Chronic Changes in Skeletal Muscle Histology and Function in Peripheral Arterial Disease

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Background. Peripheral arterial disease (PAD) is associated with an impairment in exercise performance and muscle function that is not fully explained by the reduced leg blood flow during exercise. This study characterized the effects of PAD on muscle function, histology, and metabolism.

Methods and Results. Twenty-six patients with PAD and six age-matched control subjects were studied. Ten of the PAD patients had unilateral disease, which permitted paired comparisons between their diseased and non-diseased legs. All PAD patients had a lower peak treadmill walking time and peak oxygen consumption than controls. Vascular disease (diseased leg in unilateral patients and the most severely diseased leg in bilateral patients) was associated with decreased calf muscle strength compared with control legs. In patients with unilateral disease, the diseased legs had a greater percentage of angular fibers (indicating chronic denervation) and a decreased type II fiber cross-sectional area (expressed as percent of total fiber area) compared with the non-diseased, or control, legs. In diseased legs, gastrocnemius muscle strength was correlated with the total calf cross-sectional area (r=0.78, p<0.05) and type II fiber cross-sectional area (r=0.63, p<0.05). Activities of citrate synthase, phosphofructokinase, and lactate dehydrogenase in all 26 PAD patients (most diseased leg) did not differ from control values. Despite a wide range in citrate synthase activity in PAD patients, activity of this enzyme was not correlated with muscle strength or treadmill exercise performance.

Conclusions. In patients with PAD, gastrocnemius muscle weakness is associated with muscle fiber denervation and a decreased type II fiber cross-sectional area. In contrast, the PAD patients displayed substantial heterogeneity in muscle enzyme activities that was not associated with exercise performance. Denervation and type II fiber atrophy may contribute to the muscle dysfunction in patients with PAD and further confirm that the pathophysiology of chronic PAD extends beyond arterial obstruction. (Circulation 1993;87:413-421)

KEY WORDS: peripheral arterial disease, muscle, skeletal, exercise, claudication, enzymes, denervation, muscle, peripheral vascular diseases

Patients with peripheral arterial disease (PAD) develop intermittent claudication with walking exercise because extremity blood flow is limited and inadequate to meet the metabolic demand of the muscle.1 This results in an objective impairment in exercise performance, such that the peak oxygen consumption during treadmill testing is 50% lower than values in age-matched controls.2 The etiology of the exercise impairment in patients with PAD is multifactorial. Although muscle ischemia is a major contributor, the hemodynamic severity of the disease (assessed by measurements of peripheral blood flow or ankle pressure) is poorly correlated with exercise performance.3-4 Patients with severe ischemic disease develop structural changes in skeletal muscle consisting of denervation, fiber atrophy, and a selective loss of type II fibers relative to type I fibers that may contribute to the muscle dysfunction.5-7 Ambulatory patients with mild PAD also have muscle weakness and reduced muscle endurance,8 but it is not established whether denervation and fiber atrophy also contribute to their impairment in muscle function.

In patients with PAD, an increase in skeletal muscle oxidative enzyme activity may be an adaptive response to the reduced skeletal muscle blood flow.9-11 However, other investigators have observed a decrease in oxidative enzyme activity in PAD, particularly in more severe forms of the disease.12,13 Thus, changes in skeletal muscle enzyme activities in patients with PAD may be heterogeneous, and the functional significance of these changes is not well established.

To further define the structural and metabolic changes that occur in skeletal muscle of ambulatory
Table 1. Characteristics of Patients With Peripheral Arterial Disease and Control Subjects

<table>
<thead>
<tr>
<th>Demographics</th>
<th>PAD Unilateral</th>
<th>All PAD</th>
<th>Controls</th>
</tr>
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<tr>
<td>No. of patients</td>
<td>10</td>
<td>26</td>
<td>6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64±10</td>
<td>65±7</td>
<td>63±5</td>
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</tbody>
</table>

Peak treadmill performance

Walking time (minutes) | 9.8±2.9* | 8.1±4.1* | 18.6±2.3 |

VO2 (ml/kg/min) | 15.1±1.8* | 14.5±2.6* | 27.4±3.8 |

Ankle/brachial index

Resting

Diseased | 0.72±0.10* | 0.66±0.21* | 1.10±0.07 |
Nonsymptomatic | 0.97±0.07* |

After exercise

Diseased | 0.34±0.14* | 0.27±0.14* | 1.04±0.09 |
Nonsymptomatic | 0.76±0.16* |

Patients with unilateral peripheral arterial disease (PAD) and unilateral combined with bilateral diseased patients (all PAD) and control subjects performed graded treadmill exercise protocol to define peak exercise performance. Measurements of the ankle/brachial systolic blood pressure index were performed at rest and immediately after exercise. Values are mean±SD.

*p<0.05 for the value in patients compared with control subjects.

PAD patients as well as the role of these changes in muscle function and exercise performance, we evaluated muscle histology, biochemistry, and exercise performance in 26 patients with PAD and in six normal controls. Ten of the patients had vascular disease confined to one leg, which facilitated comparisons between diseased and nonsymptomatic patients, permitting the non-ischemic metabolic and histological states of the individual's muscle to be defined in the nonsymptomatic leg. The results demonstrate PAD-associated muscle denervation and atrophy of type II fibers that correlate with muscle dysfunction in the underperfused muscle.

Methods

Subjects

Twenty-six male patients with PAD were studied, of which 10 had intermittent claudication in only one leg (unilateral patients), and 16 had claudication in both legs. Unilateral PAD was confirmed by an abnormal ankle-to-brachial systolic blood pressure indexes (ABIs) in the symptomatic leg (see below). In the unilateral patients, four had aortoiliac disease, whereas all 10 had femoropopliteal disease as identified by segmental limb pressures and pulse volume recordings using established criteria. Six age- and activity-matched control subjects were also enrolled (Table 1). All participants were sedentary, reporting that they did not exercise on a regular basis. Patients were excluded if they were diabetic or had ischemic rest pain, ulceration, or gangrene. Patients also were excluded if their exercise capacity was limited by symptoms of angina, congestive heart failure, chronic obstructive pulmonary disease, or arthritis. Chronic medications were continued at the same dosages. Control subjects had no clinical evidence of chronic or active disease, received no medications, and had an unremarkable medical history. The study was approved by the University of Colorado School of Medicine Human Subjects Committee, and informed consent was obtained from all enrolled subjects.

Vascular Testing

All subjects were initially studied at rest in the supine position with cuffs on each ankle and arm. At each ankle, systolic blood pressures were measured by Doppler ultrasound (model 841, Parks Medical Electronics, Beaverton, Ore.) in duplicate and averaged for the dorsalis pedis and posterior tibial arteries. Systolic blood pressures were also measured in duplicate in each arm by auscultation. The right and left ABIs at rest were calculated from the highest arm and ankle pressures. One minute after the completion of the exercise test, systolic blood pressures were measured in the ankles and arm to determine the postexercise ABI. The criteria for vascular disease were a resting ratio of <0.94 that decreased to <0.73 after exercise. Data from both legs are presented for the unilateral patients, but only data from the most diseased leg of 16 patients with bilateral PAD were used (most diseased leg was determined from the lowest rest and postexercise ABIs).

Treadmill Testing

In patients with PAD, a graded treadmill protocol was performed to maximally tolerated claudication pain using previously described and validated methods. Controls exercised to exhaustion using the same protocol. Rates of oxygen consumption (VO2) and carbon dioxide production (VCO2) were measured at rest and during treadmill exercise with an Ametek metabolic system (Ametek Thermox, Pittsburgh, Pa.). Arm blood pressure (by auscultation) and heart rate (by 12-lead ECG) were obtained every minute during exercise. Cardiac status was monitored throughout the treadmill test by 12-lead ECG. The exercise test began at 2 mph and 0% grade, and the grade was increased by 3.5% every 3 minutes to peak exertion. In all subjects, peak exercise performance was characterized by the longest walking time and the highest VO2 attained during the treadmill test.

Muscle Strength and Endurance Testing

Strength testing was performed in all subjects on a Cybex Dynamometer (Lumex Inc., Ronkonkoma, N.Y.). Testing of the gastrocnemius and anterior tibial muscles was performed with the subject in the supine position with the leg fully extended. Joints not being tested were stabilized to prevent recruitment of other muscle groups. Each subject flexed and extended the foot with maximal effort on a pedal at a regulated speed of 60° sec⁻¹ for a total of five repetitions. This sequence was performed a second time, and the single maximal value for each muscle group (expressed in foot-pounds of peak torque) was used for analysis. No patient experienced claudication during strength testing. After a 3-minute rest, muscle endurance was measured in the gastrocnemius muscles. The subjects were in the same positions as for strength testing, but each muscle contraction was performed at a rate of 240° sec⁻¹. Muscle endurance was recorded as the number of repetitions performed using a particular muscle group until a 50% decrement in peak torque was observed. Endurance
Muscle claudication pain (as defined by reference 17) before the exercise was terminated.

**Calf Cross-sectional Area**

To estimate the maximal calf muscle cross-sectional area, calf circumference (in centimeters) was measured at the largest part of the muscle and converted to cross-sectional area by the following formula: circumference²/4π (assuming the calf to be nearly circular). In addition, a subset of six unilateral PAD patients and three controls underwent computed tomography (CT) scanning (model CT-900S series, Toshiba Corporation, Nasu, Japan) of each calf. A single 1-cm scan was obtained simultaneously through both calves at the level of the biopsy sites with the legs parallel. The perimeter of the calf musculature was manually traced (excluding the tibia, fibula, and subcutaneous tissue), and the cross-sectional area (in centimeters squared) of the muscle was calculated.

**Muscle Biopsy**

Bilateral biopsies of the medial head of the gastrocnemius muscles were performed at rest in both legs of the patients and controls. After a subcutaneous injection of 3 ml lidocaine, a 5-mm biopsy needle (Bergstrom muscle biopsy cannula, DePuy Inc., Warsaw, Ind.) was used to remove approximately 40–50 mg of tissue. Samples to be analyzed for enzyme activities were immediately frozen in liquid nitrogen and stored at −80°C. For histological analyses, tissue was stored in saline-soaked gauze immediately after the biopsy for ≤30 minutes. Subsequently, muscle tissue was oriented in gum tragacanth on a wooden block and flash-frozen in liquid nitrogen–cooled isopentane. The muscle samples were stored at −70°C in closed containers. In patients with bilateral PAD, only data from the most diseased leg were used for analysis.

**Histological Analysis**

Serial 10-μm cryostat sections of muscle tissue were analyzed for both histopathological changes and fiber-type specific characteristics. Sections of tissue were stained for myosin ATPase (pH 9.4 and 4.6) and nicotinamide adenine nucleotide dehydrogenase-tetrazolium reductase (NADH-TR). These stains were used for qualitative analysis, which included a general histological description as well as evaluation for the presence of target or grouped fibers, which indicate denervation and reinnervation, respectively. In addition, oil-red O was used to stain for extracellular fat, periodic acid–Schiff for glycogen, Verhoeff–van Gieson for fiber

### Table 2. Muscle Strength, Endurance, and Calf Cross-sectional Area in Peripheral Arterial Disease Patients and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>PAD (Unilateral)</th>
<th>All PAD</th>
<th>Controls</th>
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<tbody>
<tr>
<td><strong>Muscle performance</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Gastrocnemius strength</strong> (foot-pounds)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased</td>
<td>31±11†</td>
<td>30±9*</td>
<td>53±11</td>
</tr>
<tr>
<td>Non symptomatic</td>
<td>38±14*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gastrocnemius endurance</strong> (no. of repetitions)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased</td>
<td>8±4†</td>
<td>8±5*</td>
<td>13±4</td>
</tr>
<tr>
<td>Non symptomatic</td>
<td>13±5</td>
<td></td>
<td></td>
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<tr>
<td><strong>Anterior tibial strength</strong> (foot-pounds)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Diseased</td>
<td>9±4*</td>
<td>9±4*</td>
<td>13±3</td>
</tr>
<tr>
<td>Non symptomatic</td>
<td>12±7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Muscle cross-sectional area</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Calf measurement (cm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diseased</td>
<td>94.5±18.4†</td>
<td>95.2±16.2</td>
<td>99.7±11.9</td>
</tr>
<tr>
<td>Non symptomatic</td>
<td>99.5±20.2</td>
<td></td>
<td></td>
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<tr>
<td>Computed tomography (cm²)</td>
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<td></td>
</tr>
<tr>
<td>Diseased</td>
<td>78.5±6.1†</td>
<td>80.5±14.0</td>
<td></td>
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<tr>
<td>Non symptomatic</td>
<td>82.6±4.3</td>
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**PAD,** peripheral arterial disease. Muscle strength (peak torque at 60°/sec) and endurance (50% reduction in peak torque at 240°/sec) were measured on a Cybex isokinetic dynamometer. Muscle cross-sectional area was estimated from calf circumference and, in a subset of six unilateral patients and three controls, from computed tomography (excluding subcutaneous tissue and bone). Values are mean±SD.

*tp<0.05 for the value in patients compared with control subjects. \p<0.05 for the value in the diseased leg of unilateral patients compared with their non symptomatic leg.

**Figure 1. Top panel:** Gastrocnemius muscle strength in control subjects (○) and patients with unilateral peripheral arterial disease (PAD) (●). Each subject is shown as a point, and for the PAD patients the value in each individual's diseased leg and non-symptomatic leg is connected by a line. *p<0.05 for the value in patients compared with control subjects, and +p<0.05 for the value in the non-symptomatic leg of unilateral patients compared with their diseased leg. **Bottom panel:** Gastrocnemius muscle type II fiber area measured from cryostat sections stained for myosin ATPase at pH 9.4.
morphology and connective tissue, and acid phosphatase for necrosis.21

The diameter, number, and percent cross-sectional area of each fiber type were calculated using standard morphometric techniques from cryostat sections stained for myosin ATPase (pH 9.4) activity. The optimal number of fields that needed to be sampled was determined by an iterative process that minimized the within-subjects standard deviation of each measurement but without oversampling.22 To calculate fiber diameter and number, every eighth microscopic field (field size, 0.293 mm²) was photographed and projected to a final magnification of ×364. The diameter of each fiber was determined as the maximum measure of the lesser fiber axis that would correct for any fibers cut at an oblique angle. The number of type I and II fibers were counted in the same fields and are expressed as a percent of the total number. The percent of angular fibers (indicating denervation) was also determined from these fields.

The cross-sectional area of each fiber type was estimated using the point-grid method.23 For cross-sectional area calculations, every fifth microscopic field (field size, 0.113 mm²) was photographed and projected onto a grid to a final magnification of ×590. The points (line intersections) falling over type I and II fibers were recorded for each microscopic field. The total number of line intersections from all fields of a single cryostat section for each biopsy were then summed for each fiber type. The data are expressed as the percent of the total fiber cross-sectional area (sum of the number of points for both type I and II fibers) occupied by each fiber type.

Biochemistry

Samples for noncollagenous protein determinations were prepared as described by Lilienthal et al.24 Protein quantitation was performed by the method of Lowry et al.25 using bovine serum albumin as a standard. Lactate dehydrogenase,26 citrate synthase,27 and phosphofructokinase28 activities were quantified using established spectrophotometric methodologies. Enzyme activities were normalized to grams of wet weight and grams of noncollagen protein. Citrate synthase activity was also normalized to the lactate dehydrogenase activity. The variability of the assay methods in our laboratory was initially assessed in animal studies. For citrate synthase activity, four separate homogenates from two rats yielded a coefficient of variation (standard deviation divided by the mean) of 18%.
Statistical Analysis

Student's t test for paired data or a within-subjects ANOVA was used for within-subjects comparisons, and an unpaired t test was used for between-subjects comparisons. Linear regression was used for calculating correlations between variables. Categorical data were analyzed using the $\chi^2$ or Fisher's exact probability tests. Values are given as mean±SD and considered significant when $p<0.05$ in a two-tailed test. In control subjects, the functional, histological, and biochemical data were similar between legs; therefore, the results were averaged from both legs.

Results

Patient Characteristics

Patients with PAD were well matched to the control subjects for age but had a reduced peak exercise performance as defined by treadmill walking time and peak oxygen consumption (Table 1). Patients reported symptoms of intermittent claudication for 6±8 years with a range of 1–45 years. In all patients with PAD (unilateral and bilateral subjects), the most diseased leg had an abnormal ABI at rest that decreased further after exercise (Table 1). The average resting and postexercise ABIs in the unilateral patient's nonsymptomatic legs were in the normal range as previously defined and significantly greater than in their diseased legs (Table 1). However, postexercise ABIs in the nonsymptomatic legs were significantly lower than in control subjects, suggesting the possibility of mild arterial disease in the nonsymptomatic leg.

Muscle Strength and Endurance

In all 26 PAD patients, gastrocnemius muscle strength in their diseased legs was 43% less and anterior tibial strength was 31% less than the corresponding values in the control subjects (Table 2). Gastrocnemius muscle endurance in the diseased legs was 38% less than that observed in the control subjects. In the subset of patients with unilateral disease, gastrocnemius strength (Figure 1, top panel) and endurance were less in the diseased than in the nonsymptomatic legs, but strength in the anterior tibial muscles was not different between legs (Table 2).

Estimates of Muscle Cross-sectional Area

The maximal calf cross-sectional area was estimated by measurements of circumference and by CT scans of the calf muscles. In patients with unilateral PAD, their diseased legs had 5% reduction ($p<0.05$ using a paired analysis) in cross-sectional area compared with their nonsymptomatic legs (Table 2). In contrast, the calf cross-sectional area in diseased legs, measured by either method, was not different from control values due to the large standard deviation of the measurement in the PAD patients.

Muscle Histology

Histological analysis of the muscle samples was performed only in the unilateral PAD patients and in controls. Representative micrographs of gastrocnemius muscle are shown as follows: from a control subject using myosin ATPase stain (Figure 2A), from the diseased leg of a unilateral PAD subject using myosin ATPase stain (Figure 2B), and the diseased leg of a unilateral PAD subject stained with NADH-TR (Figure 2C). Qualitatively, the muscle samples from patients and controls showed no evidence of inflammation, fiber necrosis, or unusual accumulations of intracellular fat, connective tissue, or glycogen. However, all diseased legs of unilateral patients had histological evidence of denervation consisting of angular (Figure 2C), or target, fibers. In addition, fiber-type grouping, suggesting reinnervation, was seen in five of the 10 diseased legs (Figure 2B). Taken together, histological evidence of denervation or reinnervation was observed less often in the nonsymptomatic legs (40%, $p<0.05$) or in control legs (36%, $p<0.05$) than in the diseased legs (100%).

Fiber type distribution, diameter, and area were also determined in biopsies from the unilateral PAD patients and control subjects. Type I fiber diameter and the average number of type I fibers per field did not differ among diseased, nonsymptomatic, and control legs (Table 2). Type II diameter and the average number of type II fibers per field were also similar for the diseased and control legs. However, type II fiber area in the diseased leg (measured independently of diameter or number) was 38% of the total fiber area, which was less than the type II area in the nonsymptomatic (49%) and control (48%) legs (Figure 1, bottom panel). In the nonsymptomatic leg, type II fiber diameter was less, and type II fiber number was greater than in the diseased leg. However, type II fiber area in the nonsymptomatic legs was not different from type II fiber area in the control legs.

Muscle Biochemistry

The noncollagenous protein contents (expressed as grams per gram wet weight) were similar for all diseased legs, the nonsymptomatic legs of unilateral patients, and control subjects (Table 4). Activities of citrate synthase, lactate dehydrogenase, and phosphofructokinase in the diseased legs of unilateral patients did not differ from the values in their nonsymptomatic legs or from control values (Table 4), whether normalized to grams of noncollagenous protein or grams of wet weight (data not shown). However, there was a large variability in enzyme activities in the diseased legs of the unilateral patients. To determine if this heterogeneous response was characteristic of the PAD population, enzyme activities were also assessed in the most diseased leg of 16 additional patients with bilateral PAD. This analysis confirmed that there was no difference for citrate synthase, phosphofructokinase, and lactate dehydrogenase activities between diseased legs of PAD patients and control subjects. Finally, when citrate synthase activity was normalized to lactate dehydrogenase activity (to control for any changes in enzyme expression), there remained no difference between the diseased legs of all PAD patients and control subjects (data not shown).

Predictors of Muscle Strength and Exercise Performance

The ABI is a marker of the hemodynamic severity of the vascular disease.29 In the diseased legs of the unilateral PAD patients, the ABI was not correlated with any changes in muscle histology or enzyme activities. In all 26 PAD patients, there was no correlation of the ABI at rest or after exercise with gastrocnemius muscle strength or peak exercise performance on the
treadmill. In the patients with unilateral disease, gastrocnemius muscle strength was correlated with total cross-sectional area of the calf (Figure 3, top panel) and type II fiber area (Figure 3, bottom panel), but similar correlations were not observed in the nonisometric legs. Finally, gastrocnemius strength in the diseased legs of all patients was correlated with peak walking time on the treadmill \( r = 0.41, p < 0.05, y = 0.18x + 2.75 \).

Despite the large population variation in citrate synthase activity of all diseased legs in the PAD subjects, there was no correlation between citrate synthase activity and peak treadmill walking time \( r = -0.23, p = 0.26 \) or with gastrocnemius muscle strength or endurance in the diseased legs. Phosphofructokinase and lactate dehydrogenase activities were also not correlated with muscle strength or treadmill exercise performance. In contrast, citrate synthase activity in control legs was correlated with peak walking time \( r = 0.86, p < 0.05, y = 0.09x + 13.87 \).

**Discussion**

PAD is associated with chronic changes in affected muscle morphology and function. Histological analysis of gastrocnemius muscle from the diseased legs of patients with unilateral PAD demonstrated denervation and a reduction in the cross-sectional area of type II fibers compared with healthy, age-matched controls. These histological changes were associated with muscle

<table>
<thead>
<tr>
<th>Muscle fiber characteristics</th>
<th>Unilateral peripheral arterial disease</th>
<th>Controls</th>
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<tbody>
<tr>
<td>Type II fiber diameter ((\mu m))</td>
<td>71±11</td>
<td>76±5</td>
</tr>
<tr>
<td>Type II no. of fibers/field</td>
<td>3.5±1.6</td>
<td>3.7±1.0</td>
</tr>
<tr>
<td>Type II fiber area (% of total fiber area)</td>
<td>38±13*†</td>
<td>48±8</td>
</tr>
<tr>
<td>Type I fiber diameter ((\mu m))</td>
<td>72±12</td>
<td>76±9</td>
</tr>
<tr>
<td>Type I no. of fibers/field</td>
<td>5.8±2.9</td>
<td>4.4±1.3</td>
</tr>
<tr>
<td>Angular fibers (% of total fiber number)</td>
<td>9.7±1.2*†</td>
<td>13±1.6</td>
</tr>
</tbody>
</table>

Muscle fiber histology determined from cryostat sections stained for myosin ATPase at pH 9.4, with the field size described in “Methods.” Values are mean±SD. *p<0.05 for the value in patients compared with control subjects. †p<0.05 for the value in the nonisometric leg of unilateral patients compared with their diseased leg.

PAD, peripheral arterial disease; NCP, noncollagen protein. Values are mean±SD. There were no significant differences in enzyme activities between patients and controls.

**Table 3.** Histological Characteristics of Gastrocnemius Muscle in Patients With Unilateral Peripheral Arterial Disease Compared With Control Subjects

**Table 4.** Enzyme Activities of Gastrocnemius Muscle in Patients With Peripheral Arterial Disease Patients and Control Subjects

Weakness in the diseased legs, and the muscle weakness contributed to the impairment in exercise performance. As another control, results from the nonisometric legs confirmed that the histological and functional changes in the diseased legs were not the result of deconditioning, aging, or environmental factors.

Previous studies have shown that PAD is associated with alterations in skeletal muscle histology. Necrotic and regenerating fibers as well as inflammation have been seen in the diseased legs of patients with unilateral PAD hospitalized for surgical evaluation\(^{30}\) and in patients with severe ischemic disease.\(^{5}\) In this study, ambulatory patients with less severe symptoms of claudication had no findings of fiber necrosis, inflammation, or accumulations of glycogen, fat, or connective tissue in their diseased legs, and the muscle fibers appeared normal. Thus, milder forms of the disease are not associated with generalized morphological changes in skeletal muscle. In contrast, signs of denervation (angular fibers) and reinnervation (grouped fibers) occur both in ambulatory patients with claudication\(^*\) and, to a greater degree, in patients with ischemic rest pain or gangrene.\(^{5,31}\) In this study, histological evidence of denervation was found in all diseased legs of PAD patients. Another study, using electrophysiological techniques, has confirmed that the muscle denervation in patients with unilateral claudication was limited to the distal motor axons, and the degree of denervation was correlated with the severity of the vascular disease.\(^{32}\) Thus, skeletal muscle denervation in PAD may be due to ischemic damage of the distal motor nerves and may contribute to the muscle weakness and dysfunction observed in patients with claudication.

Type I and type II skeletal muscle fibers are distinguished by twitch characteristics (slow versus fast) and metabolic profiles (oxidative versus glycolytic).\(^{21}\) Type I fibers have a high mitochondrial content and are well adapted for prolonged work. Type II fibers, with their
Muscle weakness has been reported in PAD patients, but the decrease in strength was only weakly correlated with walking tolerance. In our study, gastrocnemius strength correlated with peak treadmill walking time, but the low correlation coefficient suggests that other factors are more important to walking ability than muscle strength in patients with PAD.

In the unilateral patients, the nonischemic historical state of the individual's muscle was determined in their nonsymptomatic legs. Although type II fiber diameter in patients' nonsymptomatic legs was less than in the diseased or control legs, the average number of type II fibers in the nonsymptomatic leg was increased compared with the diseased leg (Table 3). Therefore, type II fiber area was not different between the non-symptomatic and control legs. The cause of the decreased type II fiber diameter in the nonsymptomatic legs is not known but may reflect changes related to inactivity or other environmental factors distinct from the effects of ischemia.

In controls, the activity of skeletal muscle oxidative enzymes reflects the level of habitual physical activity. For example, exercise training results in an increase in several oxidative enzyme activities that is directly related to the improvement in exercise performance. In contrast, deconditioning is associated with decreased skeletal muscle oxidative enzyme activities. In patients with PAD, increased activities of skeletal muscle oxidative enzymes have been observed in some studies, and the amount of increase in citrate synthase activity was correlated with the hemodynamic severity of the occlusive disease. The increase in one oxidative enzyme, cytochrome oxidase, was only weakly correlated with treadmill exercise performance, but in other studies changes in oxidative enzyme activities were not correlated with exercise performance.

Decreases in oxidative enzyme activity in PAD have also been reported. In these studies, the more severe the occlusive disease, the lower the oxidative enzyme activity. A decrease in oxidative enzyme activities with increasing disease severity may simply reflect loss of ambulation and immobility. Also, denervation of skeletal muscle (as observed in the patients in this study) is associated with a decrease in oxidative enzyme activities and loss of muscle function. Finally, the higher oxidative enzyme activities in the control group (compared with the PAD patients) could also reflect the level of fitness in the control group.

In the present study, nonsurgical, ambulatory patients with unilateral PAD were enrolled, with the nonsymptomatic leg providing a control for the effects of activity and other environmental factors on muscle metabolism. A healthy, sedentary control group allowed for the determination of any absolute differences between normal subjects and the patients with vascular disease. The results showed that citrate synthase activity in the diseased legs of unilateral patients was not different from values in their nonsymptomatic legs or in the control group. Furthermore, the citrate synthase activities were very heterogeneous in the unilateral PAD population, ranging from 25 to 115 μmol/min/g noncollagen protein. This variability could reflect the assay methods; however, when citrate synthase activity was normalized to grams of wet weight, grams of noncollagenous protein, or lactate dehydrogenase (as a

Figure 3. Top panel: Relation between cross-sectional area of the calf and gastrocnemius muscle strength in the diseased legs of patients with unilateral peripheral arterial disease. These two parameters were correlated by linear regression (y = 0.46x - 10.83). Bottom panel: Relation between the cross-sectional area of type II fibers from histological analysis and gastrocnemius muscle strength in the diseased legs of patients with unilateral peripheral arterial disease. These two parameters were correlated by linear regression (y = 0.56x + 9.70).

Taken together, the findings of denervation and a selective atrophy of type II fibers may account for the decrease in skeletal muscle cross-sectional area and the muscle weakness in the diseased legs of unilateral PAD patients. The likelihood of these relations is strengthened by the correlations of type II fiber area and muscle cross-sectional area with muscle strength (Figure 3).
marker enzyme not expected to change\textsuperscript{9–11,12}, there remained no differences between diseased and non-symptomatic or control legs. Finally, given the variability in enzyme activities, additional patients with bilateral PAD were enrolled. The results confirm that PAD was not associated with either an increase or decrease in enzyme activities but that the population displays substantial heterogeneity. This heterogeneity is not unexpected in ambulatory PAD subjects given the multiple influences of vascular disease severity, denervation, and level of fitness on enzyme activities, as discussed above. Finally, regardless of any absolute changes in oxidative enzyme activities previously reported, the functional significance has not been well established. The lack of correlation between citrate synthase activity and muscle strength or exercise performance in the PAD subjects suggests that any adaptations in oxidative enzymes do not compensate for their muscle dysfunction or decreased exercise performance. In contrast, the expected correlation between citrate synthase activity and exercise performance was observed in the control group.

Thus, the chronic response of skeletal muscle in PAD is characterized by denervation and a decreased type II fiber area. These changes are associated with muscle atrophy and a loss of muscle strength that may contribute to the functional impairment in this population.

Acknowledgments

We thank Laura Ruff, Kimberly Masterson, Diana Wolf, Katherine Barriga, and Robert Ferrill for excellent technical support. Kenneth Schneider produced the figures in their final form.

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Circulation. 1993;87:413-421
doi: 10.1161/01.CIR.87.2.413
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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