 at 60 TPM (Fig 3). Muscle oxygen consumption of the trained group increased, similar to that of the sedentary group over the lower frequencies, but then proceeded further to a higher peak oxygen consumption of 5.68±0.34 μmol·min-1·g-1 (P<.01) at 90 TPM. The peak oxygen consumption, identified from the highest value obtained irrespective of contraction frequency, was also greater (P<.05) in the trained (5.71±0.29 μmol·min-1·g-1) as compared with the sedentary (4.37±0.39 μmol·min-1·g-1) group.

Since oxygen delivery to the contracting muscle was similar in both groups, the higher peak oxygen consumption of the trained groups could only have occurred by a greater oxygen extraction (Fig 4). Although the oxygen extraction of the trained animals proceeded to a greater value (63.6±4.7%, P<.05) than that of the sedentary animals at 90 TPM, it was not significantly different (P<.10) from the oxygen extraction of the sedentary animals (50.4±6.5%) observed at their peak oxygen consumption at 60 TPM, owing to the greater variability inherent in this parameter.

Oxygen Cost of Tetanic Contractions

The excellent linear curve fit, illustrated in Fig 5, indicates that the energy cost per newton of force development was essentially constant for each group, over the wide range of contraction frequencies used, whereas fatigue was very different (Fig 2). The oxygen cost per contraction was significantly less (P<.01) in the trained (0.14±0.01 μmol O2·g-1·contraction-1) as compared with the sedentary (0.20±0.02 μmol O2·g-1·contraction-1) group. These values correspond to 13.5±0.69 and 19.1±1.85 nmol O2·N-1·contraction-1 for the trained and sedentary groups, respectively.

Fiber Composition and Muscle Capillarity

Muscle fiber area was marginally less (=10%, .20>P>.10) in the trained as compared with sedentary animals. Muscle fiber composition shifted with training (Table 3). The percent of type Iib fibers (and fiber area) decreased (P<.001), whereas the percentage of type Ila fibers (and fiber area) increased (P<.001) with training. Similar to the biochemical evidence of citrate synthase activity (Table 2), the darkened NADH stain in the trained plantaris muscle suggests that the oxidative

TABLE 2. Citrate Synthase Activity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Quadriceps</th>
<th>Gastrocnemius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>10.6±0.61</td>
<td>18.1±1.64</td>
</tr>
<tr>
<td>Trained</td>
<td>21.4±1.53*</td>
<td>36.4±1.58*</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
P<.01 vs corresponding sedentary value.
TABLE 3. Plantaris Fiber Population, Area, and Capillarization

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Type I % of Total Fiber Types</th>
<th>Type Ila % of Total Fiber Types</th>
<th>Type Iib % of Total Fiber Types</th>
<th>Muscle Fiber Area, μm²</th>
<th>Capillaries per mm²</th>
<th>Capillary to Fiber Ratio</th>
<th>Capillary Contacts per Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>13.9±1.35</td>
<td>5.9±0.56</td>
<td>17.2±1.18</td>
<td>66.0±1.11</td>
<td>137±108</td>
<td>677±28</td>
<td>4.68±0.11</td>
</tr>
<tr>
<td>Trained</td>
<td>14.0±1.37</td>
<td>7.7±0.81</td>
<td>25.3±1.99*</td>
<td>59.8±2.27*</td>
<td>2200±102</td>
<td>859±26*</td>
<td>5.70±0.10</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *P<.05 vs corresponding sedentary value.

Enzymes in mitochondria were increased by training (Fig 6).

It is apparent from histochemical sections illustrated in Fig 6A and 6D that muscle capillarity was increased by training. Quantitative comparisons of the capillary to fiber ratio and the average number of capillary contacts per fiber (capillaries surrounding a fiber) demonstrated significant increases (P<.001) due to the exercise training program (Table 3). The change in the distribution of capillary contacts per fiber in the mixed-fiber plantaris muscle is indicated in Fig 7. An increase in capillarity is apparent among the poorly vascularized and the highly vascularized fibers within the plantaris muscle.

Discussion

The novel and significant findings of the present study demonstrate that peripheral adaptations, induced by physical activity in aged animals with peripheral arterial insufficiency, serve to improve vascular-tissue exchange properties and lead to functional increases in muscle performance. Thus, an improvement in muscle function is imparted without obligating an increase in blood flow. These findings are particularly relevant, since they were observed in a population of aged animals similar to the elderly population of patients that are most affected by peripheral vascular disease. The animal model of intermittent claudication used in the present study involved an abrupt surgical imposition of femoral stenosis sufficient to impair exercise hyperemia but not limit resting blood flow. Although this may not be characteristic of all patients that present with intermittent claudication, it did provide a reproducible and constant proximal obstruction of a large supply vessel with which to assess subsequent adaptations.16,19,21

To appropriately evaluate the functional significance of peripheral adaptations within muscle, it is essential to have equivalent blood flows, and therefore oxygen deliveries, to the contracting muscles. Even though equivalent oxygen deliveries (≈10 μmol · min⁻¹ · g⁻¹) were achieved between groups, muscle performance was markedly improved in the physically active animals, especially when the energy demands were high (Fig 2). Thus, specific changes must have occurred within the muscle to modify the balance between energy supply and energy demand. This cannot be attributed to sufficient changes in anaerobic metabolism, since we have previously shown that the contribution of ATP from glycolysis is small (<6%) during the sequential contraction protocol where oxygen consumption is high.24 Rather, the primary reason is an increase in aerobic energy supply. The peak oxygen consumption of the trained muscle was increased ≈30% (P<.01) above that of the sedentary animals, which were limited to cage activity. Since oxygen deliveries were similar, there was a greater oxygen extraction across the contracting muscle. This indicates that factors important in vascular-tissue oxygen exchange have been modified by training.

Morphometric and biochemical analyses of muscles obtained from the sedentary and trained animals provide some insight. Muscle capillary density was markedly greater in the trained group, as evident by an ≈25% increase (P<.001) in capillaries per square millimeter (Table 3). This increase in capillary density was due, in part, to a marginal reduction in muscle fiber size of ≈10% and, in part, to an expansion of the existing capillary network. The apparent reduction in muscle fiber size (.20>P>.10) is not typically a feature of aerobic-type exercise training but has been reported in
The diaphragm of healthy aged animals involved in a treadmill running program similar to that used in this study. Smaller individual fibers would increase the number of capillaries per cross-sectional area and result in a smaller average distance between the capillary and center of the fiber. More importantly, an expansion of the existing capillary network due to angiogenesis is evident by the increase in capillary to fiber ratio ($P<.001$) and capillary contacts per fiber ($P<.001$). This increase in capillary network surrounding each fiber should be most effective in enhancing capillary-tissue nutrient exchange. Further, the greater capillary volume should lengthen red blood cell transit time and, for a given blood flow, increase the time for oxygen exchange, since capillary recruitment is essentially complete during contractions. Both factors could have contributed to the greater oxygen extraction by the trained muscle. In addition, the greater mitochondrial density within the fibers of the trained muscle, characterized by the higher activity of the marker enzyme citrate synthase, could have reduced the diffusion path length for oxygen. Thus, the muscle of aged animals is capable of developing exercise-induced adaptations, similar to those found in young adults, which serve to more effectively support the energy needs of active muscle.

Our novel finding of an exercise-induced angiogenesis within fast-twitch muscle has not been observed in normal healthy aged animals or elderly humans. The lack of vascular changes reported previously could have been due to an inadequate adaptive stimulus associated with the exercise programs. On the other hand, the imposition of exercise in the presence of arterial insufficiency may have been the critical factor prompting angiogenesis. Fujita et al found that an exercise challenge in the presence of coronary artery insufficiency was a necessary combination to induce coronary vascular expansion.

The increase in peak oxygen consumption in the trained animals of $\approx 30\%$ is actually smaller than would be expected from the improvement in muscle performance. At the time of peak oxygen consumption, the accumulative muscle tension development each minute for the trained group ($395 \pm 21 \text{ N} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$) was far greater ($\approx 80\%$) than that for the sedentary group ($219 \pm 13 \text{ N} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$). This implies that some other change has occurred. The other factor that contributed to the improved performance was an $\approx 35\%$ reduction in the energy cost per contraction (Fig 5). This improved economy of contraction has not been a common finding of exercise training but can be found when appropriate shifts in fiber-type composition are induced within muscle. Although a shift in fiber-type distribution, to decrease type IIb and increase type IIa myosin isoform composition, was observed in the trained muscle (Table 3), it is unlikely that this shift was sufficient to account for the large change in energy cost per contraction. A relatively large increase in slow type I isoform would be needed, since the largest difference in contraction energetics of approximately threefold is between slow (type I) and fast (type IIa and IIb) isoforms. However, an increase in the common expression of both fast and slow myosin isoforms within individual fibers has been observed in muscle during challenges that transform fiber types. Thus, more careful electrophoretic and/or specific antibody analyses of myosin isoforms are required to assess the possible extent of myosin isoform shifts that may have occurred but were not evident in our conventional histochemical analysis.

The precise stimulus prompting the increase in mitochondrial content within muscle is presently unknown. The suggested importance of local tissue hypoxia has been bolstered by the increase, although quantitatively small, in mitochondrial content found in patients with peripheral vascular disease, even when they do not exercise. The elevation in mitochondrial enzyme diminishes with successful revascularization when blood flow improves but remains unchanged if revascularization is unsuccessful. Whereas this evidence argues that tissue ischemia is implicated, the increase in mitochondrial content is quantitatively much greater when exercise is involved. Cellular events related to both exercise intensity and duration are important modifiers. Thus, conditions determined by energy supply relative to energy demand may be more critical. Elevating the energy demand by muscle contractions, in the presence of ischemia, is an effective means to stimulate the adaptation. This hypothesis is further supported by the large increase in mitochondrial content of $\approx 75\%$ in the mixed-fiber muscle of our femoral-stenosed trained animals as compared with healthy aged rats ($\approx 50\%$) with normal limb blood flow that exercised at the same treadmill speed. Further, the appreciable increase in mitochondrial content (25% to 110%) found in the red and white sections of the gastrocnemius in these aged animals is contrasted to the smaller responses (15% to 40%) of femoral-stenosed young adult animals that were similarly trained. Unfortunately, the present results are not definitive in establishing the critical
Fig 6. Photomicrographs of plantaris muscle of sedentary (A through C) and trained (D through F) animals histochemically stained to illustrate the following: capillarity (A and D), oxidative enzyme distribution of NADH tetrazolium reductase (B and E), and fiber-type distribution of myofibrillar ATPase after preincubation at pH 4.5 (C and F; type I, dark; type IIA, light; and type IIB, intermediate intensity). All photos are at the same magnification. Bar=100 μm.
Factor(s) inducing mitochondrial adaptations, since complexities exist. For example, same walking speed represents a relatively greater challenge for aged animals\(^7\) and therefore should lead to an enhanced adaptation.\(^8\) In addition, the muscle mitochondrial content of aged animals is significantly reduced compared with young adults.\(^9\) This could expand the stimulus for adaptation, since mitochondrial enzyme capacity does influence metabolic control signals\(^37\) and aerobic energy provision.\(^38\) Further, the red and white sections of the upper leg quadriceps muscle exhibited increases in mitochondrial contents larger than those induced in the corresponding sections of the gastrocnemius (Table 2), whereas flow deficits are greatest during exercise in the muscles of the lower limb with impaired femoral artery blood flow.\(^10\) Thus, additional studies are needed to evaluate the relative importance of ischemia and energy demand in causing the remodeling of muscle to increase its mitochondrial content.

The increased in exercise tolerance found with training has been reported previously for healthy aged animals,\(^7,10\) for young adult animals with peripheral arterial insufficiency,\(^19-23\) and for patients with peripheral vascular disease.\(^39,40\) The finding that most patients with peripheral vascular disease improve their exercise tolerance without any change in limb blood flow has led to the recognition that peripheral adaptations are important.\(^10,18,20,21\) The results of the present study demonstrate that improved vascular-tissue oxygen exchange properties, attributed to the microvascular and biochemical adaptations within the muscle, enhance muscle performance. The adaptations are especially robust in aged animals even though the exercise program involved extended walking at a well-tolerated pace without increasing treadmill speed. Similar adaptations in patients could improve exercise tolerance and account for the enhanced oxygen extraction observed across the active limbs of physically active patients with claudication.\(^17,18\) A further benefit would be derived if training also induced an increase in collateral blood flow\(^14-16\) and/or led to a more effective distribution of the limited flow within the limb to better supply the active fibers.\(^17,18\)

The increased mitochondrial content within the trained myocyte should impart another advantage in exercise tolerance on the basis of its expected influence in the control of energy metabolism. During a moderately elevated rate of energy expenditure, the higher the mitochondrial enzyme capacity within the fiber, the less displaced are important cytosolic metabolic control signals from rest.\(^37,41\) This should improve the ability of the muscle fiber to meet and sustain the aerobic energy needs of the contractile effort. As a result, the muscle should be better able to sustain an exercise task that is within the flow capacity of the muscle.

It should be recognized that the peripheral arterial insufficiency established in the present study is not representative of the diverse population of elderly patients with peripheral vascular disease, especially those suffering from severe diffuse disease that involves the distal vasculature. Rather, our experimental condition involved acute-onset occlusion of a large proximal vessel, similar to that found in many patients who present with intermittent claudication.\(^15,39\) Our results showing a significant improvement in muscle performance that does not obligate an increase in blood flow adds support to those advocating exercise as an adjunct in managing patients with peripheral vascular disease.\(^14,15,17,18,39,40\)

### Acknowledgments

This study was supported by National Heart, Lung, and Blood Institute Grant HL-37387 and a pilot grant for the National Institute of Aging. Dr H.T. Yang was a postdoctoral fellow of the American Heart Association, New York Affiliate. The excellent technical assistance of Judy Freshour and David Barrett is gratefully acknowledged.

### References


Peripheral adaptations in trained aged rats with femoral artery stenosis.
H T Yang, R W Ogilvie and R L Terjung

doi: 10.1161/01.RES.74.2.235

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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