Activity-Rest Regimen of Latissimus Dorsi Stimulation for Cardiomyoplasty: Anatomy, Isomyosins and Sustained Power of Sheep LD up to One Year

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Abstract

A prudent explanation of the clinical effect of dynamic cardiomyoplasty is that a minimal systolic assistance enhances the chronic elastic girdle effect of the transposed Latissimus Dorsi (LD). Slowness of the contraction-relaxation cycle and reduced power output of a fully conditioned LD limit its systolic support. Steady partial transformation of LD could increase power output by taking advantage of a faster contraction-relaxation cycle. To avoid full fast-to-slow transformation of LD, we chronically tested a daily activity-rest regimen of muscle stimulation in a simplified experimental model. To mimic loss of resting tension which occurs in cardiomyoplasty, sheep LD after tenotomy of distal aponeurosis were resutured in shortened position and ITREL neurostimulators (Medtronic) connected to intramuscular electrodes were implanted according to the Medtronic Protocol. From two weeks after surgery shortened LD were burst-stimulated either 10 or 24 hr per day, the stimulators being programmed to the settings that elicited just fatiguing contractions in the shortened LD. Full-day activated LD were stimulated six months and then left unstimulated for additional six months, while the half-day activated muscles were stimulated up to one year. Two weeks after surgery and two, four, six and twelve months after stimulation, fusion frequency of tetanic contraction, power output, and fatigue resistance of LD were assessed. To allow histological and molecular characterization of the two groups of stimulated muscles, LD were biopsied at six months of stimulation, and sheep sacrificed at twelve months to collect macrosopic anatomical records and perform molecular and histological analyses of proximal, intermediate and distal muscle specimens. After one year of 10 hr/day electrostimulation the gross anatomy of the LD were substantially conserved in comparison with contralateral, normal muscles (about 10% atrophy accompanied by minor fat infiltration and fibrosis). Isomyosin analysis shown that even after one year of stimulation the 10 hr/day stimulated LD contained large amounts of fast type myosin, in particular MHC2A, the isoform of fast-oxidative fibers, less prone to fatigue than the type 2B fibers of which normal LD of adult sheep is very rich. Though after six months of 24 hr/day stimulation LD were fully converted to type 1 myosin, after additional six months of resting these LD were white in appearance, atrophic (about 40%), fibrotic, and their isomyosin pattern as mixed as the LD stimulated 10 hr/day for twelve months. Accordingly, after four and six months of stimulation the frequency of tetanic fusion was higher (i.e., the contraction-relaxation cycle was faster) in 10 hr/day stimulated LD than in 24 hr/day stimulated LD; the difference disappeared at one year since the fusion frequency of the rested LD recovered to values of the one-year 10 hr/day stimulated LD. Of foremost importance is the fact that from two-month up to one-year of stimulation the sustained power output per muscle of the 10 hr/day stimulated LD (that is of the daily rested muscle) is three to four times higher than that of the 24 hr/day activated LD. From two and at least up to twelve months of stimulation the sustained power of the “daily-rested” LD become higher than that of the heart at rest. In conclusion, results of our activity-rest daily regimen are encouraging: sheep LD loses very low contractile mass, and its power is equal or bigger than that of the left ventricle, since it seems to achieve a stable intermediate state of fast-to-slow transformation when stimulated.
If these results will be confirmed and extended to human muscle, we are confident that they could be the experimental basis for a demand cardiomyostimulator, whose discontinuous activity could offer to cardiomypoplasty patients the long-standing advantage of a faster and more efficacious muscle contraction.

**Key words:** activity-rest daily regimen, discontinuous electrostimulation, dynamic cardiomypoplasty, heart failure, latissimus dorsi, muscle conditioning to fatigue resistance, sheep, skeletal muscle cardiac assistance, sustained power.

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Experiments in sheep aimed to investigated the use of Lattisimus Dorsi (LD) as an energy source for a skeletal muscle ventricles allowed us to conclude that the power generated by a fully conditioned LD could provide no better than partial assistance for a failing heart [1, 3, 4, 6, 7, 9, 16, 24, 51]. On the other hand, those 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 [10, 11]. 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 active systolic assist. To demonstrate beat-to-beat assistance a very critical approach is needed [50], but load independent measurements demonstrate a real amelioration of the heart energetic when analyses are compared before and after cardiomyoplasty [33]. Using a relevant analogy, if one compares the pictures of a young girl which uses an orthodontic device, at the beginning her teeth look crooked with and without the apparatus, and again she beautifully smiles with and without the device at the end of three years of continued use. Only if one compares the pictures taken the first and the last day of use the difference is evident.

At our knowledge the limiting factors of LD-heart interactions in cardiomypoplasty are: i) loss of resting tension due to LD mobilization; ii) circumferential wrapping around the failing heart; and iii) muscle performance after full conditioning. Both the mobilization of the muscle and the need of not interfer with heart diastole reduce LD resting tension thus decreasing its work potential. In cardiomypoplasty only a portion of LD is circumferentially wrapped around the heart, and since 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. 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. After a few weeks of chronical stimulation, LD mitochondrial content and capillary/myofiber ratio increase, but intracellular calcium handling becomes less efficient and therefore the contraction-relaxation cycle significantly slows; finally slow myosin substitutes fast myosins, so that a fast, powerful (but early fatiguable) LD is transformed in a slow contracting muscle which is fatigue resistant at moderate power [12]. 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 a fully transformed LD is low in cardiomyoplasty.

 Clinically, it is fully accepted that LD benefits the patient's quality of life only if its activation is critically delayed after sensed QRS to avoid mitral regurgitation [25, 30, 49]. Since maximum instant power of a fully conditioned LD is smaller than the peak power of the left ventricle [1, 3, 4, 6, 7, 9, 16, 24, 51], we suggest that the grafted muscle could assist the heart only during late end-systole, just before closure of the aortic valve. Of course, such a short window asks for a fast, powerful contraction which is not delivered by a fully transformed LD. 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 [22, 42].

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 (over one watt of power per LD). We here present one-year 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. Furthermore, a daily intermittent stimulation of the LD could also be less detrimental for the muscle tissue since such a protocol gives the muscle time to recover in between activity periods.

**Materials and Methods**

Surgical procedures and analyses of dynamic characteristics of sheep LD were performed at the Experimental
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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. The pilot study was conducted in six adult sheep. To mimic dissection effects of cardiomyoplasty on muscle vascularisation and loss of resting tension, after tenotomy of distal aponeurosis the sheep LD was resutured in a shortened position; an ITREL stimulator (Medtronic) and intramuscular electrodes were implanted according to the Medtronic Protocol as previously described [7]. Two weeks after surgery, to allow wound healing, tetanic fusion frequency, power output and fatigue tests were assessed according to [5, 7, 16]. Then stimulators 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 three impulses lasting about 140 msec at 20 Hz. Tetanic fusion frequency, power output and fatigue tests were repeated at two-, four-, six- and twelve-month stimulation. Since fatigue appeared just above the conditioning settings (but of course at higher sustained power outputs), stimulation parameters were not changed during the first six months of the experiment. In terms of muscle performance, endurance has a similar connotation to fatigue resistance. 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 (sustained power). To measure sustained power, the average force during the duty cycle was multiplied by the velocity of shortening, then the average power during the duty cycle was averaged over the total duty-rest cycle and expressed per muscle (W). The measures were repeated using bursts at higher frequency (in-burst frequency of 10, 20, and 30 Hz) or more frequent (either 30 or 100 bursts per min) until muscle fatigue appeared. The protocol is analogous to the graded exercise test commonly used in work physiology. To allow repetitive measurements of the contractile characteristics of the shortened LD in the same sheep, the leg and not the muscle tendon was secured to the force transducer. Since in these conditions isometric tests could not be performed at optimal muscle length, tetanic fusion frequency was used as an index of duration of contraction-relaxation cycle of the shortened LD. At twelve months the sheep were sacrificed by excessive anesthesia. LD were dissected, perimysial fat and connective tissue carefully removed, and muscles weighted and photographed. Three muscle specimens were cut out from proximal, intermediate and distal portions of the LD, quenched in liquid nitrogen and stored at -80°C until use. Myosin ATPase, immunohistochemistry, isomyosin profile by SDS PAGE of MHC, and quantitation of molecular markers of muscle damage/regeneration (total protein, collagen, myosin/actin ratio and embryonic myosin) were performed as described in [13, 38, 39, 46, 47]on serial cryostat sections of the twelve-month specimens and on distal biopsies performed only at six months of stimulation to limit the surgical muscle damage during the experiment [7].

Results

Figure 1 shows gross anatomy of representative experimental muscles removed after twelve months of 10 hr/day stimulation or six-month 24 hr/day stimulation followed by six-month rest. The biggest of the three muscles is the right contralateral normal LD of sheep Bo95-6, while the muscle immediately below is the contralateral LD stimulated twelve-month 10 hr/day; though slightly diminished in size and weight the chronically stimulated muscle appears trophic and as red as the normal muscle. Similar were the aspects of all the twelve-month 10 hr/day electrostimulated LD, that is of sheep Bo95-1, Bo95-4 and Bo95-5. The third muscle in figure 1 is the LD of sheep Bo95-3 which had been stimulated six-month daily and then rested for additional six months. Likewise LD of sheep Bo95-2...
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which had been also six-month stimulated 24 hr/day and then rested six months, LD of sheep Bo95-3 appears pale and fibrotic. Table 1 shows the wet weights of the contralateral and experimental muscles after careful removal of perimysial fat. Muscles stimulated twelve months 10 hr/day show about 10% atrophy in comparison of their normal contralaterals, while the LD stimulated six-month daily and then rested for additional six months present about 40% atrophy.

Table 1 shows the results of the isomyosin analysis performed on LD at twelve-month. Myosin heavy chains of the experimental LD are very different from normal LD due to their high content of MHC1, the isoform peculiar of slow myofibers, but again they are not fully transformed by activity-rest regimens of stimulation either discontinued every day or after six months of daily stimulation. To interpret these results it is essential to remind that at six months of stimulation LD biopsies of the all day stimulated muscles contained only MHC1 (the slow type isoform peculiar of fatigue-resistant muscle fibers), while the muscles stimulated 10 hr/day contained large amounts of fast type myosins, in particular MHC2A, the isoform peculiar of fast-oxidative fibers, less prone to fatigue than the type 2B isoforms, of which is rich the normal LD of adult sheep [7, 12].

Accordingly, Table 3 shows that the tetanic fusion frequency is at one year similar in the two groups of muscles, while at six months the daily stimulated LD were slower than the half-day stimulated LD. It is worth stressing that the tetanic fusion frequency (that is, the contraction-relaxation cycle) of the 10 hr/day stimulated muscles is very similar after four, six and twelve months of stimulation.

Finally, Table 4 shows power outputs and fatigue tests of LD muscles four, six and twelve months after surgery. Up to six months sheep LD stimulated 24 hr/day sustain about 0.5 watts of external power, while the 10 hr/day stimulated muscles delivers without fatigue about 2 watts of external power up to twelve months of stimulation. It is interesting to note that one of the muscles rested for six months sustains more power than after six months of 24 hr/day stimulation regimen.

Discussion

The first concept we would like to stress is that fatigue-resistance of skeletal muscle is a relative concept. Muscles are fatigable 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 it is energetically low demanding, indeed our diaphragm sustains ventilation 24 hr/day! Previously [7], we showed that after shortening but before

<table>
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<th>Table 1. Cardiomyoplasty-like mobilization of sheep LD and daily continuous or activity-rest stimulation. Wet weight of sheep LD after either twelve months of 10 hr/day stimulation or six-month of 24 hr/day stimulation followed by six-month rest. Mean [range] (number of observations).</th>
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<tbody>
<tr>
<td>Sheep</td>
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<tr>
<td>LD wet weight (gr)</td>
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<tr>
<td>Contralateral normal LD</td>
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<td>6-month 24 hr stim &amp; 6-month rest</td>
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<td>12-month 10 hr stim</td>
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<th>Table 2. Cardiomyoplasty-like mobilization of sheep LD and daily continuous or activity-rest stimulation. Myosin heavy chains complement of sheep LD after either twelve months of 10 hr/day stimulation or six-month of 24 hr/day stimulation followed by six-month rest. Mean [range](number of observations).</th>
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<tr>
<td>Fatigue-resistant fibres</td>
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<tr>
<td>MHB2B</td>
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<tr>
<td>Normal sheep (15)</td>
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<tr>
<td>6-month 24 hr stim (2)</td>
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<tr>
<td>6-month 24 hr stim &amp; 6-month rest (2)</td>
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<td>6-month 10 hr stim (4)</td>
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<td>12-month 10 hr stim (4)</td>
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Table 3. Cardiomyoplasty-like mobilization of sheep LD and daily continuous or activity-rest stimulation. Tetanic fusion frequency (Hz) of sheep LD after either twelve months of 10 hr/day stimulation or six-month of 24 hr/day stimulation followed by six-month rest. Mean [range] (number of observations).

<table>
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<tr>
<th>Condition</th>
<th>Tetanic fusion frequency (Hz)</th>
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<tr>
<td>Two weeks after LD mobilization (6)</td>
<td>&gt;30 [&gt;30 - &gt;30]</td>
</tr>
<tr>
<td>6-month 24 hr stim (2)</td>
<td>&lt;5 [&lt;5 - &lt;5]</td>
</tr>
<tr>
<td>6-month 10 hr stim (4)</td>
<td>10 [10-10]</td>
</tr>
<tr>
<td>6-month 24 h stim &amp; 6-month rest (2)</td>
<td>20 [20-20]</td>
</tr>
<tr>
<td>12-month 10 hr stim (4)</td>
<td>10 [10-20]</td>
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conditioning the sheep LD was able to deliver about 0.1 watt of extracted power by stimulating it with single impulses at 2 Hz (120 events per minute), which is the higher frequency of a heart pace-maker. Of course, when tetanic contractions were elicited by bursts of impulses, the shortened LD delivered 0.2-0.3 watts of external power without signs of 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 then external power either levelled-off at about 0.2 watts per muscle, or even ceased when work-loads were near to maximal values. An implication of our original observations is that after cardiomyoplasty LD could deliver sustainable power immediately after the healing period by setting the stimulator at very low muscle demands [7].

Of course after chronic stimulation the working capacity of the LD increases, but only if the LD is rested several hours every day the sustained power exceeds the value of the left ventricle at rest, since by such an activity-rest regimen it seems possible to maintain an intermediate state of myofiber transformation in the sheep LD.

The biological basis of such an approach is that "intermediate" myofibers do exist in nature: several different fiber types with intermediate characteristics between very fast- and very slow-contracting fiber exist in skeletal muscles of mammals, man included, and their characteristics are induced and maintained by different level of activity against load [2, 12, 14, 27, 36, 41, 43, 48, 50, 51]. By selecting the proper work-load it is probably possible to make pressure on muscle genes to transform all the fibers to a desired type. Independent groups of physiologists and biochemists studying muscle plasticity in long-term stimulated muscles reported apparently conflicting results for years only because one group performed the experiments using implantable devices to stimulate rabbit muscles 24 hours per day [31, 40], while the other group, using external devices, discontinued muscle stimulation during every night [35, 36]. Available data of experiments in rabbit, rat and mouse show the results we are collecting in sheep. We are confident that results of our pilot study will be confirmed by independent experiments, and that they will be validated by clinical trials in humans, since in rodents and

Table 4. Cardiomyoplasty-like mobilization of sheep LD and daily activity-rest or continuous stimulation. Sustained external power (W) and fatigue (-D%) of sheep LD after either twelve months of 10 hr/day stimulation or six-month of 24 hr/day stimulation followed by six-month rest. Mean [range] (number of observations).

<table>
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<tr>
<th>Daily stimulation</th>
<th>24 hr/day</th>
<th>10 hr/day</th>
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<tr>
<td>Sheep Hz 30, Events per min</td>
<td>95-2, and 95-3 36 (3 pulses/burst)</td>
<td>95-1, 95-4, 95-5, and 95-6 28 (3 pulses/burst)</td>
</tr>
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</table>

| 4-month stimulation | Mean external power, (W) | 0.6 [0.4 - 0.8] (2) | 2.4 [1.0 - 5.9] (4) |
|                     | Fatigue(-D%)             | 0 (2)               | 0 (4)               |

| 6-month stimulation | Mean external power, (W) | 0.5 [0.4 - 0.6] (2) | 2.7 [2.2 - 3.9] (4) |
|                     | Fatigue(-D%)             | 0 (2)               | 0 (4)               |

| 6-month stim & 6-month rest | Mean external power, (W) | 0.8 [0.4 - 1.2] (2) | 1.7 [0.5 - 3.2] (4) |
|                            | Fatigue(-D%)             | 0 (2)               | 0 (4)               |

| 12-month stimulation | Mean external power, (W) | 1.7 [0.5 - 3.2] (4) | 0 (4)               |
|                      | Fatigue(-D%)             |                     |                     |
rabbit results were validated after long-term chronic electrostimulation.

To allow repetitive measurements of the contractile characteristics of the shortened LD in the same sheep, the leg and not the muscle tendon was secured to the force transducer. Since in these conditions of measurement isometric tests could not be performed at optimal muscle length, tetanic fusion frequency was used as an index of duration of contraction-relaxation cycle of the shortened LD. Since the values are lower than those expected for sheep LD under optimal length, we have an indirect evidence that the LD are really shortened by our procedure. Furthermore, it is worth stressing that after four and six months of stimulation the frequency of tetanic fusion was higher (i.e., the contraction-relaxation cycle was faster) in the 10 hr/day than in the 24 hr/day stimulated LD, and that this difference disappeared at one year since the fusion frequency of the six-month rested muscle recovered to values of the 1-year 10 hr/day stimulated LD. Though performed under suboptimal conditions, changes in frequency of tetanic fusion are evidence of a training-detraining effect of our stimulation regimens.

Convincing results are collected by analysis of isomyosin gene expression in the experimental muscles. Analyses of myosin heavy chain isoforms by SDS-PAGE in serial sections of muscle biopsies taken six-months after stimulation showed that the 24 hr/day stimulated LD contain only MHC1, i.e., they are fully transformed, while the muscles stimulated 10hr/day still contain large amounts of fast type MHC, in particular of the 2A type [7]. Furthermore, we here show that after twelve months of stimulation, the 10 hr/day stimulated LD contain substantial amounts of fast type myosin heavy chains, and that after six months of discontinued stimulation the fast type MHC are reexpressed in the 24 hr/day stimulated LD, which was shown to be fully transformed at six-month [7]. These observations explain the different dynamic characteristics of the two groups of LD, since calcium uptake/release is faster in type 2A than in type 1 myofibers. Indeed, at two, four and six months of stimulation frequency of tetanic fusion was higher in 10 hr/day stimulated muscles than in LD fully conditioned by 24 hr/day electrostimulation. At one year the contraction-relaxation cycle of the 10hr/day stimulated LD is as fast as at six month, while the six-month discontinued stimulation made faster the LD fully transformed by six-month of 24 hr/day activity. Interestingly the complement of MHC are very similar in the two groups of muscles, suggesting that the total amount of muscle contractions have a main role in driving gene expression in the myofibers. These results are in full agreement with results of long-term training and detraining experiments in rodents, rabbit, goat, sheep and man. [2, 5, 6, 12, 26, 28, 31, 36, 40, 41, 50].

Comparison between various regimens of stimulation, such as daily amount of treatment or frequency, are rare. It is likely that 10 hr stimulation per day will produce different results than 24 h. Firstly, 10 hr of stimulation cover about one third of the 24 hr 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 (i.e., after several years), 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). Furthermore, cessation of stimulation has pronounced effects on the mRNA pattern leading to a rapid reversal (hours) of the stimulation-induced changes [36]. Biochemical changes, may be acidosis, AMP, inorganic phosphate which accumulate during muscle fatigue, and/or cytosolic calcium are probably the intracellular messengers of muscle plasticity. The actual clinical stimulation protocol of cardiomyoplasty are very demanding, so it is not surprising that the LD is transformed in a pure slow-type muscle by long-term continuous stimulation. Indeed nine months and two years after cardiomyoplasty histological analyses revealed only type 1 fibers in the LD flap stimulated every cardiac cycle with 30 Hz bursts lasting 185 msec [26].

In summary, it is conceivable that in our pilot experiment in sheep an intermediate state of LD transformation is maintained at least up to 1 year by daily modulation of the working periods. A second issue in dynamic cardiomyoplasty is if muscle damage is induced by the chronic abnormal stimulation, 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 per se could be a trauma to muscle fibers [8, 23, 37, 44, 45, 53]. Cardiomyoplasty is a complex procedure and it is difficult to even identify the relevant variables [6, 7, 21, 28, 29]. Since the controlled environments of a physiologists’ experiment are not applicable, we have variable results in our few sheep. In any case the biopsies of 10 hr/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. This result is in agreement with data previously reported on goat LD surgically dissected and stimulated two months either 24 hr/day or 16 hr/day [28], and long-term studies in rabbit and rodents whose continuous stimulation is known to decrease the surface/diameter and muscle mass to blood perfusion ratios to favor oxidative metabolism of the myofibers [40]. If the muscle is daily rested homeostasis seems to be near to normal values. Indeed after one year of stimulation atrophy is only 10% in 24 hr/day stimulated muscles, while the LD stimulated six-month 24 hr/day and then rested six months shows a 40% atrophy. Results of the histologic analyses performed on biopsies taken after six months of stimulation strongly suggest that the decreased weight of the LD stimulated six-month 24 hr/day and then rested for additional six months is more than the consequence.
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of the six months of daily stimulation than of the six-month of rest. Indeed the myofibers of those LD were atrophic and the tissue heavily infiltrated by fat and connective tissue after six months of 24 hr/day stimulation [7]. Furthermore, we reminded that, though shortened, the LD are properly innervated and therefore possibly activated by standing and walking activity likewise the contralateral normal LD.

Of course the real question is if in cardiomyoplasty the unusual work performed after grafting 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; furthermore, direct histological evidence of muscle damage had been collected in sheep and goat experiments [21, 29, 32, 34, 49, 52]. On the other hand, two autopic 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 endothelial and smooth muscle walls; interestingly, in these two cases LD graft was activated every second or fourth sensed QRS, and clinical results were excellent [15, 17].

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. [21, 22, 28, 29]. 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 [15, 17].

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 which could be accepted in clinical practice. For heart transplant too, the surgical problems were solved several years earlier than the rejection problem. Carpentier and Chachques established the basic surgical procedure twelve years ago [10], now the knowledge exists to overcome remaining problems of cardiomyoplasty. Acker and colleagues and several others are collecting scientific evidence on the mechanisms and effectiveness of cardiomyoplasty [33, 49]. Risks of "damage" of LD may be reduced and muscle performance increased: i) by using pre- and post-cardiomyoplasty different work-rest stimulation regimens; ii) by testing nerve vs intramuscular electrostimulation; iii) by optimizing the surgical procedure; and iv) by administrating local anabolic agents to the LD flap [6, 7, 18-21]. 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 the patients.

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