

Daily Work-Rest Stimulation Regimen of Sheep LD for Cardiomyoplasty: Rationale and Results up to Six Months

Giorgio Arpesella^(1, 2), Piero M. Mikus⁽²⁾, Alessandro Giannoni⁽³⁾, Pierluca Lombardi⁽²⁾, Angelo Pierangeli⁽²⁾, Sandra Zampieri⁽⁴⁾, Claudia Catani⁽⁴⁾ and Ugo Carraro⁽⁴⁾

(1) Cardiovascular Surgery, University of Chieti, (2) Experimental Surgery Unit, Cardiovascular Surgery, University of Bologna, (3) Biomedical Technologies Service of The S. Orsola Hospital, Bologna and (4) C.N.R. Unit for Muscle Biology and Physiopathology, Department of Biomedical Sciences, University of Padova, Italy

Abstract

A realistic explanation of the clinical effect of dynamic cardiomyoplasty is that active muscle contraction can chronically reverse remodelling of the dilated failing heart. While the beat to beat analysis shows hemodynamic effects only when critically optimized, a minimal systolic assistance explains the chronic elastic girdle effect of dynamic cardiomyoplasty. Slowness of the contraction-relaxation cycle of the conditioned Latissimus Dorsi (LD) limits the systolic support. A stable partial transformation of the LD could deliver a higher power by taking advantage of a relatively faster contraction-relaxation cycle. To avoid full fast-to-slow muscle transformation which occurs with the actual clinical protocols, we suggest a discontinuous protocol of muscle stimulation. To test this approach in a simplified model, the sheep LD was resutured in a shortened position after tenotomy of distal aponeurosis to mimic loss of resting tension which occurs in cardiomyoplasty. LD was either stimulated half day daily or all day around. Two weeks after surgery and two, four, and six months after stimulation fusion frequency of tetanic contraction, power output, and fatigue resistance were assessed. After six months of 10 h daily electrostimulation the mean fiber diameter is almost twice than in full day stimulated muscle. The isomyosin analysis shows that the 24 h/day stimulated muscles contains almost only MHC1 (the slow type isoform peculiar of fatigue resistant muscle fibres). Half-day daily stimulated LD contains significant amount of fast type myosin, in particular MHC2A, the isoform of fast-oxidative fibers, less prone to fatigue than the type 2B fibres of which normal LD of adult sheep is rich. Frequency of tetanic fusion is lower in 24 h/day stimulated LD than in 10 h daily stimulated LD, whose behaviour seems to level off at four months. The power output of the half-day stimulated LD is at least twice the amount of the all day stimulated muscle. Results are encouraging: sheep LD loses less mass and power, and seems to achieve a stable intermediate state of fast-to-slow transformation when stimulated 10 h daily during six months. If confirmed after a year of stimulation and extended to human muscle, we are confident these results could be the sound experimental basis of a demand-cardiomyostimulator, whose discontinuous activity could offer the long-standing advantage of a faster and powerful muscle contraction.

Key words: discontinuous electrostimulation, dynamic cardiomyoplasty, Latissimus Dorsi, muscle conditioning to fatigue resistance, sheep, skeletal muscle cardiac assistance, sustainable power output.

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From 1987 to 1992 we investigated the use of conditioned Latissimus Dorsi (LD) as an energy source for skeletal muscle-powered cardiac assistance by means of a skeletal muscle ventricle. Our conclusion after a series of experiments in sheep was that the power generated by a

fully conditioned LD could provide no better than partial assistance for a failing heart [1, 3, 4, 6, 8, 15, 23, 48]. On the other hand, the analyses and results are useful for a critical evaluation of the energetic contribution of the LD to dynamic cardiomyoplasty, a procedure in which the

patient's own left LD is wrapped circumferentially around the failing heart, conditioned and stimulated to augment cardiac contractility [9, 10]. At our knowledge three are the main factors influencing the LD-heart interactions in cardiomyoplasty: i) muscle performance after conditioning; ii) LD mobilisation and loss of resting tension; iii) circumferential wrapping around the failing heart.

In a few weeks muscle conditioning by chronical stimulation transforms a fast short-term powerful muscle in a slow contracting muscle which is fatigue resistant at moderate power. In the preexisting myofibers slow myosin substitutes fast myosins, mitochondrial content and capillary/myofiber ratio increase, intracellular calcium handling is less efficient and the contraction-relaxation cycle significantly slows [11].

Mobilisation of the muscle and the need of not interfering with heart diastole modifies LD resting tension. The relevance of the resting tension of the muscle on work performance is clearly illustrated by the fact that in LD, which is 25-30 cm long, two cm of shortening from the optimal resting length halves isometric force of the tetanus and therefore power output. If we take into account that in cardiomyoplasty only a portion of LD is circumferentially wrapped around the heart, and that, according to Laplace's law, doubling the radius of the heart, the muscle mass ought to be four time bigger to maintain the same pressure, it is conceivable that in dilated heart the muscle contribution to systolic work is difficult to be demonstrated by beat-to-beat analysis. Though heart delivers only 1.3 watts of power to maintain basal metabolism, all together the above mentioned factors explain why the systolic contribution of LD is low in Cardiomyoplasty. During contraction-relaxation cycle instant power peak from zero to zero. LD may contribute to hemodynamic work of the heart, if its peak power is equal or bigger than the instant power of the ventricle during its own contraction-relaxation cycle. Since instant power of the left ventricle (at least of an almost normal ventricle, as in most cases of actual series of patients enrolled for dynamic cardiomyoplasty) is bigger than the peak power of a fully conditioned LD, the grafted muscle could assist the heart only during the late end-systole, just before aortic valve closure.

Clinically, it is fully accepted that LD contributes to heart hemodynamic work (or at least it benefits the patient's quality of life) only if its activation is critically delayed after sensed QRS [24, 27, 46].

For reason of completeness we remind that conditioned LD is also used as an energy source for counterpulsation, e.g., in aortomyoplasty. Even in this less energy demanding approach, we have to take into account that muscle relaxation is a passive process which dissipates some of the energy accumulated during muscle contraction: in skeletal muscle it accounts for 10% of total power, not a trivial amount when power is limited.

To many authors, cardiomyoplasty is a clinical reality, which founds its basis more on a girdle effect which limits and/or reverse the progressive dilatation of a failing heart,

than on an easy-to-demonstrate beat-to-beat systolic assistance. Using an irreverent but pertinent analogy, if we compare the pictures of a young girl which uses an orthodontic device, at the beginning her teeth look crooked with and without the apparatus, and then she beautifully smiles at the end of three years with and without the device. Only comparing pictures taken the first day of use and three years later, we see the difference! In cardiomyoplasty to demonstrate beat-to-beat assistance a very critical approach is needed [46], while load independent measurements demonstrate a real amelioration of the heart energetics when analyses are compared before and after cardiomyoplasty [30].

Therefore we re-evaluated the concept of "muscle conditioning" and its goal in cardiomyoplasty. Actual clinical protocol makes the LD very resistant to fatigue, but meanwhile its dynamic characteristics are suboptimal, at best. Indeed with a 185 msec stimulation train of six impulses, the contraction-relaxation cycle of a conditioned LD lasts longer than the heart systole [21, 39].

Following our analysis, we are testing whether an intermediate state of muscle transformation could be indefinitely maintained to better sustain by faster contractions a cardiac-like amount of averaged external power (about one watt of power per LD). We here present mid-term results of a pilot study based on the hypothesis that resting the LD several hours per day allows to maintain an intermediate state of transformation as a result of daily training-detraining effect. Our results are also relevant to the question if an intermittent stimulation of the LD is less detrimental for the muscle tissue. If results of this pilot study will be confirmed, they could be the basis for a clinical testing of a demand-cardiomyostimulator for Cardiomyoplasty.

Materials and Methods

Surgical procedures and analyses of dynamic characteristics of sheep LD were performed at the Experimental Surgery Unit, S. Orsola Hospital, University of Bologna; histochemical and molecular analyses on muscle specimens were accomplished at the Department of Biomedical Sciences, University of Padova. A pilot study was conducted in six sheep. To mimic dissection effects on vascularisation and loss of resting tension of cardiomyoplasty, the sheep LD was resutured in a shortened position after tenotomy of distal aponeurosis; intramuscular electrodes were implanted according to the Medtronic Protocol as previously described [6]. Two weeks after surgery, power output and fatigue tests were assessed according to [5, 15]. Then ITREL stimulators (Medtronic) were programmed to settings that just elicited fatiguing contractions in the shortened LD. Four sheep were stimulated 10 hours daily, while two sheep were stimulated all day around. In both cases LD was stimulated about 30 times per minutes with bursts of impulses lasting 140 msec at 20 Hz. Power output and fatigue tests were repeated at two-, four- and six-month stimulation. In terms of muscle performance, endurance has a similar connotation to fatigue resistance.

Daily work-rest stimulation for cardiomyoplasty

Table 1. Cardiomyoplasty-like mobilization of sheep LD. Myosin Heavy Chain complement of sheep LD after six months of either 24 h or 10 h per day stimulation

	Fatigue-resistant fibres		
	Fast-types		Slow-type
	MHB2B	MHC2A	MHC1
Normal sheep (15)	73	15	12
24 h stimulation			
Bo 95-2	-	-	100
Bo 95-3	-	-	100
12 h stimulation			
Bo 95-1	13	66	21
Bo 95-4	44	29	27
Bo 95-5	60	18	22
Bo 95-6	-	45	55

It can be measured directly as the time in which a specified task can be performed, or indirectly as the magnitude of the power that can be sustained over time. To measure the sustained power, the average force during the duty cycle was multiplied by the velocity of shortening. The average power during the duty cycle was averaged over the total duty-rest cycle and expressed per muscle. The protocol is analogous to the graded exercise test commonly used in work physiology. Since the sheep LD is expected to weight about 250 g (a conservative overestimation of the muscle weight in adult sheep) the external power output normalized per kg of muscle mass (W/kg) is four-five time higher than the one measured per muscle. Tetanic fusion frequency is used as an index of duration of LD contraction-relaxation cycle. Immunohistochemistry, electrophoretic isomyosin profile, and quantitation of molecular markers of muscle damage/regeneration (total protein, collagen, myosin/actin ratio) were performed on serial cryostat sections of small biopsies only at six months of stimulation to limit the surgical muscle damage during the experiment. Methods are detailed in: [12, 35, 36, 43, 44].

Results

The isomyosin analysis shows that while the all day stimulated muscles contain only MHC1 (the slow type isoform peculiar of fatigue-resistant muscle fibers), the muscles stimulated half-day still contain significant amounts of fast type myosins, in particular MHC2A; this isoform is peculiar of fast-oxidative fibers, less prone to fatigue than the type 2B isoforms, of which is rich the normal LD of adult sheep (Table 1).

Immunohistochemistry for MHC1 confirms that all the myofibres of the all day stimulated muscles are positive

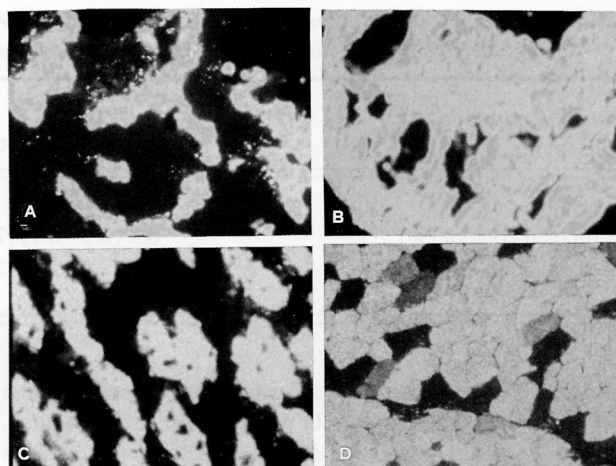


Figure 1. Cardiomyoplasty-like mobilization of sheep LD. Fiber typing of LD by MHC1 immunolocalization. A, normal LD; B, 24 hr daily stimulation, C and D, 10 hr daily stimulation. Analysis by anti MHC1 staining of cryostatic sections shows only type 1 myofibers in the all day stimulated LD, while the half-day stimulated LD muscles show a mosaic pattern.

(Fig 1, B), and that half-day stimulated muscles maintain a mosaic appearance after six months of stimulation (Fig 1, A and D).

Table 2 shows that after six months of daily 10 h electrostimulation the mean fiber diameter is almost twice than in all day stimulated muscles, whose myofibres are dispersed among interstitial tissue. On the other hand, total protein, collagen content and myosin/actin ratio do not show consistent differences between the two groups (Table 3). Thus, all the muscles seem to have reached a stable extent of transformation.

In accordance with the histological and molecular results, Table 4 shows that the tetanic fusion frequency for discontinuously stimulated LD is higher than for continuously stimulated muscles, indicating that their contraction-relax-

Table 2. Cardiomyoplasty-like mobilization of sheep LD. Mean muscle fiber diameter after six months of either 24 h or 10 h per day stimulation.

	24 h stimulation	12 h stimulation
Bo 95-2	21.3 ± 2.1	
Bo 95-3	12.4 ± 1.4	
Bo 95-1		20.8 ± 1.2
Bo 95-4		30.3 ± 2.5
Bo 95-5		29.2 ± 2.3
Bo 95-6		41.2 ± 9.7
Mean	16.8 ± 1.6	30.4 ± 3.9

Daily work-rest stimulation for cardiomyoplasty

Table 3. Cardiomyoplasty-like mobilization of sheep LD. Total protein, collagen content and myosin/actin ratio after six months of either 24 h or 10 h per day stimulation. Mean, (range) and (number of sheep).

Daily stimulation Sheep	24 hrs 95-2, and 95-3	10 hrs 95-1, 95-4, 95-5, and 95-6
Total protein (%)	7 (1)	15 (7 - 22) (4)
Collagen content (%)	0.9 (0.9 - 0.9) (2)	0.6 (0.2 - 0.9) (3)
myosin/actin ratio	2.4 (2.4 - 2.4) (2)	2.0 (1.9 - 2.1) (4)

ation cycle is much slower. Furthermore, transformation of the all day stimulated LD proceeds up to six months, while the behaviour of the 10 h daily stimulated muscles levels off four months after stimulation.

Table 5 shows the results of power output and fatigue tests of LD muscle two weeks after distal aponeurectomy and suture in shortened position. Before conditioning, 2 Hz

continuous electrostimulation yields less than 0.1 watt per LD and fatigue is present after 10 min of low-frequency continuous electrostimulation. When the shortened LD are stimulated with 28 per min 10 Hz bursts of pulses lasting 130 msec each (two pulses of 0.2 msec per burst) an external power of 0.2 watts is sustained without fatigue. If the bursts are delivered at 20 Hz (three impulses per train)

Table 4. Cardiomyoplasty-like mobilization of sheep LD and daily continuous or discontinuous stimulation. Tetanic fusion frequency (Hz).

Daily stimulation Sheep	Tetanic fusion frequency (Hz)					
	24 hrs		10 hrs			
	95-2	95-3	95-1	95-4	95-5	95-6
Two weeks after LD mobilization	> 30	> 30	> 30	> 30	> 30	> 30
Two-month stimulation	10	10	20	30	20	20
Four-month stimulation	10	10	10	20	10	20
Six-month stimulation	< 5	< 5	10	10	10	10

Table 5. Sustainable power output of normal LD of adult sheep two weeks after distal aponeurectomy and suture in shortened position. Power, watts per muscle (W).

Sheep	Continuous	28 events/min (130 msec)			
	2 Hz	10 Hz	20 Hz		
	Fatigue W (10 min)	Fatigue W (10 min)	W	Fatigue (10 min)	
Bo 95-1		0.1 absent	0.4		present
Bo 95-2		0.2 absent	0.2	absent	
Bo 95-3		0.5 absent	0.5	absent	
Bo 95-4	0.1 present	0.2 absent	0.4		present
Bo 95-5		0.1 absent	0.5	absent	
Bo 95-6		0.3 absent	0.7		present
Mean		0.2 absent	0.4	absent (3)	
			0.5		present (3)

Daily work-rest stimulation for cardiomyoplasty

Table 6. Cardiomyoplasty-like mobilization of sheep LD and daily continuous or discontinuous stimulation. Sustained external power and fatigue test. Mean, (range) and (number of sheep).

Daily stimulation	24 hrs	10 hrs
Sheep	95-2, and 95-3	95-1, 95-4, 95-5, and 95-6
Hz 30, Events per min	36 (3 pulses)	28 (3 pulses)
Two-month stimulation		
Mean external power, (W)	0.4 (0.3 - 0.5) (2)	1.2 (0.4 - 3.1) (4)
Fatigue - Test (min 10)		
-Δ% of time zero	-6 (0 - -12) (2)	-13 (0 - -26) (4)
Four-month stimulation		
Mean external power, (W)	0.6 (0.4 - 0.8) (2)	2.4 (1.0 - 5.9) (4)
Fatigue - Test (min 10)		
-Δ% of time zero	0 (2)	0 (4)
Six-month stimulation		
Mean external power, (W)	0.5 (0.4 - 0.6) (2)	2.7 (2.2 - 3.9) (4)
Fatigue - Test (min 10)		
-Δ% of time zero	0 (2)	0 (4)

in three sheep a mean power of 0.4 watts is sustained without fatigue, while in the three other sheep, which yield 0.5 watts of external power, fatigue appears during 10 min of stimulation. Of course, when fused tetani are produced by over than 50 Hz stimulation and/or frequency of train is over 100 per min, power increases more than ten times during the first min, but fatigue appears and power decreases near to zero after a few minutes.

Table 6 shows that after two, four and six months of all day stimulation the sheep LD sustain around 0.5 watts of external power, while all the 10 h daily stimulated muscles deliver more than one watt after both four and six months of stimulation. Sheep Bo 95/6 which presents the larger myofiber sizes, consistently showed the higher values of sustained power throughout the experiment.

Discussion

Two questions were the goals of our study: Is it possible to maintain an intermediate state of transformation to obtain fatigue-resistance, but avoid the slowness which is an unwanted consequence of full fiber type transformation? Is the unusual activity performed by LD in cardiomyoplasty damaging the muscle?

Independent groups of physiologists and biochemists analyzing extent of transformation in long-term stimulated muscles reported apparently conflicting results for years only because one group performed the experiments using implantable devices to stimulate rabbit muscle 24 hours per day [28, 37], while the other group using external devices was obligated to discontinue muscle stimulation during

every night [32, 33]. Available data of experiments in rabbit, rat and mouse show the results we are collecting in sheep. We are confident that our preliminary results will be confirmed by additional data, and that they will be validated by clinical trials in humans, since experiments in rodents and rabbit were validated after long-term chronic electrostimulation. The biological basis of this approach is that "intermediate" myofibers exist in nature: several different fiber types with intermediate characteristics between very fast- and very slow-contracting fiber exist in muscle of mammals, man included, and their characteristics are induced and maintained by activity against load [2, 11, 13, 33, 38, 40, 45, 47]. By selecting the proper work-load it is possible to make pressure on muscle genes to transform all the fibers to a desired type. Of course, this is not the answer to all cardiomyoplasty problems, but it is at least a testable hypothesis. The first concept to stress is that muscle fatigue-resistance is a relative concept. Muscles are fatiguable or fatigue-resistant to a given work-load and for a given period of time: normal muscles are able to sustain indefinitely a task if energetically low demanding. We here showed that even before conditioning, the shortened LD was able to perform about 100 mWatts of extracted power by stimulation with single impulses at 2 Hz (120 events per minute), which is the higher frequency of heart pace-makers. Such a result may explain while pioneering cardiomyoplasty performed in Latin America using unsynchronized normal pace-maker to stimulate the pedicled LD could improve patients' quality of life.

Of course, when tetanic stimulation was elicited by bursts of impulses in shortened muscles, the sheep LD delivered 0.2-0.3 Watts of external power per LD without fatigue. After increasing the frequency of tetani or inducing more powerful tetani by bursts at higher frequency, the external power reached 0.5-1.0 Watt per muscle, but the muscles fatigued in a few minutes and the external power either levelled-off at about 0.2 Watts per muscle, or even ceased when work-load was near to maximal values. An interesting implication of these preliminary observation is that LD could be used immediately after the healing period by setting the stimulator at very low muscle demands.

After 6 months of stimulation, analysis by anti MHC1 staining of cryostatic sections shows only type 1 myofibers in the all day stimulated LD, while the half-day stimulated LD is a mixed muscle. Graded amounts of staining are present in different myofibers of the half-day stimulated LD suggesting a partial transformation of pre-existing myofibers, while selection of fibers or cycles of damage/regeneration seem not to occur.

The mean diameter of the half-day myofibers is twice the diameter of the all day stimulated LD. The latter result is in good correlation with data previously reported, since continuous stimulation is known to decrease the surface/diameter and muscle mass to blood perfusion ratios to favour oxidative metabolism of the myofibers [37]. If the muscle is daily rested homeostasis is near to normal values. In the more powerful sheep of the half-day stimulated group, the fibers are normotrophic.

The analysis of myosin heavy chain isoforms in serial sections by SDS-PAGE confirms that the all day stimulated LD muscles contain only MHC1, i.e. they are fully transformed, while the muscles stimulated 10 hours daily still contain significant amount of fast type MHC, in particular of the 2A type. This observation could explain the different dynamic characteristics of the two groups of LD, since calcium uptake/release is faster in 2A myofibers. Indeed, in 10 h daily stimulated muscles frequency of tetanic fusion was higher than in LD fully conditioned by all day electrostimulation. Comparison between various regimens of stimulation, such as daily amount of treatment or frequency, are rare. It is likely that 10 h stimulation per day will produce different results than 24 h. Firstly, 10 h of stimulation cover about one third of the 24 h stimulation period and, secondly, this protocol gives the muscle time to recover in between. Though the possibility exists that final outcome of changes using either method may be, ultimately, similar after long-term periods of stimulation, it is well established that in animals stimulated 12 h/day, mRNA of MHC1 becomes detectable after stimulation periods exceeding 20 days, while continuous stimulation (24 h/day) leads to an earlier appearance (9 days). Cessation of stimulation has pronounced effects on the mRNA pattern leading to a rapid reversal of the stimulation-induced changes [33]. Biochemical changes, may be acidosis, AMP and inorganic phosphate which accumulate during muscle fatigue are probably among the intracellular

messengers of muscle plasticity. The actual clinical stimulation protocol of cardiomyoplasty is very demanding, so it is not surprising that the LD is transformed in a pure slow-type muscle by long-term continuous stimulation. Nine months and two years after cardiomyoplasty the LD flap stimulated every cardiac cycle with 30 Hz bursts lasting 185 msec showed preservation of muscle structure, and histochemical analyses revealed only type 1 fibres [25]. On the other hand, in the sheep LD an intermediate state of transformation is maintained at least up to 6 months by daily modulation of the working periods.

A second issue in dynamic cardiomyoplasty is if muscle damage is induced by chronic stimulation *per se*, in particular when a muscle-to-heart contraction ratio of 1 : 1 is applied. Exercise may induce muscle damage, and physiologists, sport scientists and physiatrists are well aware that spontaneous exercise could be *per se* a trauma to muscle fibers [7, 22, 34, 41, 42, 50]. Physiologists are hardly working on this, but to allow statistical analysis of the collected data their experiments are tailored according to experimental requirements, not to every day variable conditions. Cardiomyoplasty is a complex procedure and it is difficult to even identify the relevant variables. Since the controlled environments of a physiologists' experiment are not totally applicable, we have variable results in our few sheep, but at least we are collecting data. In any case the biopsies of half-day stimulated LD present a well preserved muscle structure with moderate and non-specific changes: myofiber size is much larger, and interstitial tissue is smaller than in biopsies of all day stimulated LD. Of course the real question is if the unusual work performed after grafting in cardiomyoplasty damages human LD. There are reports explaining long-term ceased effect of the procedure with indirect evidence of major muscle atrophy, fibrosis and fat infiltration [20, 29, 31, 46, 49]. On the other hand, two autoptic cases directly show that this is not an obligatory event: 15 months or even 8 years after cardiomyoplasty morphological and molecular analyses of the pedicled LD showed preserved muscle mass and patent vessels with normal walls; interestingly, in these two cases LD graft was activated every second or fourth sensed QRS, and clinical results were excellent [14, 16].

Of course, several independent factors may damage the muscle beside the pattern of activation, i.e., lesions of nerves, arteries, or veins during or after operation, loss of resting tension, etc. [20, 26]. Our histopathological observations of grafted LD up to 8 years after cardiomyoplasty demonstrate that damage is not a mandatory consequence of the unusual activity the muscle performs to assist failing heart [14, 16, 26].

Eleven years after the first clinical case, we may hope that cardiomyoplasty is at the stage the heart transplant was after immunopharmacologists solved the problem of rejection of autologous transplant by immunosuppressive drugs. For heart transplant too, the surgical problems were solved several years earlier than the rejection problem. Carpentier and Chachques solved the basic surgical prob-

lems of cardiomyoplasty eleven years ago [9], now the knowledge exists to overcome remaining problems of cardiomyoplasty. Risks of damage of LD may be reduced, and muscle performance increased by work-rest regimens and by administration of local anabolic agents [17-19].

Acker and colleagues and several others are collecting scientific evidence on the mechanisms of cardiomyoplasty effectiveness [30, 46]. We are confident that our pilot experiment will attract attention, and reinforcing the concept of a lighter and demand stimulation of the grafted LD, it will contribute to a larger acceptance of the procedure, to a better management of pharmacologically intractable heart failure, and to a better quality of life of patients.

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Address correspondence to:

Prof. Giorgio Arpesella, via S. Stefano 16, 40121 Bologna, Italy.

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