Latissimus Dorsi Vascular Delay Improves Muscle Function for Use in Cardiomyoplasty

Katia Rossini, Elena Giardini[(1)], Andrea Donà, Abdul El Messlemani and Francesco Mazzoleni[(1)]

Department of Biomedical Science, and (1) Institute of Plastic Surgery, University of Padova, Italy

Abstract

The aim of our study was to investigate the effectiveness of vascular delay of a LD muscle flap in an experimental rat model of Dynamic Cardiomyoplasty. We hypothesized that muscle function would be improved by a vascular delay procedure that increases distal muscle perfusion of the Latissimus Dorsi (LD) muscle. In the first group of 6 rats the right LD were subjected to a "vascular delay" procedure, the left LD were tenotomized and resutured in the shortened position it spontaneously attained to mimic transposition effects of cardiomyoplasty, which result in LD distal devascularization and decreased resting tension. After 7 days the LD muscle flaps were studied. In a second experimental group, the right LD muscles of 6 rats were subjected to a vascular delay procedure. One week later both right (vascular delay) and left (control) LD muscles were subjected to a tenotomy procedure. Seven days later the LD flaps were studied. In both group the LD muscles were excised and fixed in liquid nitrogen at resting length. Muscle damage was graded by histological morphometry (H-E) on distal, medial, proximal cryostat sections. Muscle damage in distal vascular delay LD was 9% ± 1.87 (SE), in distal tenotomized LD was 79% ± 9.97, and in distal muscle tenotomized after vascular delay was 41.67% ± 7.03. Muscle damage in medial vascular delay LD was 17% ± 7, in medial tenotomized LD was 30% ± 7.85, and in distal muscle tenotomized after vascular delay was 18.34% ± 4.59. Muscle damage in proximal vascular delay LD was 18%±4.63, in proximal tenotomized LD was 25.42% ± 7.4, and in proximal muscle tenotomized after vascular delay was 12.5% ± 2.81. In conclusion we found that "vascular delay" procedure of LD tenotomized and resutured in the shortened position, that mimics the Dynamic Cardiomyoplasty significantly reduces muscle damage and improves LD muscle flap perfusion and function particularly in the distal portion of muscle.

Key words: dynamic cardiomyoplasty, tenotomy, vascular delay.

Basic Appl Myol 11 (3): 139-142, 2001

The surgical procedure known as dynamic cardiomyoplasty has attracted worldwide interest as a treatment for selected patients with end-stage heart failure [9].

In a single stage the entire LD muscle is mobilized and trasposed from the position on the chest wall into the thorax and then wrapped around the heart. The muscle is then stimulated to contract in a training protocol [3, 4, 8, 16, 19].

An important LD muscle damage is well recognized, in particular of the distal part of the flap, both during the early post-operative phase [5], and following chronic stimulation [7, 18].

Many histological changes are documented such as replacement of muscle fibers by fibrous tissue and fat. These changes obviously reduce the mechanical effectiveness of cardiomyoplasty. The ischemia of the distal part of the muscle has anatomical basis.

The pattern of circulation of the human LD is a type V in the Mathes and Nahai classification [11], with a dominant pedicle that normally supplies the proximal one-third – one half of the muscle, and two secondary segmental pedicles which supplie the distal part. The dominant pedicle is the Thoracodorsal artery and venae comitantes. It arises from the subscapular artery, has a length of 8 cm and a diameter of 2.5 mm; it enters the deep surface of the muscle in the posterior axilla 10 cm inferior to the muscle insertion into homerus.

One of the secondary segmental pedicles are the lateral row of four to six perforating arterial branches and venae comitantes. They have as regional source the posterior intercostal artery and vein, a length of 2-3 cm and a diameter of 0.6 mm. Their location is lateral to lumbosacral fascia.

The other segmental pedicles are the lateral row of four to six perforating vessels and venae comitantes. They arise from the lumbar artery and vein, have a length of 1-2 cm a diameter of 0.5 mm and are located adiacent to site of muscle origin into lumbar vertebrae [11, 12].

During surgical trasposition of the muscle the perforators of the two secondary segmental pedicles are obviously divided and the thoracodorsal artery supplies alone the entire muscle with a consequent ischemia of the distal portion of the muscle.

We hypothesized that muscle and morphology and function would be improved by a vascular delay procedure in an experimental LD muscle flap model.

Materials and Methods

Twelve wistar rats weighting 250-300 gr were used in this study. Rats were divided into two groups. The animals were housed in cages in a temperature (22°C) and light (12 hour/day) controlled room and they were provided with the same commercial rat food and tap water. The guidelines for the care and use of laboratory animals were followed.

Anaesthesia was induced by intraperitoneal administration of rompun and ketalar as described in Dalla Libera et al. [6].

In the first group (N = 6) the right LD were subjected to a "vascular delay" procedure, the left LD were tenotomized. The operative field on each side was prepared with iodine scrub. In detail: RIGHT SIDE: A 3 cm incision was made over the anterior border of the muscle. The anterior border of the muscle was identified and the dissection extended under the muscle into the submuscolar plane. All the perforanting branches were identified and divided. The thoracodorsal pedicle was identified and preserved. A 4/0 non adsorbible suture was used to close the incision. LEFT SIDE: A 3 cm incision was made over the anterior border of the muscle. The muscle was identified and the dissection was extended under the muscle. The medial and lateral rows of the perforating arterial branches were divided. Care was taken to preserve the dominant pedicle. An incision was made along the distal and paravertebral border of the muscle in order to allow LD to become shorter. Using a 5/0 non adsorbible suture the left LD muscle was resutured in the shortened position it spontaneously attained to mimic effects of cardiomyoplasty. After 1 week the animals were sacrified and the LD muscle was excised, fixed in liquid nitrogen and stored at -80°C until use.

In a second experimental group (N = 6) the right LD muscles were subjected to a vascular delay procedure with the technique described above. After 1 week the animals were returned to the operating room and a tenotomy procedure was made in both right and left side. Seven days later the animals were sacrified and the LD muscle was excised to be studied, fixed in liquid nitrogen and stored at -80°C until use.

Muscle damage was graded by histological morphometry (H-E) on distal, medial, proximal cryostat sections. Morphometric analyses were performed on glass slides or photographs using an image analyser IBAS 2000 (Kontron, Zeiss). Measurements of the profile area were obtained by a television scanner and a grey level detector that allowed us to identify, to select and to measure single features [15, 17]. Result are expressed as mean ± SE. Student's t-test was used for statistical analysis and data were considered statistically significant when $p < 0.05$.

Results and Discussion

Dynamic Cardiomyoplasty utilises skeletal muscles' entire length, consequently the thoracodorsal artery remains the only blood supply of the flap. Furthermore the Latissimus Dorsi muscle flap is stimulated electrically and contracted dinamically. In these clinical conditions the demands on its blood supply is obviously increased. We focused our work on methods of increasing blood supply to muscle flaps. In our study we wanted to verify the effectiveness of a vascular delay technique in an experimental model of Dinamic Cardiomyoplasty [2, 13, 14, 21].

We decided to utilized the Latissimus Dorsi muscle of Wistar rat because its anatomy is very closed to that in humans: a single neurovascular pedicle, the Thoracodorsal artery and venae comitantes, and secondary segmental pedicles. The second reason is due to its anatomical structure. The muscle is very thin and in future it will be possible to obtain direct videomicroscopy and measurement of skeletal muscle microcirculation throughout different delay periods.

In our experiments muscle damage in distal vascular delay LD was 9% ± 1.87 (SE), in distal tenotomized LD was 79% ± 9.97 and in distal muscle tenotomized after vascular delay was 41.67% ± 7.03. In the distal part of the muscle the surgical tenotomy alone, that mimics the Cardiomyoplasty procedure, produces a percent of muscle damage very high, with a significant difference ($p < 0.005$) from the percent of muscle damage after a vascular delay procedure (see tables 1 and 2, figures 1, 2, 3).

When tenotomy is performed after the vascular delay procedure the percent of damage measured in the distal portion of the muscle has a significant difference ($p < 0.0005$) from the damage measured after the tenotomy alone. We find apparently different results in comparison with Stremel and collaborators [20] but we have different experimental conditions. In fact Stremel

Table 1. % muscular damage.

	delay	tenotomy	delay+tenotomy
distal	9 ± 1.87	79.17 ± 9.97 a	41.67 ± 7.03 a,b
medial	17 ± 7	30 ± 7.85 c	18.34 ± 4.59 c
proximal	18 ± 4.63	25.42 ± 7.4 c	12.5 ± 2.81 d

a, significant difference ($p<0.005$) from distal delay; b, significant difference ($p<0.05$) from distal tenotomy; c, significant difference ($p<0.0005$) from distal tenotomy; d, significant difference ($p<0.01$) from distal delay+tentomy. Mean ± SE.

Table 2. % muscular tissue.

	delay	tenotomy	delay+tenotomy
distal	91 ± 1.87	20.83 ± 9.97 a	58.33 ± 7.03 a,b
medial	83 ± 7	70 ± 7.85 c	81.66 ± 4.59 c
proximal	82 ± 4.63	74.58 ± 7.4 c	87.5 ± 2.81 d

a, significant difference ($p<0.005$) from distal delay; b, significant difference ($p<0.05$) from distal tenotomy; c, significant difference ($p<0.0005$) from distal tenotomy; d, significant difference ($p<0.01$) from distal delay+tentomy. Mean ± SE.

et al. studied vascular delay periods of 3, 5, 7, 10, and 14 days followed by tenotomy of 4-days in original position while in our experiment tenotomy is performed in shortened position for 7 days, this condition mimics the Dynamic Cardiomyoplasty. Tenotomy in shortened position produced myofibrillar derangement, loss of myofilaments and streaming of Z bands [1], increase in both the endomysial and the perimysial collagen networks, with a simultaneous decrease in intramuscular capillary density. The relative volume of connective tissue increased in parallel with the duration of immobilisation or after tenotomy. There was slightly more increase after immobilisation in a shortened rather than in a lengthened position [10]. These data can explain greater muscle damage in our study in comparison with Stremel et al.'s results.

Muscle damage in medial vascular delay LD was 17% ± 7, in medial muscle tenotomized was 30% ± 7.85 and in medial muscle tenotomized after vascular delay was 18.34% ± 4.59.

Muscle damage in proximal vascular delay LD was 25.42% ± 7.4 and in proximal muscle tenotomized after vascular delay was 12.5% ± 2.81 (see tables 1 and 2, figures 1, 2, 3).

Muscle damage produced by a surgical tenotomy after a vascular delay procedure is higher in the distal part than in the medial part. The severity of muscle damage in both groups after tenotomy and vascular de-

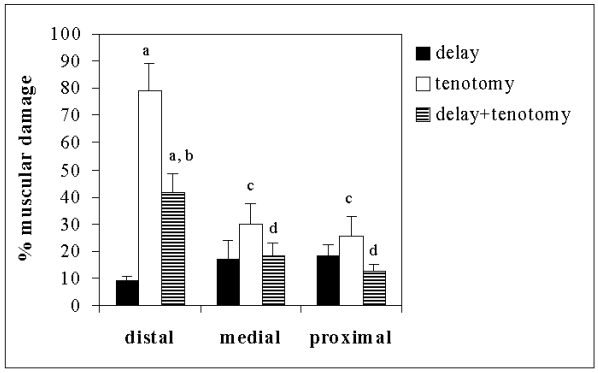

Figure 1. Graphic representation of % muscular damage a, significant difference ($p < 0.005$) from distal delay; b, significant difference ($p < 0.05$) from distal tenotomy; c, significant difference ($p < 0.0005$) from distal tenotomy; d, significant difference ($p < 0.01$) from distal delay+tentomy. Mean ± SE.

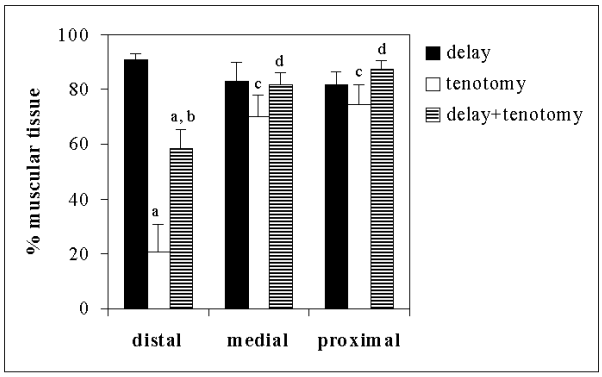

Figure 2. Graphic representation of % muscular tissue. a, significant difference ($p < 0.005$) from distal delay; b, significant difference ($p < 0.05$) from distal tenotomy; c, significant difference ($p < 0.0005$) from distal tenotomy; d, significant difference ($p < 0.01$) from distal delay+tentomy. Mean ± SE.

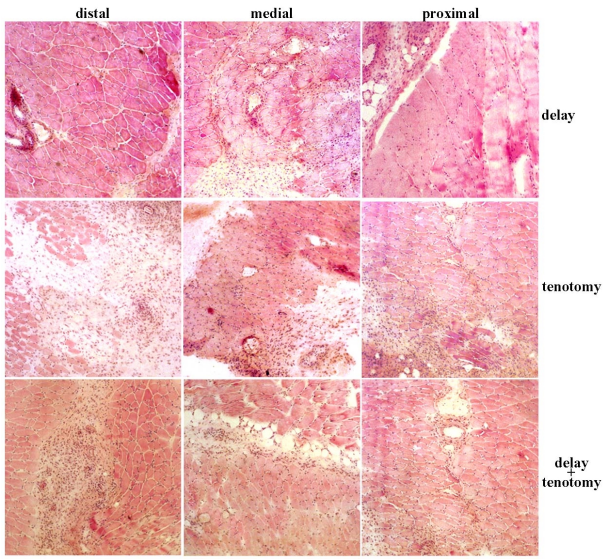

Figure 3. Hematoxylin and eosin of experimental muscles. Magnifications 100X.

lay+tenotomy increases in this order: proximal-middle-distal. It is according to expected results because the thoracodorsal arterial supply does not normally perfuse the distal two-thirds of LD muscle [16]. In fact LD muscle receive the majority of its vascular supply from thoracodorsal neurovascular pedicle and only a small portion from the peripheral perforating branches of the intercostal vessels.

In conclusion we found that vascular delay procedure of LD tenotomized and resutured in the shortened position, that mimics the Dinamic Cardiomyoplasty, significantly reduces muscle damage and improves LD muscle flap perfusion and function particularly in the distal portion of the muscle. In our opinion these results seem to be very important and could find possible and immediate clinical applications.

Address correspondence to:

Francesco Mazzoleni, Institute of Plastic Surgery, University of Padova, Italy, Via Giustiniani 2, 35128 Padova, Italy, tel. +39 0498212701, fax +39 0498758088, Email francesco.mazzoleni@unipd.it.

References

[1] Bruce-Gregorios J, Chou SM: Core myofibers and related alterations induced in rats' soleus muscle by immobilization in shortened position. *J Neurol Sci* 1984; 63 (2): 267-75.

[2] Callegari PR, Taylor GI, Caddy CM, Minabe T: An anatomic review of the delay phenomenon: I. Experimental Studies. *Plast Reconstr Surg* 1991; 89 (3): 397-407.

[3] Carpentier A, Chachques JC: Miocardial substitution with a stimulated skeletal muscle: first successful clinical case (Letter). *Lancet* 1 1985; 1267.

[4] Chachques JC, Carpentier A: Postoperative management in Carpentier A, Chaques JC, Grandjeau P (eds): *Cardiomyoplasty*. New York, Futura, 1991, 131-138.

[5] Chachques JC, Grandjeau PA, Carpentier A: Latissimus dorsi dynamic cardiomyoplasty. *Ann Thorac Surg* 1989; 47: 600-604.

[6] Dalla Libera L, Zennaro R, Sandri M, Ambrosio GB, Vescovo G: Apoptosis and atrophy in rat slow skeletal muscles in chronic heart failure. *Am J Physiol* 1999; 277(5 Pt 1): C982-6.

[7] El Oakley RM, Jarvis JC, Barman D, Greenhalgh DL, Currie J, Downham DY, Salmons S, Hooper TL: Factor affecting the integrity of latissimus dorsi muscle graft: implications for cardiac assistance from skeletal muscle. *J Heart Lung Transplant* 1995; 14: 359-365.

[8] Furnary AP, Chachques JC, Moreira LFP, Grunkmeier GL, Swanson JS, Stolf N, Haydar S, Acar C, Starr A, Jatene AD, Carpentier AF: Long term outcome, survival analysis, and risk stratification of dynamic cardiomyoplasty. *J Thorac Cardiovasc Surg* 1996; 112: 1640-1650.

[9] Hooper TL, Salmons S: Skeletal muscle assistance in heart failure. *Cardiovasc Res* 1993; 27: 1404-1406.

[10] Jozsa L, Kannus P, Thoring J, Reffy A, Jarvinen M, Kvist M: The effect of tenotomy and immobilisation on intramuscular connective tissue. A morphometric and microscopic study in rat calf muscles. *J-Bone-Joint-Surg-Br* 1990; 72(2): 293-7.

[11] Mathes SJ, Nahai F: Classification of vascular anatomy of muscles: Experimental and clinical correlation. *Plast Reconstr Surg* 1981; 67: 177.

[12] Mathes SJ, Nahai F: Clinical applications for muscle and musculocutaneous flaps. St. Louis, MO, Mosby 1982, 20.

[13] Milton SH: The effects of "delay" on the survival of experimental pedicled skin flaps. *Br J Plast Surg* 1969; 22: 244.

[14] Mussini I, Favaro G, Carraro U: Maturation, dystrophic changes and the continuous production of fibers in skeletal muscle regenerating in the absence of nerve. *J Neuropathol Exp Neurol* 1987; 46 (3): 315-31.

[15] Myers MB, Cherry G: Mechanism of the delay phenomenon. *Plast Reconstr Surg* 1969; 44: 52.

[16] Rigatelli G, Carraro U, Barbiero M, Rigatelli G: Correlations between time, Latissimus Dorsi wrap properties and systolic assistance in demand dynamic cardiomyoplasty? *Basic Appl Myol* 2000 ; 10 (5): 249-251.

[17] Rossini K, Donà A, Sandri M, Destro C, Donà M, Carraro U: Time-course of exercise and apoptosis in dystrophin-deficient muscle of mice. *Basic Appl Myol* 2000; 10 (1&2): 33-38.

[18] Santamore WP, Ali A, Stremel R, Chiang B, Kashem M, Tobin NA-E, Carroll OS, Barker PJ, Unger L, Slater AD, Gray L, Tobin G: Strategies for preserving muscle function for improved systolic assist in dynamic cardiomyoplasty *Basic Appl Myol* 1998; 8 (1): 51-58.

[19] Scelsi R, Scelsi L, El Messlemani AH, Carraro U: Pathological findings of latissimus dorsi muscle grafts after short and long-term dynamic cardiomyoplasty. Review of published autopsies and a recent post-mortem study of and unconditioned graft. *Basic Appl Myol* 2000; 10 (3): 131-136.

[20] Stremel RW, Santamore WP, Tobin GR, Barker JH. Vascular delay, angiogenesis, and cardiomyoplasty. *Basic Appl Myol* 1999; 9 (4): 155-15.

[21] Taylor GI, Corlett RJ, Caddy CM, Zelt R: An anatomic review of the delay phenomenon: II. Clinical applications. *Plast Reconstr Surg* 1991; 89 (3): 408-416.