



Effects of supervised treadmill-walking training on calf muscle capillarization in patients with intermittent claudication

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Effects of supervised treadmill-walking training on calf muscle capillarization in patients with intermittent claudication

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Abstract

The aim of this study was to evaluate the effects of supervised treadmill-walking training on the calf muscle capillarization in patients with intermittent claudication. The first 12-week period was a non-exercise, within-subject control stage and the second 12-week period was an exercise training stage. Calf muscle biopsy samples were obtained at baseline, pre- and post-exercise training. Functional capacities were measured after each biopsy. Eleven subjects completed all procedures. Their average age was (mean \pm SD) 73.9 ± 5.5 years, resting ankle-to-brachial index (ABI) 0.57 ± 0.11 and post-exercise ABI 0.40 ± 0.14 at baseline. After exercise training, there was no significant change in calf muscle capillarization, though walking capacity and peak oxygen uptake were increased significantly. The difference between the pre- and post-training capillaries in contact with type IIX and IIA muscle fibers for each subject was significantly correlated with an improved pain-free walking time, $r=0.69$ and $r=0.62$ (both $p<0.05$), respectively. This finding suggests that the change in calf muscle capillarization might contribute to improved walking capacity following exercise training in subjects with intermittent claudication.

Introduction

Peripheral arterial disease (PAD) is one of the major atherosclerotic diseases with stenosis and occlusion of the peripheral arteries, mostly in the lower limb.^{1,2} The characteristic complaint of PAD is intermittent claudication (IC), a severe muscular pain in the leg in response to walking a reproducible distance.^{3,4} IC seriously limits walking capacity of the patients. Supervised treadmill-walking training has been demonstrated as an effective treatment for patients with IC. The training benefits the patients by improving walking capacity, delaying occurrence of claudication and increasing tolerance to pain.⁵⁻⁸ Research has examined whether the improved walking capacity was related to the changes in skeletal

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muscle histology,⁹ muscular enzyme activities,⁹ walking economy,^{6,10} and muscle strength or endurance.^{7,11} However, the exact mechanism for the beneficial effects is still unclear.

Walking induced claudication has been speculated to be a result of an inadequacy of regional blood flow to meet metabolic demands of the leg muscles.^{2,3} It can be hypothesized that an improved blood supply to the muscles would be one of the mechanisms for the delayed onset of claudication and improved walking capacity after exercise. Previous studies have measured blood flow^{5,6,12-14} and systolic blood pressure^{5-7,12,14-16} in the leg in patients with IC. However no conclusive evidence has been obtained with respect to the changes in blood circulation after exercise training. Another potential mechanism for the improved walking capacity is the capillarization in the muscle. A review of muscle blood flow and aerobic capacity in healthy older people has demonstrated that the increase in muscle capillarization after endurance training would enhance oxygen and nutrient exchanges within the tissue by providing a larger surface area and shorter diffusion distance.¹⁷ To the best of our knowledge, there has been no study that has examined calf muscle capillarization in adaptation to supervised treadmill-walking training in patients with IC. In the present study, we aimed to evaluate the effect of 12 weeks of supervised treadmill-walking training on muscle capillarization and the relationship between the changes in walking capacity and muscle capillarization in subjects with IC.

Methods

Design

An original purpose of the study was to evaluate whether exercise training combined with a treatment of ginkgo biloba (a natural herbal) would produce better results in improving walking capacity and other physiological variables than exercise training plus placebo. The study was designed with two 12-week stages. Subjects were randomly allocated into a ginkgo group or a placebo group by a third-party. During the first stage, subjects took either ginkgo

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3 or placebo tablets in a double-blinded manner and were instructed to make no major changes
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5 in their diet and exercise habits. During the second stage, all subjects participated in a
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7 supervised treadmill-walking program while continued with the ginkgo or placebo treatment.
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9 Functional tests and muscle biopsy for histochemical analysis of muscle fiber types and
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11 capillarization were performed at the baseline, and the end of each 12-week stage. The
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13 performance tests detected no significant changes in the measured variables between the
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15 combined treatment and exercise training plus placebo.¹⁸ Therefore, the data of the two
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17 groups were collapsed to evaluate the effects of exercise training on the measured variables.
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19 The first stage was defined as the control stage and the second stage as the training stage. The
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21 focus of this paper was to examine the effect of the 12-week supervised treadmill-walking
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23 training on calf muscle capillarization in subjects with IC and the correlations between the
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25 potential changes in walking capacity, peak oxygen uptake (peak $\dot{V}O_2$), ankle-to-brachial
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27 systolic blood pressure index (ABI), and calf muscle capillarization after exercise training.
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34 **Subjects**

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36 Volunteer subjects were recruited from the local population in the Northern Rivers region of
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38 New South Wales, Australia. The inclusion criteria were 1) 50-80 years of age; 2) resting
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40 ABI was lower than 0.9 and decreased to lower than 0.8 after a treadmill-walking test; and 3)
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42 a stable history of IC for at least six months at the initial interview. The exclusion criteria
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44 were 1) resting ischemic pain, ulceration, or gangrene in the legs; 2) inability to walk on the
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46 treadmill at a speed of 3.2 km/h; and 3) exercise capacity limited by symptoms of angina,
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48 congestive heart failure, chronic obstructive pulmonary disease, or arthritis. This study was
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50 approved by the Human Research Ethics Committee of Southern Cross University, Australia.
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52 Written informed consent was obtained from each subject before the baseline test.
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58 **Functional measurements**

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3 A graded treadmill test was used to determine walking capacity.¹⁹ The time to the onset of
4 claudication was recorded as the pain-free walking time (PFWT) and the time until exercise
5 was ceased due to maximal claudication was recorded as the maximal walking time (MWT).
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10 A 5-point scale was applied to identify the intensity of pain,⁷ in which 1 = no pain; 2 = onset
11 of claudication; 3 = mild pain; 4 = moderate pain; 5 = maximal claudication. Peak $\dot{V}O_2$ was
12 assessed during the treadmill test using a MedGraphics CPX/D System (Medical Graphics
13 Corporation, USA) and was defined as the average of the two highest and connected 15-
14 second $\dot{V}O_2$ achieved during the test.²⁰

22 **Peripheral hemodynamics**

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24 A bi-directional Doppler unit MD5 (Hokanson, USA) was used to measure blood pressures.
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27 The ABI was calculated as the systolic pressure at the posterior tibial or dorsalis pedis
28 arteries (the higher data was used) divided by that at the brachial artery. Resting and post-
29 exercise ABI (within two minutes after the treadmill test) were measured in both legs. All
30 measurements were performed by the same researcher.

36 **Muscle biopsy**

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38 Needle muscle biopsies were taken from the medial head of the gastrocnemius muscle in the
39 worse leg (with lower ABI). Type I, IIa, and IIx fibers were classified by staining of myosin
40 ATPase isoforms.²¹ An average of 230 muscle fibers (range 120-500) was identified in each
41 sample. The Amylase-Periodic Acid Schiff (PAS) method was used to visualize capillaries in
42 muscle tissue.²² An average of 370 capillaries (range 210-700) was identified in each sample.
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51 The number of capillaries around the muscle fibers (up to 50 randomly selected fibers for
52 each type) was assessed. The capillarization was expressed as capillary-to-fiber ratio (C/F;
53 total number of capillaries divided by total muscle fibers in the investigated area) and the
54 number of capillaries in contact with each different type of muscle fibers (CC). The
55 calculation of CC followed Pyley's method.²³

Supervised treadmill-walking program

Subjects trained in the laboratory one hour per session, three sessions per week, for 12 weeks following an individualized exercise program. They started walking on a treadmill at the speed of 3.2 km/h with an individualized incline. This combination of speed and incline elicited a moderate claudication (level 4 on the 5- point scale) after about five minutes of walking. When they felt moderate claudication in their legs, subjects were allowed to recover in a sitting position and resumed walking when the pain disappeared. When the subject could walk seven minutes without moderate claudication, treadmill incline was increased by 0.5% each time. When the incline had been increased as high as 5%, the speed was then increased 0.5km/h each time for further increase of exercise intensity. This progressive procedure aimed to continuously provide overloading while the walking capacity of the subject was improving. All training sessions were supervised by the researcher and ECG (modified lead II configuration) was monitored to ensure subjects' safety when exercise intensity was increased.

Statistical analysis

Analysis of variance (ANOVA) with repeated measures was performed to detect the overall effect of the treatment among the baseline, 12-week and 24-week tests for PFWT, MWT, peak $\dot{V}O_2$, ABI, muscle fiber type distribution and capillarization. Post-hoc analysis with Bonferroni adjustment was applied to reveal where a significant difference presented if there was a significant overall effect. Pearson product moment correlation coefficient was calculated to assess the relationships between changes in the measured variables following exercise training. All variables were presented as means \pm SD. An alpha level of 0.05 was set for significant difference. All statistical analyses were performed using the SPSS statistical software package (SPSS Version 14.0 for Windows, SPSS Inc.).

Results

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Seventeen subjects completed the 24-week program. Six of them who had only one or two muscle biopsies were not included in this paper: four of them missed the second biopsy for their personal reasons. Another two took the first two biopsies but refused the third one.

Therefore 11 subjects volunteered for all three muscle biopsies and their data was analyzed and reported in this paper. The average age of this group was 73.9 ± 5.5 years, with resting ABI 0.57 ± 0.11 and post-exercise ABI 0.40 ± 0.14 at baseline.

The PFWT, MWT, and peak $\dot{V}O_2$ did not change significantly during the control stage, but significantly increased after the training stage. Neither the resting nor the post-exercise ABI showed a significant change following exercise training (Table 1).

There was no significant difference in the muscle fiber type composition over the three muscle biopsies. For this group of subjects, nearly half of the muscle fibers in the gastrocnemius muscle were type II fibers and more type Ix fibers than type Iia fibers (Table 2). The C/F and CC with each type of muscle fiber were not significantly different between the three biopsies (Table 2).

In the hypothesis generating analysis, the difference between the pre- and post-training CC with type Ix and Iia fibers for each subject was significantly correlated with an improved PFWT, $r = 0.69$ and $r = 0.62$ (both $P < 0.05$), respectively. There were no significant correlations between the differences in CC with type I muscle fibers and C/F with the increase in PFWT following exercise training. The differences in CC with the three types of muscle fibers and C/F did not show significant correlations with the changes in MWT and peak $\dot{V}O_2$ after the training.

Discussion

The present study did not find significant changes in the calf muscle capillarization in this group of subjects, though their walking capacity and peak $\dot{V}O_2$ were increased significantly following 12 weeks of supervised treadmill-walking training. This result indicated that the

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3 substantial improvement in walking capacity was not matched by substantial improvement in
4 muscle capillarization. It is noteworthy, in the hypothesis generating analysis, that the
5 changes in capillary supply to type II fibers in the calf muscles were significantly related to
6 the increase in PFWT following training. It was found that approximately 50% of all muscle
7 fibers in the calf muscles were Type II fibers in this group of subjects. Previous research in
8 healthy older populations has reported that increased muscular capillarization can enhance
9 oxygen and nutrient exchanges within the tissue¹⁷ and is positively associated with leg blood
10 flow.²⁴ Considering the increased PFWT following training in the present study, the
11 correlations show that improved capillary supply to the calf muscles is likely to be a reason
12 for the improvement in walking capacity.
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Studies on healthy older people have reported that endurance training can significantly
increase capillary supply to skeletal muscles, which are associated with increased peak $\dot{V}O_2$ ²⁵
or metabolic enzyme activities.²⁶ It has been reported that the increased capillarization
provided a larger surface area for exchange of oxygen and other substance between the
capillaries and muscle fibers.^{17,26} Our results appeared to be in agreement with that report.
The ratio of capillaries to muscle fibers might affect oxygen and nutrient supply and delay the
mismatch between supply and demand.

The changes in calf muscle capillarization did not show significant correlation with the MWT
after training. It has been reported that local perfusion is not the only factor controlling
MWT.⁶ Other factors, such as pain tolerance, gait adaptation to treadmill walking, or
psychological factor, might have contributed to the improved maximal walking capacity.
Previous study indicated that peak $\dot{V}O_2$ of patients with PAD is more likely to be limited by
claudication and does not necessarily reflect the limit in metabolic function.²⁷ Our results
agreed with these statements and we did not find significant relationship between the
increases in MWT and peak $\dot{V}O_2$ of and the change in calf muscle capillarization. Resting or

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post-exercise ABI did not show any significant changes following training. It has been suspected that ABI may not reflect blood flow to muscle tissue during walking and this may explain its weak relationship with walking performance.²⁸

The major limitation of this study might be the relatively small number of subjects, which did not provide enough statistical power to detect significant changes in muscle capillarization following exercise training. In addition, the exercise program employed in our study may not produce sufficient training stimulus to significant adaptation in the muscle tissues. It has been reported that resistance training significantly increased capillary density in the calf muscles of patients with IC.²⁹

Conclusion

We observed that the 12-week supervised treadmill-walking training did not change calf muscle capillarization significantly, though the training resulted in a significant increase in walking capacity in this group of subjects. Significant correlations were found between the increase in PFWT and the changes in CC with type II muscle fibers. Even though with a small sample size, this result shows a trend in which the change in muscle capillarization may be a mechanism through which supervised treadmill-walking training effectively improved walking capacity in subjects with IC. In order to confirm this point of view, a larger experimental group and longer duration of exercise training may be needed in the future studies.

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Table 1 Changes in walking capacity, peak oxygen uptake and ankle-to-brachial index (ABI) at the three tests

	Baseline	12-week	24-week
PFWT (s)	116 ± 65	130 ± 62	348 ± 264**
MWT (s)	308 ± 135	323 ± 161	714 ± 191***
Peak $\dot{V}O_2$ (ml/kg/min)	12.93 ± 2.10	12.76 ± 2.35	14.90 ± 2.56*
Resting ABI	0.57 ± 0.11	0.60 ± 0.18	0.59 ± 0.15
Post-exercise ABI	0.40 ± 0.14	0.45 ± 0.29	0.41 ± 0.19

All data are presented as means ± SD

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, the 12-week test vs. the 24-week test

PFWT: pain-free walking time; MWT: maximal walking time.

Table 2 Changes in muscle fibre types and the number of capillaries in contact (CC) with each fibre at the three tests

	Baseline	12-week	24-week
Type I (%)	55.1 ± 16.5	55.4 ± 22.5	51.4 ± 12.9
Type IIa (%)	19.3 ± 9.8	16.9 ± 7.8	18.2 ± 8.2
Type IIx (%)	25.7 ± 18.9	27.6 ± 21.0	30.4 ± 15.9
Capillary-to-fiber ratio	1.63 ± 0.33	1.70 ± 0.45	1.73 ± 0.32
CC with type I	1.86 ± 0.29	1.95 ± 0.35	2.14 ± 0.36
CC with type IIa	1.49 ± 0.34	1.53 ± 0.42	1.67 ± 0.19
CC with type IIx	1.13 ± 0.27	1.17 ± 0.33	1.34 ± 0.31

All data are presented as means ± SD