Pre-Operative Physical Training Effects on Latissimus Dorsi Muscle in Patients Undergoing Dynamic Cardiomyoplasty: a Preliminary Report

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Abstract

The deleterious effects of long-standing heart failure on skeletal muscle structure have been recently documented. Nevertheless skeletal muscles have surged in the latest years as potential tools to support a chronically impaired dilated heart. The risk of adopting a suboptimal assist device led us to investigate the changes induced by a physical training on the Latissimus Dorsi (LD) muscle in 3 patients undergoing Dynamic Cardiomyoplasty (DC) procedure. All patients were submitted to 1.5-2 month training period before surgery. Muscular biopsies were taken before and after training. Muscle fiber diameter, capillary morphometry, fiber/capillary ratio, mitochondrial size and percentage, and fiber typing were assessed in all the specimens. Moreover computerized exercise equipment allowed comparison between muscular peak torque (PT), total work done (TW), and work for repetition (W) before and after training. All patients completed the training protocol. Muscle performance clearly and significantly improved after the physical exercises. Peak torque increased from a minimal of 12% to a maximum of 83%, TW from 82% to 191%, and W from 38% to 100% according to the various exercise velocities. Electron microscopy documented the beneficial effects of such an adopted training. Fiber diameter increased from 42 ± 6 to 50 ± 6 μm ($p < 0.001$) in type 1, from 40 ± 7 to 52 ± 6 μm in type 2A ($p < 0.001$) and from 38 ± 4 to 48 ± 3 μm in type 2B fibers ($p < 0.001$). Capillary/fiber ratio improved from 1.20 ± 0.10 to 2.00 ± 0.12 ($p < 0.001$), and mitochondrial size increased from 0.045 ± 0.018 to 0.11 ± 0.05 μm ($p < 0.001$) in type 1 fibers and from 0.030 ± 0.019 to 0.055 ± 0.037 μm ($p < 0.001$) in type 2 fibers. Not surprisingly, the myosin heavy chains remained substantially unchanged, since increased trophism of muscle fibers was similar in all the three types of myofibers. This preliminary report documents the positive influence of physical training on LD structure as well as performance in patients undergoing DC procedure. Further studies are required to observe the effects of pre-operative trained LD in terms of DC outcome.

Key words: muscle trophism and strength, pre-operative physical training, latissimus dorsi muscle, dynamic cardiomyoplasty, skeletal muscle assist devices, muscle fatigue, myosin ATPase, isomyosins, MHC, capillary-fiber ratio.

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Dynamic cardiomyoplasty (DC) is a surgical technique clinically introduced in 1985 by Carpentier and Chachques [2]. Such a procedure was proposed as a new approach in the treatment of refractory heart failure to improve the chronically impaired cardiac contractility by means of a wrapped and synchronously contracting Latissimus Dorsi (LD) muscle. Many investigators have extensively documented the alterations of skeletal muscles in patients with congestive heart failure [7, 9, 27] and several have shown a wide range of muscular derangements induced by long-standing peripheral hypoperfusion related to the chronically impaired left ventricular function [18, 30]. In order to avoid suboptimal cardiac support to the failing heart following DC we designed a pilot study to thoroughly

investigate the effects of a pre-operative physical training in an attempt to restore or, at least, improve the performance of the LD muscle. Part of the results present were previously reported in abstract form (6-24).

Materials and Methods

Three male patients were included in this pilot study after informed consent was obtained. Patient age ranged from 51 to 56 years. All patients were affected by end-stage heart failure refractory to conventional pharmacological treatment (digitalis, diuretics, ACE-inhibitors). Hemodynamic parameters included left ventricular ejection fraction varying from 23% to 27%, left ventricular end diastolic diameter from 67mm to 70mm, and New York Heart Association Class III. Traditional surgery (coronary artery bypass) was not considered advisable in case of ischemic etiology due to the absence of viable myocardium in akinetic areas. Heart transplantation was contraindicated in all patients due to severe peripheral vascular disease, diabetes and pulmonary hypertension. Several episodes of acute pulmonary edema occurred in all patients despite maximal medical therapy. Dynamic Cardiomyoplasty selection criteria were fulfilled in all the cases [3].

The training protocol consisted of repetitive sessions of isokinetic exercises for a 1.5-2 month period by means of a specific equipment (Cybex-Orthotron II, Lumex-Ronkonkoma, N.Y, USA). Such a machine allowed us to exercise a desired group of muscles (arm extensor muscles) leading to circumscribed training preventing potential deleterious cardiocirculatory effects of a more generalized body training. The patients were recumbent in a supine position during exercise accomplishment. Both body sides were trained (right and left LD). All the patients underwent a pre-training evaluation to individually tailor the exercise parameters. The initial pre-training assessment was performed for 3 times and the best parameters were set up for the subsequent repetitions all along the protocol. The exercise consisted of arm extension beginning from an ante-flexion of 90° which consistently elicit LD contraction as clearly documented by concomitant surface electromyographic monitoring. The patients were submitted to 5 sessions per week, and each session included 10 series of 6 repetitions of the same exercise, with a 2 min-rest period between each series. Exercise parameters included the peak torque (foot-pounds), and muscle work for repetition (foot-pounds). The tests were performed at different velocities according to the degree scale (low velocity - 60°/sec. or 90°/sec. - medium velocity - 120°/sec- and high velocity -180°/sec. -). The final muscle performance assessment (end-training evaluation) was performed after the training period submitting the patient at two tests, recording the best response. At the highest velocity in spite of the work for repetition we recorded the total work done in 15 seconds. The test was performed according to the protocol described by the program HUMAC V 5.01 (Computer Sports Medicine, Inc, Waltham, MA, USA). During exercise accomplishment heart rate and blood pressure (cuff method) were sistematically controlled (basal, during, at peak, and recovery) to avoid any deleterious effects. Moreover ECG was constantly monitored to detect any potential arrhythmia occurrence. Mechanical data were processed by means of a personal computer (Olivetti 290-30, Ivrea, Italy) which enabled us to visualize in real time the results of the exercise. Pre-training and end-training data were compared to analyse the induced changes of muscular performance.

Pre-training muscle biopsies were taken from the right LD in order to avoid any potential source of damage to the left LD which was utilized for the wrapping procedure at surgery. Other muscle biopsies were taken at the end of the exercise protocol from the right and during surgery from the left LD (post-training sample). Muscle specimens were partially frozen in isopentane cooled in liquid nitrogen at -170° for enzyme histochemistry. Other samples were fixed in 10% neutral formalin for routine staining and other small specimens were fixed in Karnovsky fluid for electron microscopy. Serial transverse cryostat sections were treated for myofiber ATP-ase at pH 9.6, for ATP-ase EDTA reversal at pH 4.35 and for NADH. According to the staining properties for acid myofiber ATP-ase the muscle fibers were classified into type 1, type 2A and type 2B fybers.

Histological evaluation also included the assessment of fyber type diameter. Quantitative analysis of fiber diameter and of mitochondrial parameters was accomplished on micrographs adopting an automatic Interactive Image Analysis System-IBAS I, II (Kontron, Bildanalyse, Munich, Germany). Each micrograph was placed under a TV camera and the related image was shown on a screen. The image was processed according to a program properly devised and selected from the program menu of the IBAS system. Automatically the chosen structures were counted and measured. In our measurements we selected 3 parameters: area, diameter (of area-equivalent circle) and area percentage. The area percentage was automatically assessed as the ratio between the area occupied by the structures and the field where these structures were contained. This field was selected by a determined window inside the fiber. All measurements were calculated in microns (micron square for the area). The estimation of capillaries was performed on paraffin embedded transverse sections stained with Gomori silver impregnation for reticulin and on semithin transverse sections of Karnovsky fixed material. The numerical density of capillaries (CD) was evaluated with a frame in the eyepiece of the microscope at 40X objective. The CD per area was calculated as: number of capillaries within the frame/area of the frame. The capillaries/fiber ratio (C/F) was calculated as: capillary number within the frame/number of fibers within the frame.

Myosin heavy chains. We previously described in details the methods of myosin purification and quantitation by densitometry of SDS PAGE of MHC [5, 22, 23].

Muscle exercise related data were processed by a personal computer. Histology values were stored on a floppy disk for the statistical analysis by means of the IBAS I system. Histogram were computed either for the area or for the diameter. At the same time, basic statistics data (count number, number of size classes, number of counts in under and/or overflow, in case where full range as not used, minimal, maximal and mean value; standard deviation) were given.

Results

All the three patients completed the training protocol and no major complications occurred. For the patient n° 1 the training protocol was not performed according to the HUMAC program, and therefore we assessed only the peak torque at 60°, 90°, and 120°/sec.. In patient n° 2 the low velocity exercises have been accomplished at 90°/sec since the 60°/sec velocity induced some derangement of hemodynamic parameters and was considered an excessive workload.

All the patients showed improvement of muscle performance. The entity of positive changes from pre-training values encompassed a wide range of results according to the different velocities (figure 1). Indeed PT increased from 43% (pt n° 3) to 60% (pt n° 1) at 60°/sec exercise, from 15% (pt n° 2) to 68% (pt n° 1) at 90°/sec, from 22% (pt n° 2) to 75% (pt n° 3) at 120°/sec, and from 12% (pt n° 2) to 83% (pt n° 3) at 180°/sec.

The increases of the work for repetition ranged from 53% (pt n° 3) to 60% (pt n° 3) at 60°/sec., from 38% (pt n° 2) to 39% (pt n° 2) at 90°/sec, from 43% (pt n° 2) to 100% (pt n° 3) at 120°/sec.

The total work done, obtained only at the velocity of 180/sec. in pt n° 2 and pt n° 3, improved 82% and 191% respectively.

Histological findings confirmed a better muscular condition following the training program. Indeed LD type 1, 2A, and type 2B fiber diameter increased significantly after the programmed exercise, whilst only the type 2A muscle fiber percentage increased significantly (table 1 and figure 2). Since the light exercise affects at the same extent all the myofiber types it is not surprisingly that the MHC patterns remained unchanged after training: table 4 shows that the variations among trained versus untrained LD is of the same extent or smaller than the changes among right and left muscles of the patients. Trained muscles were shown to be better perfused since a significant increase of capillary/fiber ratio was documented (table 2). The reported inverse relationship with the capillary density was correlated to the augmented skeletal muscle fiber diameter. A more active and efficient aerobic metabolism was shown by means of documenting an increased NADH activity. The mitochondrial content was positively changed by exercise program with a marked increased of size and percentage in muscle fibers (table 3).

Table 1. Fiber type diameter and composition in LD muscle before and after training.

	Fiber type diameter (μm)			Fiber types (%)		
	1	2A	2B	1	2A	2B
Untrained	42 ± 6	40 ± 7	38 ± 4	44	36	20
Trained	50 ± 6**	52 ± 6**	48 ± 3**	40	44*	16

*p < 0.05 **p < 0.001

Table 2. Changes in capillary morphometry in untrained and trained LD muscles. C/F, capillary/fiber ratio; CD, capillary density.

	C/F	C D
Untrained	1.20 ± 0.10	220 ± 30.8
Trained	2.00 ± 0.12**	200 ± 22.8

*p < 0.001

Discussion

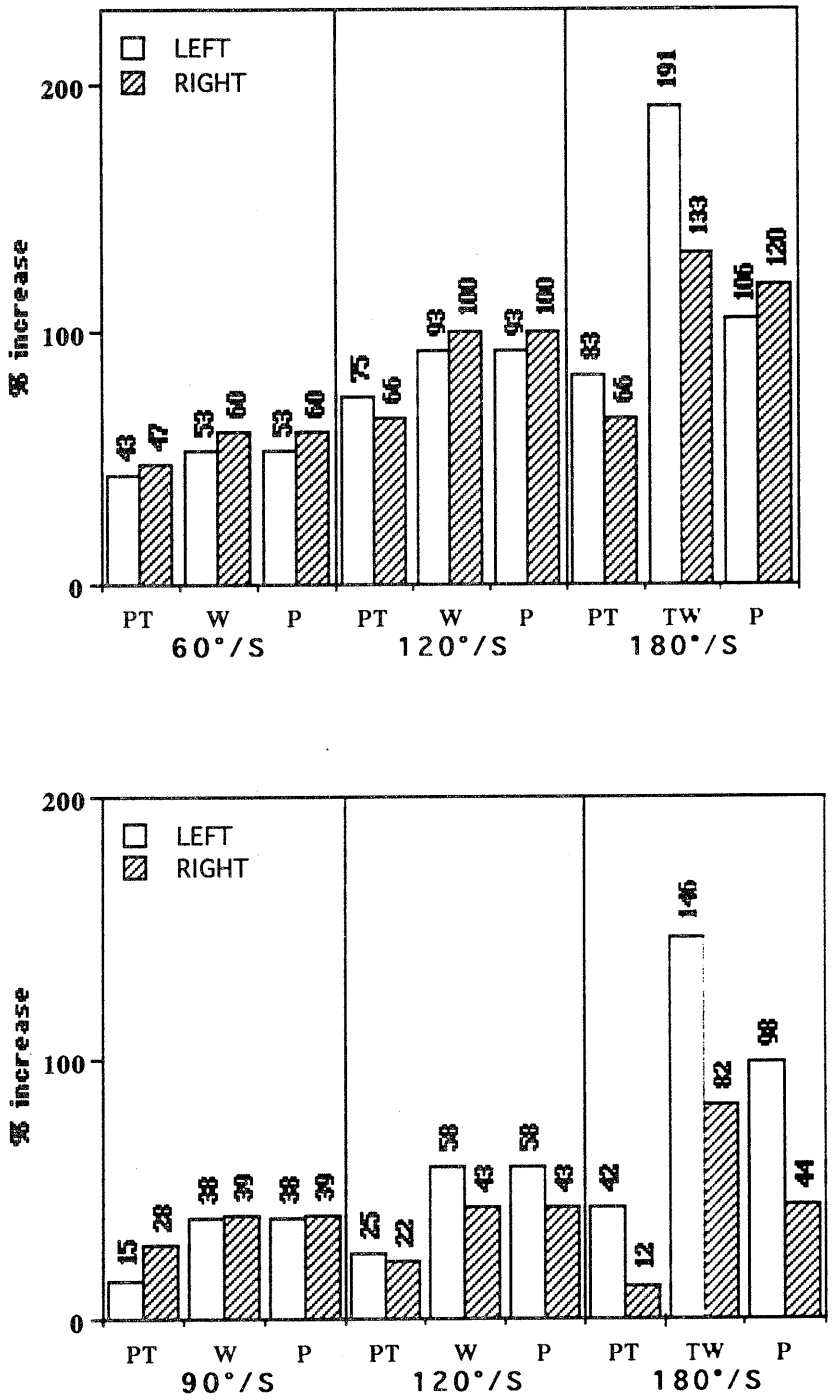
The impairment of cardiac contractility and subsequent reduction of pumped blood flow in CHF leads to a reduction of peripheral perfusion.

Chronic hypoperfusion induces some adaptive changes in the peripheral organs, and skeletal muscles have been documented to developed peculiar abnormalities and to represent one of the more affected districts in response to long-lasting cardiac dysfunction [7, 9, 27]. Mancini and coll. documented atrophy occurring in skeletal muscles of patients with chronic heart failure, even in its mild to moderate states [18]. This study furthermore demonstrated the presence of important fatty infiltration among muscle

Table 3. Mitochondrial size and percentual area in untrained and trained LD muscles.

	Size (diameter, μm)		Percentual area	
	Fiber type 1	Fiber type 2	Type 1	Type 2
Untrained	0.045 ± 0.018	0.030 ± 0.019	19.8	8.7
Trained	0.11 ± 0.05*	0.055 ± 0.037*	23.2	11.7

*p < 0.001



PT: Peak Torque. **W**: Work for repetition. **TW**: Total Work (done in 15 sec)
P: Mean Power for repetition

Figure 1. This figure reports the graphics related to muscular performance of patient n 2 (upper graphic) and patient n 3 (lower graphic) following the training protocol. The bars relate to the percentage of increase in respect to pre-training data (0 line). Peak torque, work for repetition, power, and total work done are shown. Clear beneficial effects on both muscle groups, right and left, are documented.

fibers. The authors hypothesized that muscle impairment in CHF might be related to several etiologies, namely malnutrition, inactivity, increased metabolic state secondary to high levels of circulating catecholamines, cortisol, ACTH and tumor necrosis factor. The reduction of exercise capacity of these patients was therefore correlated to the altered skeletal muscle condition, and not solely to the reduced cardiac output. Mancini moreover demonstrated a derangement of the peculiar metabolic pathways, espe-

Table 4. SDS PAGE of myosin heavy chains

MHC type	2A	2B	1
		%	
LD: left [5]	35 (27-48)	26 (12-48)	39 (24-51)
LD: right, pre-training [5]	36 (25-49)	24 (14-43)	40 (22-49)
LD: right, post-training [1]	51	19	30

Percentual content of myosin heavy chains: Mean with upper and lower figures. Number of cases in square brackets.

cially the ones related to phosphocreatine use and resynthesis [7].

An adjunctive and fascinating hypothesis has been indicated by Dunningan and Caforio who suggested that skeletal muscle abnormalities might be secondary to cardiac failure, as a part of a generalized myopathy, or an underlying genetic disease, or an autoimmune pathology [1, 10].

Drexler demonstrated that in CHF patients there is a significant reduction of volume density and surface area of mitochondrial cristae leading to a reduced aerobic activity of skeletal myocyte. Moreover he showed a direct relationship between muscle alteration and exercise capacity arguing that these negative muscular features might be reversible [9]. Wilson and Massie demonstrated a decrease of intracellular pH values by studying the CHF skeletal muscles adopting a phosphor 31 Magnetic Resonance Image [19, 31]. Other feature of chronic heart failure-related skeletal muscle derangement consists of an impaired plasma inorganic phosphate utilization.

Patients affected by CHF have been commonly treated so far by conventional pharmacological therapy in concomitance with exercise or exertion avoidance. These conductance is likely to be no longer accepted because of the most recent findings on the matter. A slight exercise train-

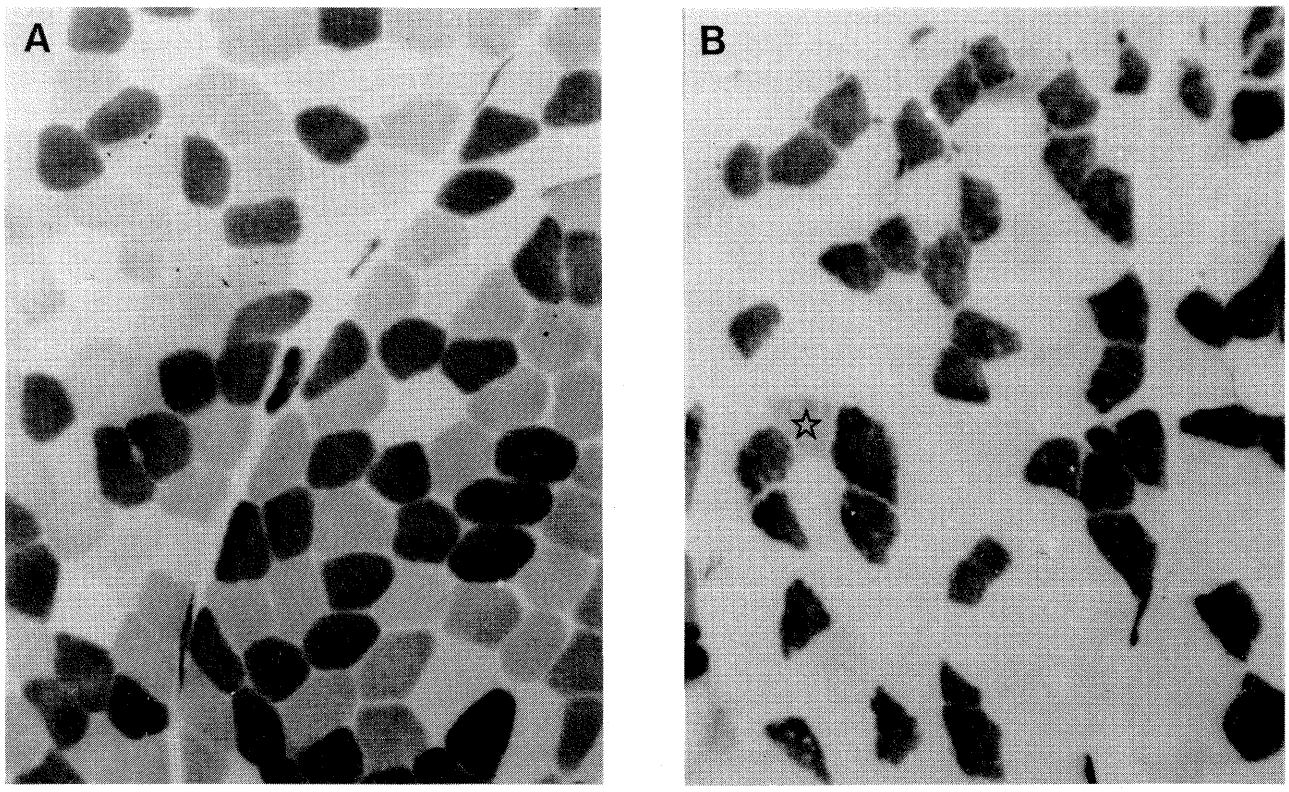


Figure 2. Myofibrillar ATPase after incubation at pH 4.60. LD before (A) and after (B) physical training. In panel B there is predominance of type 2A fibers. Only scattered type 2B fibers (star) are present.

ing has been shown to induce favourable adaptation in the skeletal muscles [19, 29]. This effect derives from metabolic "improvement" due to greater reliance on fat oxidation and reduced lactate production. The marked negative changes imposed by the muscle deconditioning due to the rest therapy are consistent with a progressive deterioration of the patient clinical status and quality of life. Recent reports have documented a shift from type 1 and type 2A fibers to type 2B fibers with subsequent marked increase of anaerobic metabolism in CHF patients [25, 28].

All these findings clearly underline the impaired condition of skeletal muscles in case of long-standing cardiac dysfunction. Nevertheless skeletal muscles have been recently proposed as potential long-term cardiac assist tools to treat medically refractory CHF patients [2, 3]. Some reports have documented clinical improvement after LD wrapping and long-term synchronous contractions [4, 11, 15, 20, 21]. Anyhow an eterogeneous range of post-operative results have been published pointing out the lack of predictors in terms of surgical results. All the investigators involved in the Cardiomyoplasty project are well aware that still little is known in respect to the skeletal muscle response and adaptation induced by long-term stimulation and by the new functional task. The factors linked to a better post-operative outcome are still unclear. Patient selection, in terms of hemodynamic criteria, has been imputed so far as the main cause of the wide range of surgical results, but other determinants, unfortunately still to be disclosed, are likely to play a major role. Muscle immobilization has been invariably linked to a reduction of oxidative enzymes, muscle mass and capillary density [12, 14]. Skeletal muscle deconditioning (secondary to reduced exercise activity, frequent hospitalizations and bed-rest) in patients affected by severe left ventricular dysfunction is considered the most likely trigger of myocyte derangement [8, 26, 32]. We can speculate therefore that the abnormalities induced by long-lasting skeletal muscle underperfusion in congestive heart failure and the concomitant phenomenon of muscle deconditioning may be deteriorated by the muscle-related effects of DC procedure (muscle immobilization in the 2 weeks after surgery). Moreover some evidences have been reported about the damage induced by surgical muscle manipulation (harvesting and transposal) and long-lasting electrical stimulation at high regimen as adopted in the clinical practice. Lucas and Kalil separately reported degeneration of wrapped skeletal muscle claiming that the actual consequences of such a prolonged stimulation with the current adopted pacing protocol (30 Hz of pacing frequency, and 1:1 or 1:2 contraction ratio) might negatively influence the muscle viability and performance at long-term [13, 16]. Thereby pre-operatively abnormal skeletal muscles are likely adopted during DC surgery and the subsequent post-operative electrical conditioning may act as a further source of muscular damage due to the highly demanding muscle function. Such a combination of negative factors might irreversibly lead to a design of an unproper assist device.

Many studies have shown us the effects of chronic electrostimulation in terms of muscle adaptation, but none focused these changes in CHF skeletal muscles affected by the above mentioned abnormalities.

These preliminaries prompted us to investigate, in a pilot study, the structural and functional changes induced by a brief training period merely in LD muscles involved in DC procedure. Our aim was meant as an initial attempt to optimize skeletal muscle condition pre-operatively. As mentioned before CHF patients are characterized by well identified muscle abnormalities which should be reversed to provide optimal condition for a long-term cardiac assistance. The pre-operative LD muscle training, which was tailored according to the individual features, enabled us to pursue the improvement of the target muscle as well as to prevent potential cardiovascular adverse effects related to strenuous exertion in such patients. Our pre-operative data did confirm that these patients were not in terminal phase of heart failure, showing some functional reserve left (New York Heart Association Class III). The programmed physical training contributed to improve dramatically the muscular performance. The muscle functional parameters improved significantly in all patients following the training program. Histology of trained muscles confirmed the beneficial impact of muscle training on the ultrastructure of the myocytes.

The adopted protocol is absolutely reproducible and feasible. It is conceivable that an improved pre-operative muscle condition might favourably influence the muscle response to the a new muscular functional demand as required following DC. The pre-operatively trained muscles are better vascularized, as documented by electron microscopy. The reduced vascular supply due to surgical harvesting at the distal part of the LD, may be counteracted by such a pre-operatively enhanced vascularization or provide more flow in response to the augmented requests, and eventually improve muscle performance.

We strongly believe that current investigation in terms of pre-operative muscle histological and functional characteristics following an exercise test will surely add more information and they might provide additional clues for a more precise patient selection.

A thorough knowledge on the correct use of skeletal muscle potentials is still far from the current clinical practice, but ongoing studies are sheding new lights on long-term muscle preservation and function.

The effects of postoperative LD recovery period (no muscle contraction) and subsequent electrical stimulation on the pre-operative physical-related muscle changes are still unknown and careful assessment is required to validate the potential clinical impact.

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