

Improvement in Walking Distance after Rehabilitation in Patients with Peripheral Arterial Disease is Associated with Changes in Skeletal Muscle Myosin Heavy Chains

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Abstract

Intermittent claudication in peripheral arterial disease limits exercise capacity. Rehabilitation can produce an improvement in absolute walking distance. Very often changes in skeletal muscle, rather than changes in blood flow, are responsible for the improved exercise capacity. We studied 8 patients with intermittent claudication (Fontaine 2 class) that underwent a 4-week treadmill mild-moderate aerobic endurance training. We analyzed the Myosin Heavy Chain (MHC) composition of the gastrocnemius muscle before and after rehabilitation by taking needle microbiopsies. The absolute walking distance increased from 394 ± 188 m to 580 ± 215 (p<0.05). No changes in peak VO₂ were found. This improvement was accompanied by a significant increase in the percent expression of the slow aerobic MHC1 isoform, which was paralleled by a decrease of the anaerobic, fast twitch MHC2b.

This finding suggests that the increased exercise capacity obtained with this specific rehabilitation protocol may be related to favorable changes in skeletal muscle composition.

Key words: claudication, exercise, myosin heavy chains, peripheral arterial disease, rehabilitation, skeletal muscle.

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Peripheral Arterial Disease (PAD) affects a large population and is very often accompanied by intermittent claudication, which limits exercise capacity. Revascularization is sometimes possible and leads to symptoms relief, but in some circumstances the surgical procedure is not possible. Rehabilitation has been proposed as a possible alternative. Different protocols have been developed with favorable outcome resulting in a significant improvement in pain threshold and therefore in walking distance [8, 9, 12]. The reason why rehabilitation can produce such a tremendous benefit is not clear yet. PAD is accompanied by skeletal muscle alterations in function, histology and metabolism as demonstrated by Hiatt and coworkers [2, 14]. Muscle weakness in PAD has been shown to be accompanied by muscle fibres denervation and atrophy, that mainly occurs in type II fibres. [14]. A mitochondrial DNA injury has been described as well [2].

We have previously demonstrated that in Congestive Heart Failure (CHF) the limitation of exercise capacity is, at least in part, due to changes in skeletal muscle

Myosin Heavy Chain (MHC) composition [17-21]. In fact in CHF there is an increased expression of MHC2a and 2b that are known to be more fatigable because, though anaerobic, they have higher ATP consumption and reach lactate threshold earlier. We have also shown that improvement in exercise capacity with pharmacological treatment [19] is accompanied by a reshift of MHCs toward the slow, more fatigue resistant isoforms.

In this paper we have tested the hypothesis that the improvement in walking distance obtained after rehabilitation is linked to changes in skeletal muscle metabolism and biochemistry. For these reason we have studied changes in MHCs composition in the gastrocnemius of patients with PAD before and after a rehabilitation program. We have used a treadmill protocol that has been shown to be superior to strength training [9].

Material and Methods

Patients

Eight male patients with PAD and intermittent claudication (Fontaine 2 class) were studied. They all had

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normal resting ECG and echo ejection fraction greater than 50%. Exclusion criteria were: diabetes, pain at rest, calf BP < 50 mmHg, beta-blockers therapy, previous diagnosis of Chronic Obstructive Lung Disease, coronary angioplasty or by-pass graft within 1 year. They underwent measurement of Ankle/brachial index at rest and after exercise, and cardiopulmonary exercise capacity at baseline and after rehabilitation. They also had a gastrocnemius microbiopsy for MHCs assessment at the same time points.

7 age and sex matched detained individuals with no history of cardiovascular disease, normal resting ECG, echo and ankle/brachial index served as controls.

Vascular testing (Ankle/brachial Index)

At rest and after 1 minute stress (repetitive extension of the foot) the ankle/arm systolic blood pressure ratios were measured simultaneously by using a photoplethysmographic method (Microlab Padua Italy). Criterion for vascular disease was a resting A/B ratio $\leq$ 0.80. Only data from the most diseased leg were used (lowest rest ABI).

Cardiopulmonary exercise test and walking distance

All the patients underwent maximal claudication-limited cardiopulmonary exercise testing with a protocol envisaging a first step at 3 km/h and 0% grade. The grade was afterward increased by 3.5% every 3 minutes to peak exertion [7]. A Schiller Cardiovit CS100 with 1308 capnograph was used [18-19]. Oxygen consumption at maximum exercise was expressed as peak oxygen consumption (peak VO_2) defined as the mean oxygen consumption of the last 30 seconds of an incremental exercise test. Ventilation (VE) and Ventilatory Threshold (VT) were measured using the Wassermann equation. Absolute walking distance or absolute claudication distance (ACD), which was estimated when intolerable cramps appeared, was calculated by multiplying exercise velocity per minutes walked.

The initial claudication distance (ICD) was defined as the symptom free distance measured on the treadmill at speed 3 km/h and 0% grade until pain threshold was reached.

Rehabilitation program

A four-week rehabilitation program was used. Every day patients underwent the following procedures:

Step A

- 5 minutes warm-up
- treadmill walk at speed 3 km/h and 0% grade. The length of the walk was 70% of the initial claudication distance (ICD). This was adjusted at the end of every rehabilitation week when the ICD was re-evaluated.
- The treadmill walk was repeated 5 times/day with 5 minutes cool-down and 3 minutes rest intervals.

Step B

- 30' of callisthenic exercise
- 15' of respiratory exercise

Step C

- 5' warm-up
- free walk on a known distance (the walking distance was 70% of ICD adjusted every week). The walk was repeated 5 times with intervals of 3' rest.

Step D

- 30' cyclette at watt 0

Skeletal muscle microbiopsy

Skeletal muscle needle biopsies were taken from the medial gastrocnemius of the more diseased leg with a 17-gauge soft-tissue Menghini needle (Strylab Histo-cut) according to the method described by Vescovo et al [17]. With this method we were able to obtain 50-200 μg of tissue that was immediately frozen in liquid nitrogen. Biopsies were taken at baseline and two weeks after completion of the rehabilitation program to allow de novo synthesis of myosin heavy chains.

Electrophoretic separation of Myosin Heavy Chains (MHC)

The method is an improvement of that published by Carraro and Catani [3] and is described in details by Vescovo *et al.* [17-21]. Biopsies were homogenized and solubilized in 2.3% sodium dodecyl sulphate (SDS), 10% glycerol, 0.5% 2-mercaptoethanol, and 6.25 mM Tris-HCl, pH 6.8. Analytical SDS page was performed on 7% polyacrylamide slabs with 37.5% vol/vol glycerol. Identification of individual MHCs was performed in a separate series of experiments by immunoblotting the gel bands with a panel of monoclonal antibodies against MHC₁ (slow isoform), MHC_{2a} (fast oxidative) and MHC_{2b} (fast glycolytic) [17].

Assessment of MHCs distribution

The percent distribution of the three MHCs was determined by densitometric scan of the stained slab gels [17]. A linear response in terms of electrophoretic bands area is attained on densitometry when 0.1-2 μg of individual MHC is analyzed. Quantitative densitometry was performed using internal MHCs standards with known percent distribution of MHCs [17].

Statistical analysis

Student t-test for paired and unpaired data was applied. $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics of patients

Control subjects mean age was 55.6 ± 9.8 years, while patients age was 63.4 ± 4.0 ($p = \text{NS}$).

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MHCs composition in control subjects

The relative percentage of MHC1 in the control subjects was 69.1 ± 10.7 , while for MHC2a and MHC2b it was 21.3 ± 8.5 and 11.5 ± 8.6 respectively.

Patients with peripheral arterial disease

Ankle/brachial index at rest and after exercise

The data are summarized in Table I. No statistically significant differences were found for any parameters before and after rehabilitation.

Cardiopulmonary exercise test

Cardiopulmonary exercise test parameters before and after rehabilitation are summarized in Table II and we did not find any statistically significant difference between baseline and post-rehabilitation.

Absolute walking distance

The absolute walking distance after rehabilitation improved significantly. In fact it increased from 394 ± 188 m to 580 ± 215 m ($p < 0.05$).

Myosin Heavy Chain composition

At baseline MHC1 was $46.7 \pm 11.5\%$, while MHC2a and MHC2b were $24.7 \pm 3.9\%$ and $34.4 \pm 13.9\%$ respectively. After rehabilitation MHC1 increased to $70.3 \pm 11.8\%$ ($p < 0.001$), while MHC2a and MHC2b decreased to $21.2 \pm 9\%$ ($p = \text{NS}$) and $14.4 \pm 1.9\%$ ($p < 0.02$) respectively (Table III). Fig. 1 shows an example of the electrophoretic pattern of MHCs before and after rehabilitation in 4 patients.

At baseline the percent distribution of MHC1 in patients with peripheral arterial disease was significantly lower when compared to controls ($p < 0.001$).

Discussion

It is known that treadmill rehabilitation can produce an improvement in walking distance in patients with PAD [6, 7, 8, 9, 12].

The reason why this occurs is not entirely clear yet. We know that patients with PAD have a skeletal muscle myopathy, characterized by denervation, fibres atrophy [14] and mitochondrial abnormalities [2].

In this paper, by comparing muscles of diseased patients with those of control subjects, we have shown for Table I. Ankle/brachial index before and after rehabilitation. $P = \text{NS}$ for all the parameters.

	REST		STRESS	
	LEFT	RIGHT	LEFT	RIGHT
BASE-LINE	0.67 ± 0.17	0.80 ± 0.2	0.54 ± 0.19	0.65 ± 0.30
AFTER REHAB	0.74 ± 0.13	0.82 ± 0.20	0.60 ± 0.17	0.70 ± 0.25

Table II. Cardiopulmonary exercise testing parameters before and after rehabilitation VO_2 (peak oxygen consumption), VT (Ventilatory Threshold), VE (Ventilation). $P = \text{NS}$ for all the parameters.

	VO_2	VT	VE
BASELINE	24.0 ± 4.4	1936 ± 607	44.3 ± 14.0
AFTER REHAB.	22.6 ± 4.7	1838 ± 857	38.2 ± 11.1

Table III. MHCs distribution in control subject and PAD patients.

	CONTROLS	BASELINE	AFTER REHAB.
MHC1	69.1 ± 10.7	$46.7 \pm 11.5^*$	70.3 ± 11.8
MHC2a	21.3 ± 8.5	24.7 ± 3.9	21.2 ± 9.0
MHC2b	11.5 ± 8.6	$34.4 \pm 13.9^{**}$	14.4 ± 1.9

* $p < 0.001$ vs after rehab. and controls; ** $p < 0.02$ vs after rehab. and controls.

the first time that the gastrocnemius of PAD patients has an increased expression of the fast MHC2b and a decreased expression of MHC1. This shift does probably have an adaptational meaning in that patients with PAD have repeated episodes of ischemia and lactate production, to which muscle fibres react by triggering the preferential synthesis of the fast, but more fatigable, anaerobic MHC2b [4, 20, 21]. Unfortunately this adaptation could be detrimental in that fibers with the prevalence of MHC2b have a faster speed of contraction, a higher ATP consumption and get earlier to energy depletion and therefore to muscle fatigue [15, 19].

Our data are complementary to those of Regensteiner et al [14] who showed a decreased cross sectional area of Type II fibers, accompanied by signs of muscle denervation-like damage. One of the most dramatic effect of denervation is in fact the marked loss of muscle fibers mass. Fibres type IIb, being the biggest one [5], are more prone to develop atrophy. Our data show that the overall effect of PAD on skeletal muscle is an increased

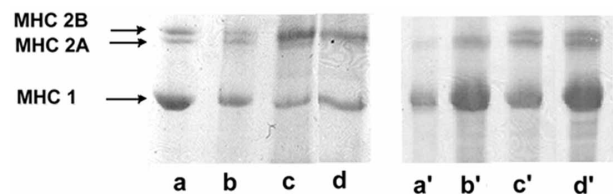


Figure 1. SDS-PAGE of MHCs. Arrows indicate the three isoforms separated on the basis of their relative mobility: in order from the fastest to the slowest, MHC1, MHC2a, MHC2b. Lanes a, b, c, d, represent biopsies taken at baseline in 4 patients with PAD. Lanes a', b', c', d' show the electrophoretic pattern in the same patients after rehabilitation.

percentage of fast MHCb that may be explained even in the presence of fibers I Ib atrophy [14], with an increased number of I Ib fibers.

Our data show that rehabilitation produces an improvement in walking capacity, that is accompanied by favorable biochemical changes. We have in fact found a reshift from the fast MHC2b toward the slow MHC1 isoform that reaches levels very similar to those of de-trained control subjects. The electrophoretic separation and relative quantitation of MHCs composition is a very reliable and reproducible method that allows precise quantitation of small changes in MHCs percentage [4, 17, 21]. In fact we have demonstrated a 5% coefficient of variation for MHC1 in the intersample evaluation. The treadmill measurement of walking distance with the protocol we used is recognized to be the most specific and reproducible one [6].

It is known that the increased muscle load which occurs during endurance (aerobic) rehabilitation produces an increase in type I fibers, as previously demonstrated in the skeletal muscle of patients with heart failure [1, 19] and in normal subjects [11]. This is accompanied by an increased expression of MHC1. This resembles the effect of muscle stimulation and training in normal subjects, where muscle loading is an important regulatory factor for the expression of specific MHC isoforms [16].

We hypothesize that muscle aerobic endurance training triggers the “de novo” preferential synthesis of MHC1, which in turn increases the walking capacity because of its peculiar biochemical profile, characterized by slow speed of shortening, low ATP consumption and greater fatigue resistance. At the same time we know that rehabilitation could induce an increase of muscle perfusion that may help to meet the metabolic demand of the oxidative fibres. The effect of treadmill training in PAD has been studied on muscle biopsies by Hiatt and coworkers [10] and they found no changes in fibers cross sectional area, while there was an increased number of denervated fibres with signs of regeneration. They also showed that different training programs can result in different histologic changes. It is also known that different patterns of fast muscle stimulation results in more or less extensive fast to slow transition [13]. Our rehabilitation protocol is rather different from that used by Hiatt in that it is a medium-intensity protocol that lasts only 4 weeks and is a combination of treadmill, callisthenic and aerobic activity. This may have different impact on muscle trophism and denervation-like damage. The severity of the disease plays also a role in the overall result of the rehabilitation program in that it has been shown by Hiatt et al. [10] that in the less diseased leg there is a more pronounced decrease of type II fibres area. Our patients, as shown by the ABI, have a moderately-severe PAD while Hiatt’s patients were more severely ill.

In our series of cases hemodynamic measurements did not show any change. In fact, though rough as a flow parameter the rest ankle/brachial index may be, this did not change after rehabilitation. This is partially in agreement with the data of Hiatt [8] who showed no correlation between changes in rest ABI, calf blood flow, and increase in peak walking time.

In our series of experiments peak VO_2 did not show significant changes after rehabilitation, while in some other previously published papers [8], after 12 weeks training there was a 30% increase in peak VO_2 . The same authors found after this period of rehabilitation a 125% increase in walking distance, with a clear discrepancy between the improvement of these two parameters. In our paper the expected increase in VO_2 may not have occurred in that the rehabilitation program lasted for only 4 weeks, while the improvement in walking distance that we obtained was “only” 65%. It may well be that a longer period of training could have sorted out an increase in peak VO_2 and a greater benefit in walking distance.

In conclusion the treadmill low-intensity endurance rehabilitation protocol that we used has brought about a clinical improvement in symptom limited walking distance. This occurred despite no hemodynamic changes were observed. There was a change in the skeletal muscle biochemical properties and metabolism. In our experience endurance training is the key step for promoting the preferential synthesis of the slow MHC1, that, because of its own characteristics, improves muscle performance and endurance.

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