Resistance Training in Patients With Peripheral Arterial Disease: Effects on Myosin Isoforms, Fiber Type Distribution, and Capillary Supply to Skeletal Muscle

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The purpose of this study was to investigate the effects of a progressive resistance training program on myosin heavy chain isoform expression, fiber type, and capillarization in patients with symptomatic peripheral arterial disease. Patients were randomized to either a training group (n = 11, mean ± SD, 70 ± 6 years, 4 men, 7 women) or a control group (n = 9, 66 ± 6 years, 5 men, 4 women). The training sessions were completed 3 times/week, using 2 sets of various exercises, each performed for 8–15 repetitions. Muscle biopsies were obtained before and after 24 weeks from the medial gastrocnemius. Following the 24-week training program, the training group had significantly decreased the percentage of myosin heavy chain type IIB. The proportion of type IIB/AB fibers as measured by using myosin adenosine triphosphatase histochemistry decreased significantly in the training group. There were significant increases in type I and type II fiber areas, and capillary density also increased significantly in the training group. There were significant increases in 10 repetition maximum leg press and calf press strengths in the trained subjects. There were no significant changes in any of the measurements in the control group. It is concluded that progressive resistance training results in significant increases in muscle strength and alters skeletal muscle composition of subjects with peripheral arterial disease.

Exercise has been used as an effective method of improving walking ability in patients with peripheral arterial disease (PAD) (1). The observation that patients with PAD have muscle weakness provides a strong rationale for strength training of lower extremity muscle groups, in order to improve walking ability (2). A decreased muscular strength in the lower extremities has been associated with an increased prevalence of PAD (3). It is unclear to what extent the disease status affects the training responses of symptomatic subjects with PAD at the cellular level.

The physiological mechanisms that contribute to the improvements in subjects with PAD with exercise are not fully understood. It is well documented that muscle metabolism of subjects with PAD is altered, but the role that this plays in response to chronic exercise is unclear (4). Potential mechanisms that have been suggested include changes in peripheral blood flow, altered muscle metabolism, and/or alterations in gait (1). The observation that improvements in exercise tolerance occur without significant changes in peripheral blood flow suggests that mechanisms distal to the site of the arterial occlusion contribute to the improvements (4).

Alterations in myosin heavy chain (MHC) isoforms have been found with patients with PAD (5). These changes in MHC isoforms may be important in terms of understanding how skeletal muscle adapts to chronic ischemia. To our knowledge, there is no reported data providing any functional correlation to changes in MHC isoforms in diseased populations. These data would provide important information as to whether changes in muscle isoforms are associated with reduced muscular strength and performance. Previous research suggests that it is chronic ischemia resulting from the disease, rather than disuse, that is the major factor causing fiber atrophy (6). Fiber area has been shown to be reduced in patients with symptomatic PAD compared to healthy age-matched controls (2,7). Resistance training in patients with PAD provides a unique model of how skeletal muscle responds to chronic ischemia. As far as we know, no investigators have studied skeletal muscle changes in response to long-term (i.e., 24 week) resistance training in this population. Previous studies involving both human and rodents have shown that heavy resistance training induces adaptations in MHC isoforms (8–11). We hypothesized that a transformation from MHC IIB toward MHC IIA would occur in subjects with PAD, although this relationship has not yet been verified with long-term progressive resistance training. It also appears that no training studies have evalu-
ated the possibility of changes in muscle capillarization in subjects with PAD.

The findings of decreased muscle strength and size of the plantar flexor muscles in patients with PAD indicates that resistance training has potential as a successful intervention, mediating positive benefits for walking and strength. However, the efficacy of long-term (i.e., >3 months) resistance training programs has not been determined in patients with symptomatic PAD. As skeletal muscle abnormalities are an important limitation to exercise tolerance in patients with PAD, and muscular strength affects their ability to perform daily tasks, the aim of this study was to investigate the effects of resistance training on the walking performance, strength, and skeletal muscle adaptation of subjects with symptomatic PAD.

**METHODS**

**Experimental Approach and Design**

Subjects underwent testing in pretraining, after 12 weeks of training, and after 24 weeks of training, representing a two-group repeated measures design. The training group performed a long-term periodized resistance training program for a total of 24 weeks and were compared with a non-exercising control group. In order to address the question of how diseased muscle adapts to resistance training, subjects with PAD were matched for age and severity of claudication before being randomly assigned to either of the two experimental groups. This long-term study design enabled us to evaluate the changes in skeletal muscle in response to resistance training as a primary mode of exercise rehabilitation for patients with PAD.

**Subjects.**—Twenty patients were recruited and randomized into the study. Patients in the training \((n = 11)\) and control groups \((n = 9)\) were of similar age, body mass index (BMI), duration of claudication, resting ankle/arm systolic blood pressure ratio (ABI) and postexercise ABI (see Table 1). Two patients from the training group and two from the control group withdrew from the study because of circumstances unrelated to the investigation.

Subjects were recruited by means of referrals from the Vascular Clinic at St. Vincents Hospital, Lismore, and local newspaper advertisements. Subjects were diagnosed by a vascular surgeon, and PAD was confirmed by an ABI of less than 0.94 at rest that decreased to less than 0.73 after exercise (12). In addition, subjects completed a medical health screening questionnaire to determine their medical history and level of physical activity. As a way to exclude patients with severe PAD and to control for confounding variables, the following exclusion criteria were used: (1) leg pain at rest; (2) ischemic ulceration or gangrene; (3) inability to walk at least 2 km/h on a treadmill; (4) limited exercise capacity, by factors other than claudication (e.g., symptoms of angina, congestive heart failure, chronic obstructive pulmonary disease, or arthritis); (5) vascular surgery or angioplasty undergone within the previous year; and (6) smoking of cigarettes. Because of the difficulty in recruiting sufficient numbers of subjects who met the inclusion criteria, both men and women were included in the study.

Prior approval by the Human Experimentation Ethics Committee of Southern Cross University was obtained for this experiment. All subjects were informed of the risks associated with participation in this study and were asked to sign an informed consent document prior to any testing.

**Resistance Training Program**

The experimental training group performed a resistance training program 3 days per week throughout the 24-week experimental period (Table 1). Training sessions were separated by a minimum of 48 hours (e.g., Monday, Wednesday, and Friday). Subjects completed two sets of each exercise to ensure adequate stimulus, and the number of repetitions was varied by using linear periodization throughout the program (Table 2). Linear periodization was used to ensure adequate recovery time and to optimize the quantity of the muscle activated (13). If the number of repetitions exceeded the designated repetition range by 2 or more, the resistance was increased for the next training session (Table 3). A rest period of 1 minute between sets was used to increase the demand on blood flow to the exercising muscles, thus promoting muscle ischemia and claudication pain.

At the beginning and end of each training session, subjects completed 3 minutes of stationary cycling followed by stretching of the major muscle groups to provide a warm-up and cooldown. Two familiarization sessions were performed at the beginning of the training program. Correct lifting techniques were explained and demonstrated to the subjects, who then practiced the exercises. A personal trainer supervised all subjects throughout the course of the training program, and continuous electrocardiogram (ECG) and blood pressure measurements were monitored periodi-

### Table 1. Physical Characteristics of Training and Control Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Training</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Age (y)</td>
<td>70 ± 6</td>
<td>69 ± 6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70 ± 13</td>
<td>81 ± 15</td>
</tr>
<tr>
<td>BMI</td>
<td>27 ± 3</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>Claudication (y)</td>
<td>2.5 ± 1.9</td>
<td>1.9 ± 1.1</td>
</tr>
<tr>
<td>ABI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>resting</td>
<td>0.61 ± 0.19</td>
<td>0.67 ± 0.17</td>
</tr>
<tr>
<td>postexercise</td>
<td>0.27 ± 0.10</td>
<td>0.34 ± 0.21</td>
</tr>
</tbody>
</table>
Partitions.

Maximum; it is the maximum load that can be lifted for a prescribed number of repetitions.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>RM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–4</td>
<td>12–15</td>
</tr>
<tr>
<td>5–8</td>
<td>10–12</td>
</tr>
<tr>
<td>9–12</td>
<td>8–10</td>
</tr>
<tr>
<td>13–16</td>
<td>12–15</td>
</tr>
<tr>
<td>17–20</td>
<td>10–12</td>
</tr>
<tr>
<td>21–24</td>
<td>8–10</td>
</tr>
</tbody>
</table>

*This is the number of repetitions performed per set. RM = repetition maximum; it is the maximum load that can be lifted for a prescribed number of repetitions.

Calf raise on leg press machine and leg extensions were always made at the start and end of each training session.

Treadmill Testing

Subjects were tested by using a graded treadmill protocol that began at an initial exercise intensity of 3 km/h at 0% grade for 2 minutes to assess their pain-free and maximal walking capacity (14). The grade was increased 2% every 2 minutes while the speed was held constant. During the treadmill test, subjects indicated when the onset and maximal claudication pain occurred. A pain scale ranging from 0 to 10 was used to assist the subjects in identifying their rate of perceived pain (RPP) during and following the treadmill test. The time in seconds was recorded for each of these events. Onset of claudication pain was described as the first feeling of pain, discomfort, or numbness in the calf, and maximal claudication pain was the maximum pain the subject could tolerate and resulted in termination of the test. The values for both the onset to claudication pain time and the maximum walking time are highly reliable when this test protocol is used (14).

Six-Minute Walk Test

The 6-minute volitional walking test was conducted over a 30 meter, flat, indoor track. This test was used to measure the distance the subjects could walk until they experienced claudication pain and the total distance that could be covered in 6 minutes. Subjects were instructed to walk from end to end, covering as much ground as they could during the allotted 6 minutes or until claudication pain forced them to stop. Subjects were allowed to rest if necessary, but they were encouraged to continue walking once the claudication pain subsided sufficiently. The distance covered until claudication pain occurred and total distances covered were recorded for analysis. The 6-minute walk test has been found to be a safe, reliable measure of functional exercise capacity in patients with symptomatic PAD (15).

Hemodynamic Measurements

Resting ABI and postexercise ABI were determined by using previously accepted methods to assess changes in peripheral blood flow in response to training and to objectively measure the severity of the disease (14). Subjects rested in the supine position for 20 minutes before any measurements were obtained. Standard size (10 cm) ankle blood pressure cuffs were placed around each subject’s ankle and arm. Systolic blood pressures were measured twice in the dorsalis pedis and posterior tibial arteries of each ankle, using a nondirectional Doppler flow detector ultrasound (Hokanson, Bellevue, WA) and pencil probe (9.3 MHz), and in each arm by auscultation. The higher of the two arterial pressures was recorded as the resting ankle systolic pressure. One minute after completion of the graded treadmill test, the ABI was recorded in both legs. The most diseased leg was determined from the lowest rest ABI and postexercise ABI.

10 RM Strength Testing

The strength evaluation included a 10 repetition maximum (RM) loading (i.e., maximum load with which the subject could perform 10 repetitions), using a Universal leg press machine (Universal Equipment, Cedar Rapids, IA). The 10 RM was chosen because the resistance training program used higher repetitions (i.e., 8–15 RM), and it was believed that this test would be more specific for this population. The test position for the leg press and calf press had the subject in a seated position with the ball of the foot placed on the footplate of the leg press machine. For the leg press the knee angle was set at 70° and the subject was instructed to extend his or her legs slowly and then return them to the start position. For the calf press the subject began the test with the knees fully extended, plantarflexed the ankle to the maximum range of motion, and then returned to the start position. Subjects were familiarized with the exercise technique and given a practice test on a different day to remove the learning effect of the test. Continuous ECG and blood pressure responses were monitored throughout the strength evaluation.

Muscle Biopsies

Percutaneous muscle biopsies were taken from the medial head of the gastrocnemius muscle before and after 24 weeks of resistance training. The biopsy samples were mounted in an embedding medium, frozen in isopentane precooled by liquid nitrogen, and stored at −80°C until analysis. Because of a possible variation in fiber type distribution from superficial to deep and from proximal to distal sites, special care was taken to extract the tissue from approximately the same location for the postraining biopsy by using the pretraining biopsy scar (~0.5 cm from scar, going from medial to lateral) and by using a marked needle depth.
The biopsies were performed approximately 1 week before beginning the training program and within 2 days of completing the training program.

Fiber Type Distribution and Area
Serial sections (12 μm) were cut in a cryostat at −23°C for histochemical analysis. A fiber type analysis was performed by using the myosin adenosine triphosphatase (mATPase) method at a pH of 9.4 after optimized acid (pH 4.6) or alkaline (pH 10.3) preincubations (16). Fibers were classified as types I, IIA, and IIB, based on staining intensities, with type IIAB fibers classified with the type IIB fibers for quantification (17). In addition, further samples were stained for reduced nicotinamide adenine dinucleotide (NADH)-tetrazolium reductase to reduce shrinkage effects from the alcohol used during the mATPase histochemical staining (18). Cross-sectional areas were determined for each major fiber type (I, IIA, IIB/AB) by measuring at least 50 fibers per type, using the Scion Image program (Scion Corp., Frederick, MD; 19).

MHC Analysis
For each biopsy sample, 3–5 additional serial cross sections (40 μm thick) were lysed for 10 minutes at 60°C in 0.5 ml of a medium containing 36.25% (wt/vol) glycerol, 6.25% (vol/vol) 2-mercaptoethanol, and 2% (wt/vol) sodium dodecyl sulfate (SDS) in Tris-HCl buffer (pH 6.8). The extracts were subsequently loaded for electrophoresis on an 8% gradient SDS-polyacrylamide gels with 4% stacking gels, run overnight (17–19 hours) at 150 V, and stained with Silver Stain. The MHC isoforms were identified according to their apparent molecular masses compared with those of marker proteins. Gels were scanned and analyzed by using the Scion Image program to determine the MHC content. In our laboratory, the coefficient of variation for repeated measurement of MHC of a given sample is <4%.

Muscle Capillarization
The amylase-PAS (periodic acid-Schiff) reagent method was used as previously described, was optimized for our assay conditions (20). Briefly, sections were cut at 12 μm and stained with PAS reagent after digestion of glycogen with 1% amylase. Sections were magnified by using a light microscope, and they were photographed (Olympus BH-2 Imaging System, Olympus America Inc., Melville, NY) to determine capillary density, capillary-to-fiber ratio, and the number of capillaries in contact with each muscle fiber. An area with at least 50 fibers was selected and used for the capillary analyses (19).

Statistical Analysis
Fiber type percentages and areas were log transformed and then analyzed. Statistical analyses of the data were performed with a general linear model for an analysis of variance (ANOVA) with repeated measures to compare within (pretraining and posttraining) and between groups (experimental, control). As there were significant main effects of trial as well as interaction of Trial × Group, probability-adjusted paired t tests and a one-way ANOVA were used for follow-up analyses. There were no significant differences between groups in any variables at week 0. Selected bivariate relationships were examined by using simple regression analysis. Values are reported as mean ± SD. Significance in this study was set at p < .05. Based on previous research investigating changes in fiber area and shifts in MHC isoforms, an effect size of 1.2 was assumed at an alpha level of .05 (two-tailed test). Therefore, the sample size required was calculated to be 18 subjects, resulting in a statistical power of 0.80 (21).

Results

Hemodynamic Measures
Resting systolic brachial pressure decreased significantly in the training group following 12 weeks of training (Table 4). There were no significant changes in any of the hemodynamic variables in the control group.

Walking Tests
For the graded treadmill test, there was a 158% significant increase in onset to claudication pain time in the training group (Figure 1). For the 6-minute walk, there was an 86% significant increase in distance to claudication pain in the training group (Table 5). No changes in walking distances and times were observed in the control group.

10 RM Strength Results
After 12 weeks, there was a significant 111% increase in 10 RM leg press strength and 79% increase in 10 RM calf press strength in the training group (Figure 2). Following 24 weeks, the 10 RM leg press strength improved significantly by 155% and the 10 RM calf press strength by 126% in the trained subjects (Figure 2). The trained group was significantly stronger than the control group in the 10 RM calf press and leg press at both 12 weeks and 24 weeks. There were no significant gains in strength measures during the study by the control group.

Changes in MHC Isoform Expression
Pretraining, there were no significant differences between the groups in expression of the MHC isoforms (Table 6). Following the 24-week training program, the training group had significantly decreased the percentage of MHC IIB. There was no significant change in the percentage of MHC I.
or MHC IIA after the training program. There were no significant changes in MHC isoform expression in the control group following the training program.

**Changes in Muscle Fiber Type and Fiber Area**

The biopsies of the control group did not change in the proportion of fiber type percentages or fiber area. In contrast, the percentage of type IIB/AB fibers was significantly decreased with resistance training, and type IIA fibers were correspondingly increased (Table 7).

No changes occurred in control subjects before and after the 24-week training program for any of the fiber areas. In contrast, all three major fiber types were significantly larger after resistance training, with types I, IIA, and IIB/AB increasing by 28%, 25%, and 24%, respectively (Table 8).

**Capillarization**

The results for the various capillary measurements before and after the 24 weeks of training are shown in Table 9. Capillary density increased significantly in the training group by 18%. The capillary-to-fiber ratio and capillaries in contact with each muscle fiber did not change significantly in the training group. There were no significant changes in any of the capillary measurements in the control group following the 24-week intervention period.

**DISCUSSION**

The aim of this investigation was to examine the effects of a long-term resistance training program on MHC isoform expression, fiber type, fiber area, and capillarization in patients with symptomatic PAD. To our knowledge, no previous research has investigated changes in skeletal muscle with resistance training in this clinical population. In addition, very few investigators have provided a comprehensive overview of resistance-training-induced changes in skeletal muscle including MHC isoforms, fiber type, and capillarization in aged populations (22,23). We demonstrated a sig-

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**Figure 1.** A, Time to onset of claudication pain during graded treadmill test at pretraining, 12 weeks, and 24 weeks in the trained and control groups. B, Maximum walking time during graded treadmill test at pretraining, 12 weeks, and 24 weeks in the trained and control groups.

**Figure 2.** A, 10 RM calf press strength at pretraining, 12 weeks, and 24 weeks in the trained and control groups. B, 10 RM leg press strength at pretraining, 12 weeks, and 24 weeks in the trained and control groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group1</th>
<th>Pretraining</th>
<th>12 Weeks</th>
<th>24 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-min claudication onset</td>
<td>T</td>
<td>131 ± 83</td>
<td>171 ± 88*</td>
<td>244 ± 119**</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>132 ± 76</td>
<td>106 ± 26</td>
<td>116 ± 53</td>
</tr>
<tr>
<td>Distance (m)</td>
<td>T</td>
<td>371 ± 76</td>
<td>425 ± 79*</td>
<td>456 ± 67</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>368 ± 105</td>
<td>358 ± 99</td>
<td>345 ± 110</td>
</tr>
</tbody>
</table>

*Significantly different from pretraining (p < .05).
**Significantly different from week 12 (p < .05).
1T = training; C = control.

**Table 5. Changes in 6-min Walk Performance in the Training and Control Groups**

**Table 6. Percentages of MHC IIB, MHC IIA, and MHC I Isoforms Before and After the 24-Week Resistance Training Program for the Training and Control Groups**

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Training Before</th>
<th>After</th>
<th>Control Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC IIB</td>
<td>24 ± 8</td>
<td>15 ± 5*</td>
<td>17 ± 8</td>
<td>20 ± 10</td>
</tr>
<tr>
<td>Male</td>
<td>24 ± 7</td>
<td>14 ± 2*</td>
<td>17 ± 8</td>
<td>24 ± 10</td>
</tr>
<tr>
<td>MHC IIA</td>
<td>30 ± 8</td>
<td>36 ± 10</td>
<td>32 ± 9</td>
<td>28 ± 9</td>
</tr>
<tr>
<td>Male</td>
<td>37 ± 1</td>
<td>43 ± 10</td>
<td>29 ± 10</td>
<td>27 ± 9</td>
</tr>
<tr>
<td>MHC I</td>
<td>45 ± 9</td>
<td>49 ± 7</td>
<td>51 ± 10</td>
<td>52 ± 12</td>
</tr>
<tr>
<td>Male</td>
<td>47 ± 10</td>
<td>52 ± 5</td>
<td>45 ± 8</td>
<td>49 ± 12</td>
</tr>
<tr>
<td>Female</td>
<td>40 ± 6</td>
<td>43 ± 9</td>
<td>55 ± 10</td>
<td>55 ± 14</td>
</tr>
</tbody>
</table>

Notes: Percentages are mean ± standard deviation. MHC = myosin heavy chain.
*Significantly different from pretraining (p ≤ .05) and significant difference between training and control group (p ≤ .05).
significant reduction in MHC IIB isoforms and a significant increase in MHC IIA isoforms. The type I, IIA, and IIB/AB fibers increased significantly following the training, and there was a significant increase in capillary density. There was also a significant improvement in 10 RM strength as well as improvements in walking capacity. It was also demonstrated that the periodized resistance training program was well tolerated by the subjects with PAD and that there were no adverse effects, most likely because of careful supervision and exercise prescription.

**Hemodynamic Measures**

No significant changes were observed in the hemodynamic measures after the training program other than systolic brachial blood pressure, which decreased significantly. During this training study we used a linear periodized program and help in producing effective exercise stimuli (28). Studies have demonstrated the superiority of periodized programs in elderly subjects (29). However, several investigations that have investigated the effects of periodized programs throughout the 24 weeks in order to reduce boredom with the same program and to allow for recovery. There are few studies that have investigated the effects of periodized programs in elderly subjects (29). However, several investigators have demonstrated the superiority of periodized pro-

### Table 7. Fiber Type Percentages Determined by ATPase Histochemistry Before and After the 24-Week Resistance Training Program for the Training and Control Groups

<table>
<thead>
<tr>
<th>Percentage</th>
<th>Training Before</th>
<th>Training After</th>
<th>Control Before</th>
<th>Control After</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIB/AB</td>
<td>21 ± 4</td>
<td>14 ± 3</td>
<td>21 ± 8</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>22 ± 5</td>
<td>15 ± 3</td>
<td>24 ± 9</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>male</td>
<td>21 ± 4</td>
<td>12 ± 2</td>
<td>20 ± 8</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>IIA</td>
<td>30 ± 4</td>
<td>36 ± 4</td>
<td>28 ± 3</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>31 ± 7</td>
<td>35 ± 3</td>
<td>28 ± 6</td>
<td>32 ± 7</td>
</tr>
<tr>
<td>male</td>
<td>25 ± 6</td>
<td>38 ± 5</td>
<td>28 ± 2</td>
<td>26 ± 2</td>
</tr>
<tr>
<td>I</td>
<td>49 ± 8</td>
<td>50 ± 8</td>
<td>51 ± 8</td>
<td>50 ± 7</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>47 ± 8</td>
<td>51 ± 4</td>
<td>48 ± 3</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>male</td>
<td>54 ± 9</td>
<td>49 ± 6</td>
<td>52 ± 10</td>
<td>53 ± 5</td>
</tr>
</tbody>
</table>

*Significantly different from pretraining (p ≤ .05) and significant difference between training and control group (p ≤ .05).

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agrees with prior findings of no improvement in hemodynamic measures following exercise rehabilitation (24,25). These findings indicate that factors that are distal to the site of arterial occlusion may be responsible for the claudication. It has been suggested by other investigators that alterations in skeletal muscle metabolism may contribute to the type of limited exercise performance (4). These changes are thought to be caused by exercise-induced ischemia and are therefore specific to the muscles that are affected by the reduced blood flow. Claudication pain is typically experienced in the calf muscle (26), and therefore specific exercises were used in our program that would target these muscles and probably helped mediate the changes observed.

**Walking Tolerance**

For the graded treadmill test, there was a significant 59% increase in maximum walking time and a significant 158% increase in onset to claudication pain time (see Figure 1). The changes in onset to claudication pain time are similar to those in previous studies that have used walking as the basis of the exercise rehabilitation (27). Most likely, differences in the variables of the program design, such as intensity, duration, and frequency of exercise sessions, account for the large variation in improvements seen with training studies. Also the severity and extent of the disease, along with differing degrees of accompanying risk factors, could be responsible for the varied responses to exercise. Studies have also shown that supervised programs result in greater improvements in walking distances compared with home-based programs, most likely as a result of the encouragement and help in producing effective exercise stimuli (28). During this training study we used a linear periodized model to vary the volume and intensity of the training throughout the 24 weeks in order to reduce boredom with the same program and to allow for recovery. There are few studies that have investigated the effects of periodized programs in elderly subjects (29). However, several investigators have demonstrated the superiority of periodized pro-

### Table 8. Fiber Areas in the Gastrocnemius Muscle Before and After the 24-Week Resistance Training Program for the Training and Control Groups

<table>
<thead>
<tr>
<th>Parameter (µm²)</th>
<th>Training Before</th>
<th>Training After</th>
<th>Control Before</th>
<th>Control After</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIB/AB total</td>
<td>3440 ± 999</td>
<td>4249 ± 1039***</td>
<td>2889 ± 884</td>
<td>3081 ± 755</td>
</tr>
<tr>
<td>female</td>
<td>2542 ± 550</td>
<td>4087 ± 866***</td>
<td>2436 ± 918</td>
<td>2926 ± 1343</td>
</tr>
<tr>
<td>male</td>
<td>3777 ± 929</td>
<td>4310 ± 1180**</td>
<td>3115 ± 854</td>
<td>3159 ± 410</td>
</tr>
<tr>
<td>IIA total</td>
<td>3421 ± 1148</td>
<td>4273 ± 1113**</td>
<td>3559 ± 1407</td>
<td>3411 ± 838</td>
</tr>
<tr>
<td>female</td>
<td>2576 ± 614</td>
<td>4047 ± 978***</td>
<td>3149 ± 463</td>
<td>2978 ± 781</td>
</tr>
<tr>
<td>male</td>
<td>3738 ± 1164</td>
<td>4358 ± 1211***</td>
<td>3764 ± 1712</td>
<td>3628 ± 843</td>
</tr>
<tr>
<td>I total</td>
<td>2695 ± 867</td>
<td>3442 ± 981*</td>
<td>3134 ± 594</td>
<td>3012 ± 742</td>
</tr>
<tr>
<td>female</td>
<td>2249 ± 726</td>
<td>3285 ± 951*</td>
<td>3026 ± 865</td>
<td>2999 ± 843</td>
</tr>
<tr>
<td>male</td>
<td>2863 ± 897</td>
<td>3501 ± 1050**</td>
<td>3189 ± 503</td>
<td>3041 ± 649</td>
</tr>
</tbody>
</table>

*Significantly different from pretraining (p ≤ .05).
**Significant difference between training and control group (p ≤ .05).

### Table 9. Mean ± SD Capillary Morphological Measurements Before and After the 24-Week Resistance Training Program for the Training and Control Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Training Before</th>
<th>Training After</th>
<th>Control Before</th>
<th>Control After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary Density (cap/mm²) total</td>
<td>287 ± 46</td>
<td>338 ± 39***</td>
<td>279 ± 35</td>
<td>282 ± 37</td>
</tr>
<tr>
<td>female</td>
<td>294 ± 51</td>
<td>334 ± 37*</td>
<td>306 ± 30</td>
<td>303 ± 50</td>
</tr>
<tr>
<td>male</td>
<td>269 ± 29</td>
<td>351 ± 51***</td>
<td>265 ± 31</td>
<td>271 ± 27</td>
</tr>
<tr>
<td>Capillary/Fiber Ratio total</td>
<td>1.65 ± 0.23</td>
<td>1.74 ± 0.09**</td>
<td>1.47 ± 0.21</td>
<td>1.52 ± 0.28</td>
</tr>
<tr>
<td>female</td>
<td>1.71 ± 0.18</td>
<td>1.75 ± 0.09***</td>
<td>1.54 ± 0.11</td>
<td>1.36 ± 0.28</td>
</tr>
<tr>
<td>male</td>
<td>1.50 ± 0.32</td>
<td>1.73 ± 0.11***</td>
<td>1.44 ± 0.24</td>
<td>1.60 ± 0.26</td>
</tr>
<tr>
<td>Capillary Contacts total</td>
<td>3.80 ± 0.22</td>
<td>4.15 ± 0.32**</td>
<td>3.48 ± 0.27</td>
<td>3.55 ± 0.39</td>
</tr>
<tr>
<td>female</td>
<td>3.85 ± 0.13</td>
<td>4.17 ± 0.31**</td>
<td>3.49 ± 0.30</td>
<td>3.88 ± 0.53</td>
</tr>
<tr>
<td>male</td>
<td>3.66 ± 0.37</td>
<td>4.09 ± 0.40**</td>
<td>3.47 ± 0.29</td>
<td>3.39 ± 0.20</td>
</tr>
</tbody>
</table>

Note: Capillary measurements are mean ± standard deviation.
*Significantly different from pretraining (p ≤ .05).
**Significant difference between training and control group (p ≤ .05).
programs over fixed programs in young subjects and therefore justified this approach (13).

Significant improvements were demonstrated in 6-minute walk distance and onset to claudication distance following 24 weeks of resistance training. The 6-minute walk test has been found to be a safe, reliable measure of functional exercise capacity in subjects with PAD, and it requires minimal equipment (30). The six-minute walk test proved highly acceptable to the patients in our studies, and reproducible results were achieved after just one familiarization test. A recent study of older adults performing strengthening exercises at home found no improvements in 6-minute walk distance (31). However, other studies with older adults have found that 12 weeks of resistance training can improve walking endurance (32). The primary factor for some studies’ having not improved walking endurance may be that the programs used low intensity (i.e., heavy enough resistance). Our data show that intensity can be moderate to heavy if conducted in a supervised program. Also, the programs are generally short term (8–12 weeks duration), which only start to stimulate early phase adaptations. Long-term programs may be required to increase walking endurance. It has been suggested by some investigators that an increase in the onset of claudication pain may be of greater practical significance, as individuals do not usually push themselves to maximal pain during normal daily activities (33).

**Muscle Fiber Area**

Our results indicate that the resistance training resulted in significant increases in muscle fiber area. Previous studies have demonstrated fiber hypertrophy in older adults with resistance training (34). Several studies have reported a more pronounced increase in the type II fiber subtypes following heavy resistance training (35). It has been indicated that the fiber-specific responses differing between studies may reflect the different training programs used and the varying duration of the programs (36). In our study there was a significant area increase in both type I and II fibers in response to resistance training. Hypertrophy of both type I and II fibers is consistent with previous studies with older subjects (23). There were similar responses to the training program in both the men and women, with large increases in fiber area from pre-training to posttraining. The wider range of resistance loads used in the present program may have resulted in recruitment of both fast and slow motor units.

Hiatt and colleagues (37) did not find fiber hypertrophy in subjects with PAD following 12 weeks of resistance training. The training program used cuff weights, and it may have been that the intensity of the training was insufficient to elicit hypertrophy. The program in the present study was designed to result in claudication pain by keeping the rest periods between sets to 1 minute. The initial 4 weeks involved 12–15 RM to help promote muscle ischemia, and then the repetitions were decreased to 10–12 RM and finally 8–10 RM in weeks 9–12 to help increase muscle hypertrophy and strength. Research has shown that performing exercises with varying repetition maximums elicits different physiological adaptations, and it has been demonstrated that manipulating the sets and repetitions during a resistance training program produces greater strength gains (13). Periodized training manipulations have been shown to be important for aiding recovery and avoiding overtraining while keeping the program interesting (38).

**MHC Isoforms and Fiber Type Composition**

To our knowledge, no previous studies have investigated changes in MHC isoforms with exercise rehabilitation in subjects with PAD. This also appears to be the first study that has investigated changes in the fiber subtypes with resistance training in patients with PAD. It is accepted that with resistance training, changes in MHC isoforms occur early in the training program (9). Following the resistance training program, there was a significant decrease in MHC IIB isoforms and a significant decrease in Type IIB/AB fibers, with a corresponding increase in IIA fibers as determined by measuring mATPase histochemistry. The shift toward the MHC IIA isoform may not necessarily be a strength adaptation; rather it may be an adaptation toward a more effective recruitment and utilization of the muscle following the high-threshold training that occurs with resistance exercise. When the type IIB fibers are recruited for activities such as resistance training, they improve in oxidative ability and changes in the MHC and histochemical profile are initiated and continue until few type IIB fibers remain (34). There were still a number of type IIB fibers remaining following the training program (~14%). It would seem that they were either not recruited by the resistance training or have higher oxidative enzyme levels and had not made the protein changes needed for isoform transformation (8,39). It could also be that these did not represent true IIB fibers but rather hybrid IIAB fibers that we did not delineate with either mATPase staining or electrophoresis. Ploutz and colleagues (39) have shown that if the presence of IIAB fibers has not been determined, the remaining type IIB fibers after heavy resistance training may not be true IIB fibers, as they have been shown to have higher concentrations of oxidative enzymes. However, our results do show that elderly diseased subjects have the ability to produce similar shifts in the expression of MHC isoforms, as has been seen in healthy older adults (23). This study highlights the plasticity of human skeletal muscle at older ages and with the presence of intermittent ischemia.

**Capillarization**

The increase in fiber size seen in the trained subjects was accompanied by a significant increase in capillary density and in the number of capillary contacts. Increased capillary density, and hence a more efficient oxygen delivery, may be a key factor in an improved walking distance. It has been indicated that changes in capillary density might contribute to the functional improvements with training. Previous studies have demonstrated increases in capillary density with resistance training (40). It appears that programs that emphasize higher repetitions (e.g., greater than 10 repetitions) may induce capillary proliferation (41). Also, it has been indicated that a base level of capillarization may exist for activated muscle fibers and, with additional overload, may be increased to address the functional demands of the fibers by way of the resistance training program (34). It does appear that, in the present study, fiber area increased as a result of
the resistance training without compromising the capillar-
ization of the muscle. Previous research with resistance
training in older adults has shown that this mode of training
provides an effective training stimulus for improving VO2
peak (40). Hepple and colleagues (40) demonstrated that 9
weeks of resistance training resulted in maintenance of the
capillary density and significantly increased the indices of
the capillary to fiber surface interface. Hagerman and col-
leagues (22) found that significant hypertrophic and fiber
conversion responses were also accompanied by an increase
in the number of capillaries per fiber, and because capillary
density remained unchanged, there was no “dilution effect.”

Conclusions

It does appear that the strength training offered signifi-
cant improvements in strength in addition to improving
walking distances. Periodized resistance training resulted in
significant shifts in MHC isoforms and fiber type deter-
mined by mATPase histochemistry. This is the first study to
measure changes in muscle fiber characteristics in PAD
muscle in response to resistance training. Specifically, there
was a significant reduction in MHC IIB and a significant in-
crease in MHC IIA. Both type I and type II fiber areas were
increased and there was a significant increase in capillary
density following the 24-week resistance training program.

It appeared that the symptomatic subjects with PAD in the
present study responded in a manner similar to that of
healthy older adults. Furthermore, there were no adverse
consequences or injuries associated with the training proto-
col. This study indicates that a long-term resistance training
program is an effective mode of rehabilitation for impro-
ving the walking ability and strength of patients with symp-
tomatic PAD. The data also indicate a dynamic plasticity of
muscle in patients with PAD in response to heavy resistance
training.

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