# Demand Dynamic Cardiomyoplasty: Two-Year Results

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**Abstract**

In Dynamic Cardiomyoplasty after stimulation by the standard clinical Protocol Latissimus Dorsi (LD) is highly fatigue-resistant, but shows undesirable dynamic characteristics. The conditioned LD could show more than fivefold reduction in shortening velocity and peak power. To obtain fatigue-resistance while preserving muscle force and velocity, we introduced the concept of daily activity-rest stimulation. LD wrap is allowed to rest during periods of hours (e.g., while the patient is asleep).

Based on experimental results obtained with burst-intermittent stimulation in animals, we implemented in a small series of patients a light regime of LD activity-rest stimulation (cardiac-rate/based demand stimulation). To determine LD contractile characteristics we developed a new noninvasive diagnostic tool (LD wrap "mechanogram"). By mechanogram and echo Doppler imaging we determine: 1) optimal synchronization delay between the contraction of the cardiac events and LD wrap; and 2) the dynamic contractile characteristics of the LD flap based on analysis of tetanic fusion frequency (TFF).

The extent of fast-to-slow transformation of contractile characteristics of the LD wrap is related to the stimulation protocols used. After Demand Dynamic Cardiomyoplasty in the eight patients who achieved at least 6-month follow-up (mean 14±3 months), there are no deaths. Quality of life is substantially improved with significant reduction of heart failure symptoms. In the subset of patients in which light stimulation started with the muscle conditioning and Demand Dynamic Cardiomyoplasty was introduced earlier than one-year after surgery, exercise capacity increases up to two-year post Demand Dynamic Cardiomyoplasty (VO2 max: pre-op 12.3±0.7 v.s. 16.6±1.7 two-year post-Demand Dynamic Cardiomyoplasty, $p = 0.05$).

In conclusion, Demand Dynamic Cardiomyoplasty is safe, well tolerated, and by maintaining an intermediate fast-to-slow LD wrap conversion provides up to two-year post-operation excellent clinical results.

**Key words**: Demand Dynamic Cardiomyoplasty, non-invasive monitoring, human LD, dynamic contractile characteristics; fiber type transformation; activity-rest stimulation; LD wrap mechanogram; echocardiography.

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To many authors, dynamic cardiomyoplasty is a clinical reality [14, 22], which founds its basis on an active girdle effect that reverses the progressive dilatation of a failing heart. Load independent measurements demonstrate a real amelioration of the heart energetic when analyses are compared before and after dynamic cardiomyoplasty [36, 49].

One of the factors limiting systolic assistance of dynamic cardiomyoplasty is muscle performance after full conditioning. After a few weeks of stimulation, Latissimus Dorsi (LD) mitochondrial content and capillary/myofiber ratio increase, but intracellular calcium handling became less efficient so that the contraction-relaxation cycle significantly slows. Finally slow myosin

substitutes fast myosins, and a fast, powerful (but early fatiguing) LD is transformed in a slow contracting muscle which is fatigue resistant at moderate power [9, 46]. On the other hand, it is well know that these changes reverse after stimulation is discontinued, the contractile characteristics typical of fast muscle returning after 3-4 weeks, while changes in fatigue resistance, capillary density and enzyme activities follow a more prolonged time-course [43].

To achieve full transformation of the LD wrap in the actual prudent management of the clinical stimulation, the conditioning period lasts two months, reducing the potential benefit during the early post-operative period. Clinically, it is fully accepted that LD benefits the patient's quality of life if its activation is critically delayed after sensed QRS to avoid mitral valve regurgitation [23, 24, 32].

Since maximum instant power of a fully conditioned LD is smaller than the peak power of the left ventricle [1, 3-7, 13, 20], we share the opinion [2] that the grafted muscle could assist the heart principally during mid and late systolic phases. Of course, such a short window asks for a fast, powerful contraction, which is not delivered by a fully transformed LD.

Monitoring is essential to take advantage of new concepts, so we developed a simple non-invasive method to analyze dynamic characteristics of LD flap during conditioning and regime stimulation. Determining tetanic fusion frequency, a traditional indicator of speed of muscle shortening and relaxation by using in-burst stimuli delivered at increasing frequency rate it is possible to estimate the contraction-relaxation cycle of the transposed LD during conditioning and long-term clinical stimulation [10, 12].

We shown that, after months of continuous daily stimulation it is possible to reverse the fast-to-slow transformation by an activity-rest stimulation Protocol based on cardiac rate (demand stimulation), so that the LD flap is rested during periods of patient's low-activity [42].

Results up to two-year follow-up are here reported.

## Materials and Methods

### *Patients, surgery, and follow-up*

Dynamic Cardiomyoplasty was performed in selected subjects according the actual international guide-lines [14, 22, 40, 49]. In the subjects that were light stimulated from the early phases of LD conditioning surgery was performed at the Institute of Cardiovascular Surgery of the University of Padova according to standard surgical procedure [14]. Four dilated cardiomyopathy patients, three men and one woman, 50 (41-57) years old, were operated between June 1996 and November 1997. In this light stimulated group (Pd01 to Pd04), after 10-14 day healing period, the LD was stimulated with a single impulse at a 1:3 synchronization ratio. Each week, one impulse was added at a 23 msec pulse interval (43 Hz) for a final burst of four impulses. After 6-12 months of this light daily stimulation, the patients were submitted to the "demand regime" to allow the LD wrap daily periods of rest.

Analyses of the clinical follow-up, including LD wrap mechanogram and echocardiography, were performed at the Division of Cardiology of The Legnago General Hospital, Legnago (Verona), Italy. These patients underwent preoperative and postoperative 24-hour Holter monitoring, and cardiopulmonary exercise testing. The subjects were followed up with respect to dynamic characteristics of the LD wrap by survival, functional class, hospital admission rate, medication used, and mechanographic analyses. Student's t-test was used for statistical analysis, and data were regarded as statistically significant when $p < 0.05$.

An additional group of subjects, operated at The Institute of Cardiac Surgery, University of Pavia, and stimulated for several years according the FDA stimula-

*Table 1. Dynamic Cardiomyoplasty: Cardiomyostimulator settings of either FDA Protocol or light (daily or demand) LD stimulation.*

| | PROTOCOLS | | |
|---|---|---|---|
| | (FDA, phase III) | Light stimulation | |
| | (Daily) | (Daily) | (Demand) |
| Cardiomyostimulator Settings | | | |
| Pulses | | | |
| Number | 6 | 4 | 4 |
| Interval (msec) | 31 | 23 | 23 |
| Synchronization delay (msec) | 5-100 | 50-80 | 8-80 |
| LD/Heart ratio | 1:2-1:4 | 1:3 | 1:3 |
| Lower limit of pace-maker function (b.p.m.) | 40 | 40 | 70 or 80 |
| Cardiac Amplitude (Volts) | >5 | >5 | <1 |
| Cardiac pulse width (msec) | 1.5 | 1.5 | 0.05 |

tion Protocol before conversion to demand stimulation, were studied in collaboration with The Cardiology Rehabilitation Center, IRCSS Fondazione "Maugeri", Montescano (Pavia), Italy. A prudent approach was also used to change from daily to demand the stimulation regime in these long-term dynamic cardiomyoplasty subjects. Usually, burst settings were first adapted to light stimulation, then six-twelve months later to stimulation at demand. Table 1 compares settings of the clinical Protocol of the FDA phase II trail used by The American Cardiomyoplasty Group [22] to those of the "light stimulation Protocol" (delivered either continuously or at demand).

A total of 22 subjects were included in the study to date, but only those who attained six-month follow-up of demand stimulation by October 1999 are here considered.

*LD wrap mechanogram*

Contractile characteristics of the LD wrap were monitored bedside using a standard polygraph (MegaCart or Mingophon, Siemens Elema, Solna, Sweden). ECG and heart sounds are recorded simultaneously with the pressure changes due to LD flap contraction. To monitor the LD wrap, the pressure transducer, which was traditionally used for apex-cardiography, is placed near the location of the rib window through which the LD enters the thoracic cavity.

We determine: i) The activation threshold, measuring the peak contraction at different amplitude of the stimulating current (from 1 to 8 Volts). A stimulation threshold slightly higher than that determined intra-operatively is usually applied [14]. The mechanographic analysis shows that when the current amplitude is increased from 2.0 to 10 V in steps of 0.5 V, the amplitude of the contraction peak level-off in some patients, but in the most of them it increases accordingly. Thus, in these cases the muscle response is not maximized because the higher voltages may cause some patient discomfort, related to sensation of wrap activity in the patient's chest. Never patients complained of pain at the higher voltages attained [10].

ii) The best synchronization between cardiac cycle and contraction of LD wrap. The optimal LD: Heart synchronization could be determined by comparing the mechanogram event with both the mitral and the aortic valve sounds as measured by the phonogram or, preferably, by connecting the mechanogram signals directly to echocardiography equipment. In this way, the mechanical event of the LD contraction-relaxation can be directly compared to cardiac events as measured by M-mode echocardiography (e.g., mitral valve closure) or echo Doppler measurements of mitral and aortic outflows.

iii) The dynamic characteristics of the LD wrap are determined from the LD response to stimuli delivered at increasing frequency rate. Tetanic Fusion Frequency is the frequency that produces a smooth contraction curve. The unfused tetanus gives a rippled slope. A safety characteristic of the cardiomyostimulator limits the number of stimuli to a burst shorter than cardiac systole. So, Tetanic Fusion Frequency can be identified by delivering three-four stimuli at intervals ranging from 8 to 75 msec (125 to 13 Hz, respectively) or doublets of stimuli at intervals ranging from 50 to 200 msec (20 to 5 Hz, respectively).

*Cardiac rate-based demand stimulation*

To provide the LD wrap with daily periods of rest, 24-hour Holter studies were first performed to determine the average heart rate during sleeping hours. Patients were considered to have no indications for bradycardia pacing. The cardiac pacing parameters of the cardiomyostimulator (Transform, Model 4710, Medtronic, Inc., Minneapolis, MN, USA) were then programmed to a rate of 70 or 80 bpm with minimum pulse amplitude and pulse width. The

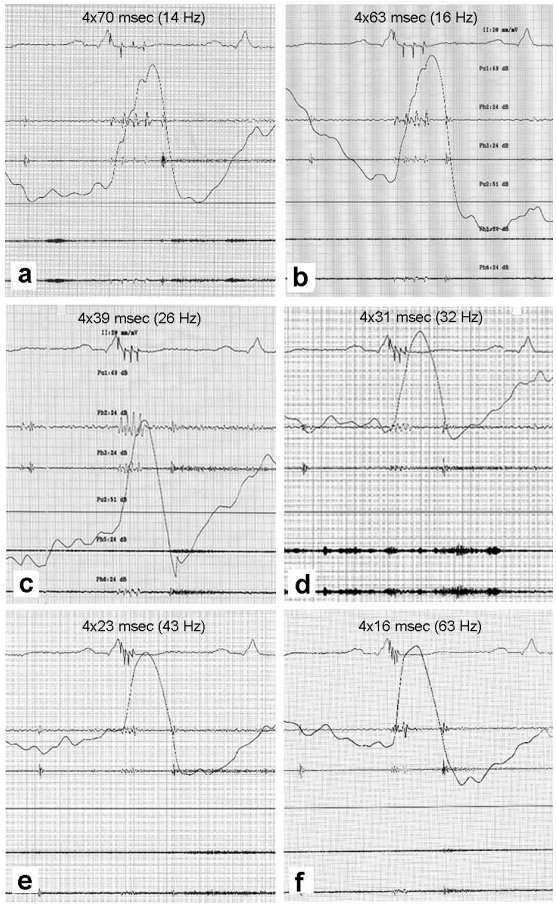


*Figure 1. Ld wrap mechanogram: Tetanic fusion frequency analysis. Four impulses are delivered at: a, 70 msec intervals (14 Hz); b, 63 msec intervals (16 Hz); c, 39 msec intervals (26 Hz); d, 31 msec intervals (32 Hz); e, 23 msec intervals (43 Hz); f, 16 msec intervals (63 Hz). The slope is clearly rippled up to 26 Hz (c). The lines of 32 Hz and 43 Hz are not as straight as those at 63 Hz. Tetanic fusion frequency is equal or higher than 43 Hz.*

muscle output was programmed to “Sense”. Programmed in this way, muscle output will occur only with sensed cardiac events, not paced events. With the lower cardiac rate set just above the average heart rate at night, the cardiomyostimulator will be pacing most of the time during resting hours but at an energy level well below that required to actually capturing the heart. During these pacing episodes the muscle output is inhibited. The net result is that muscle stimulation is primarily inhibited during resting hours and occurs regularly at the programmed synchronization ratio during active hours. Thus, an activity-rest stimulation regime is applied [10].

## Results

### *Mechanogram and mechanogram-optimized synchronization of the heart and LD contractions*

Figure 1 shows an example of the LD flap mechanogram, as recorded with the Siemens Elema MegaCart electrocardiography.

The upper trace is the electrocardiogram, which also shows the four muscle impulses. The second and the third lines are phonocardiographic traces with or without superimposed muscle impulses, respectively. The mechanogram is superimposed to the phonograms, and shows a distinct peak that starts about 50 msec after the stimulation burst.

Usually LD contraction timing is based on delay between sensed QRS and spikes of the electrical impulses delivered by the muscle stimulator [14, 22, 23, 32, 24, 40]. Our simple method allows synchronization of cardiac cycle and LD mechanical event, so avoiding any interference of muscle relaxation phase on cardiac diastole, in particular when the mechanogram is combined with echocardiography (Figure 2).

With a 8 to 100 msec delay (adjusted in each subject every three-six months) the contraction-relaxation cycle of the LD flap starts after closure of the mitral valve and ends long before aortic valve closure, i.e., it is confined in aortic outflow phase. In the shown example the mechanogram starts well after the closure of the mitral valve as determined by M-mode echocardiography. By using aortic outflow imaging the LD wrap is shown to relax during diastole, but shortening the synchronization delay from 46 to 8 msec the peak outflow and the peak contraction of the LD wrap are shown to coincide.

### *Tuning of dynamic characteristics of LD wrap by demand stimulation*

Figure 1 shows the series of mechanograms we use to determine tetanic fusion frequency. The LD wrap is stimulated with four impulses delivered at: 70 msec intervals (a, 14 Hz), 63 msec intervals (b, 16 Hz), 39 msec intervals (c, 26 Hz), 31 msec intervals (d, 32 Hz), 23 msec intervals (e, 43 Hz), 16 msec intervals (f, 63 Hz). The slope is clearly rippled up to 26 Hz, and the peak/steps are as long as the interpulse intervals (a-c). The contraction lines of the 32 Hz and 43 Hz tetani are not as straight as that of the tetanus induced by four impulses delivered at 63 Hz. Therefore, in this LD wrap the Tetanic Fusion Frequency is equal or higher than 43 Hz.

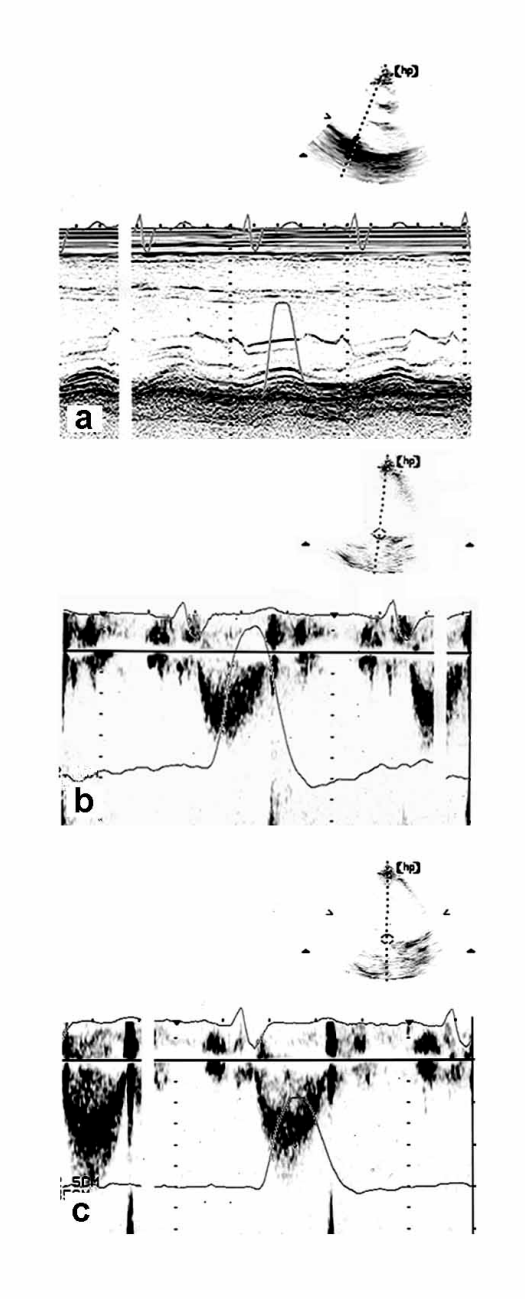


*Figure 2. Mechanogram-synchronized hearth: LD wrap interaction. M-mode echocardiography shows that the LD wrap contraction starts well after mitral valve closure (a). Aortic outflow peak and LD wrap peak contraction could be superimposed by shortening the synchronization delay from 46 (b) to 8 msec (c) taking advantage of aortic outflow imaging.*

Table 2 shows that in the small series of patients operated at The Padova University just before starting the conditioning protocols, i.e. one-two weeks after surgery, the Tetanic Fusion Frequency is 43 Hz (i.e. the tetanus is fused when pulses are delivered at 23 msec interpulse intervals). This is the expected value for human muscles [44, 50]. Tetanic Fusion Frequency of LD wrap at the end of the conditioning period by the light-stimulation protocols (4-5 weeks post-operation) is in between 43 and 32 Hz. The mechanograms show that tetanic fusion frequency slow down to around 25 Hz after several months of daily light stimulation.

When the wraps are stimulated long-term according to the FDA Protocol they are even slower. In the cohort of Italian subjects we are studying, Tetanic Fusion Frequency of LD wrap subjected to long-term standard clinical Protocol is 11 Hz, as shown in Table 2, which summarizes the results of the Tetanic Fusion Frequency analyses in the subjects stimulated more than six months by either "light" or standard FDA regime.

After six-twelve months of continuous light stimulation the LD wrap was submitted to activity-rest stimulation. A demand Protocol, based on a frequency cut-off at around 80 bpm, is determined by Holter analysis of the daily cardiac frequency, so that every day the LD flap is rested during low-activity periods both during day and night (usually two hours in the afternoon, and eight hours during night).

LD wraps after chronic activity-rest stimulation show dynamic characteristics intermediate between fast and

*Table 2. Dynamic characteristics of LD wrap after long-term daily or demand stimulation.*

| | Tetanic Fusion Frequency (Hz) | | |
|---|---|---|---|
| | Mean | SEM | cases |
| Light Stimulation according to ITDDC trial | | | |
| Before Conditioning (two-week post-op) | 43 | ±0 | (4) |
| Light Conditioning (1-month) | 35 | ±5 | (4) |
| Light Daily Stimulation (> 12-month) | 28 | ±6 | (4) |
| Demand Stimulation (> 12-month) | 33[b] | ±4 | (4) |
| All Demand Stimulation (> 12-month) | 30[c] | ±3 | (8) |
| Clinical Stimulation according to FDA trials | | | |
| >24 months (mean 29±6) | 11 | ±2 | (11) |
| Demand Stimulation >12-month | 27[a] | ±4 | (4) |

[a] $p < 0.001$ v.s. Clinical Stimulation according to FDA trials; [b] $p < 0.0001$ v.s. Clinical Stimulation according to FDA trials; [c] $p < 0.0001$ v.s. Clinical Stimulation according to FDA trials.

*Table 3. Dynamic characteristics of LD wrap after long-term daily or demand stimulation.*

| | Tetanic Fusion Frequency Analysis (Hz) | | | | | |
|---|---|---|---|---|---|---|
| | Burst-Continual Stimulation | | | | Demand Stimulation | |
| | FDA Stim. | | Light Stim. | | | |
| Subjects | (months) | Hz | (months) | Hz | (months) | Hz |
| Pd01 | 0 | | (10) | 20 | (26) | 32 |
| Pd02 | 0 | | (11) | 18 | (25) | 26 |
| Pd03 | 0 | | (12) | 43 | (13) | 32 |
| Pd04 | 0 | | (6) | 32 | (12) | 43 |
| Mean | | | (10) | 28[a] | (19) | 33[b] |
| SEM | | | | ±6 | | ±4 |
| Mi01 | (53) | 12 | (16) | 26 | (16) | 18 |
| Pv02 | (18) | 8 | (13) | 22 | (6) | 36 |
| Pv06 | (36) | 10 | (18) | 18 | (6) | 26 |
| Pv08 | (14) | 8 | (18) | 18 | (6) | 26 |
| Mean | (30) | 10 | (16) | 21[c] | (9) | 27[d] |
| SEM | ±9 | ±1 | ±1 | ±2 | ±3 | ±4 |

a, $p < 0.02$ v.s. FDA Stim.; b, $p < 0.001$ v.s. FDA Stim.; c, $p < 0.002$ v.s. FDA Stim.; d, $p < 0.004$ v.s. FDA Stim.

*Table 4. Demand Dynamic Cardiomyoplasty: heart failure symptoms.*

| | NYHA class | | | | |
|---|---|---|---|---|---|
| | | Stimulation Protocol | | | |
| Subjects | pre-op | Daily | (months) | Demand | (months) |
| Pd01 (96.06) | 3 | 1 | (10) | 1 | (26) |
| Pd02 (96.06) | 3 | 1 | (11) | 1 | (25) |
| Pd03 (97.05) | 3 | 1 | (12) | 1 | (13) |
| Pd04 (97.11) | 3 | 2 | (6) | 2 | (12) |
| Mi01 (02.93) | 3 | 4(HT) | (58) | 2 | (6) |
| Pv02 (96.11) | 3 | 2 | (13) | 1 | (6) |
| Pv06 (94.01) | 3 | 2 | (18) | 2 | (6) |
| Pv08 (96.07) | 3 | 2 | (18) | 2 | (6) |
| Mean | 3.0 | 1.9a,b | | 1.5c | |
| SEM | ±0.0 | ±0.4 | | ±0.2 | |

(year.month) of operation; HT, this patient before "light conversion", had been evaluated as a candidate to heart transplant; a, $p < 0.006$ v.s. pre-op; b, not significant v.s. demand stimulation; c, $p < 0.0001$ v.s. pre-op.

*Table 5. Light-Demand Dynamic Cardiomyoplasty. Hospitalizations (day-hospital included), and β-blockers therapy.*

| | Hospitalization (day-hospital included) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Pre-Op | | | Post-Demand Stimulation | | | |
| Subjects | Days | (Months) | Day/Month | Days | (Months) | Day/Month | Hospitalization |
| Pd01 | 40 | (18) | 2.2 | 19 | (34) | 0.6 | Decreased |
| Pd02 | 20 | (6) | 3.3 | 9 | (34) | 0.3 | Decreased |
| Pd03 | 29 | (23) | 1.3 | 27 | (23) | 1.2 | Decreased |
| Pd04 | 110 | (29) | 3.8 | 78 | (17) | 4.6 | Increased |

| | β-blockers therapy | |
|---|---|---|
| Subjects | Pre-Op | Post-Demand Stimulation |
| Pd01 | Attempted (Metoprolol), but discontinued | Carvedilol (tolerated) |
| Pd02 | Attempted (Metoprolol), but discontinued | Carvedilol (tolerated) |
| Pd03 | Attempted (Metoprolol), but discontinued | Carvedilol (tolerated) |
| Pd04 | Attempted (Metoprolol), but discontinued | Metoprolol (tolerated) |

slow human muscles. Indeed, Figure 1 displays the mechanographic analysis of a LD wrap 42 months after surgery, submitted to Demand Stimulation during the last 30 months.

We also introduced Demand Dynamic Cardiomyoplasty after years of clinical stimulation according the FDA trials. As expected, in both cases the fast-to-slow transformation of the LD wrap shifts back to faster values after months of the activity-rest regime (Table 2). The changes are highly significant ($p = 0.004$) even when only the four long-term FDA subjects are concerned (Table 3).

*Demand dynamic cardiomyoplasty: two-year follow-up*

To date all eight patients submitted to demand stimulation are alive (mean follow-up 14±3 months) and they give preference to the activity-rest regime based on their general feeling of well-being.

Table 4 shows that after several months of demand stimulation the patients maintain a sustained improvement in quality of life with reduction in heart failure symptoms (NYHA class: pre-op 3.0±0.0 v.s. 1.5±0.2 post-Demand Dynamic Cardiomyoplasty, mean±SEM, $p < 0.0001$). In three out of four patients of the "light group" hospitalization is reduced during follow-up, and β-blockers therapy could be introduced in all of them while it was attempted but not tolerated before operation (Table 5). At two-year of follow up, VO2max of the Pd subjects (submitted to light stimulation during the first year and then to demand stimulation) is significantly increased in comparison to pre operation values (Table 6, and Figure 3).

## Discussion

Stimulation Protocol of the FDA phase II trial makes the LD very resistant to fatigue, but meanwhile its dynamic characteristics ask for improvements. With a 155 msec stimulation train of six impulses delivered at 32 Hz the contraction-relaxation cycle of a fully conditioned LD may last longer than the heart systole [24, 54], so correct timing of flap contraction frequently became a challenge. Thanks to experimental results in sheep of the Arpesella's team [2-5], we planned a "light" regimen of LD stimulation and a shortened conditioning period.

The presence of a strong left axillary muscle twitch was used in the past to clinically determine the continue presence of muscle contraction and test stimulation voltage threshold of LD [15]. Results are substantiated with fluorography of heart displacement during assisted beat and shortening of the distance between intramuscular electrodes or metal clips sutured on the LD flap [54], and finally with cardiac catheterism and PV loops analyses [36, 49].

Only after introduction of M-mode echocardiography, optimization of muscle synchronization after dynamic cardiomyoplasty became rational and easily repeatable [23]. On the other hand, by echocardiography LD contraction timing is based on delay between sensed QRS and spikes of the electrical impulses delivered by the muscle stimulator, and evaluation of dynamic characteristics of the LD flap needs tissue imaging approach and off-line calculations [24].

By the new non-invasive method we are able to follow at bedside the changes of the dynamic contractile characteristics of the LD flap, sensing LD displacements with the probe of the apicocardiogram of a polygraph. Our simple method allows synchronization of cardiac cycle and LD mechanical event, avoiding any interference of muscle relaxation phase on cardiac diastole, in particular when the mechanogram is combined with echocardiography.

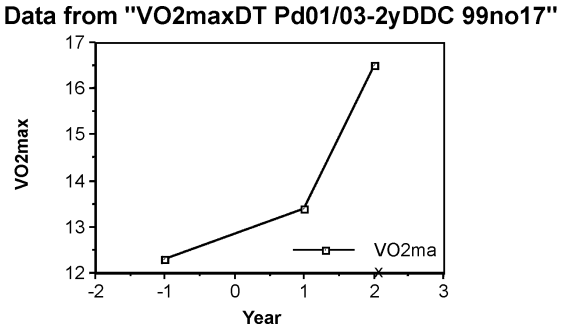


*Figure 3: VO2max.*

A major advantage of the mechanogram is that it provides information regarding the contractile characteristics of the LD wrap and how they change during conditioning and long-term stimulation. Tetanic fusion frequency is a physiologic indicator of muscle contraction and relaxation speed, and thus of fiber type composition [9, 44, 46].

In Dynamic Cardiomyoplasty, tetanic fusion frequency can be determined for the LD wrap by delivering stimuli at increasing frequency (i.e., by decreasing interpulse intervals in the burst), and by recording the contraction event by means of the pressure transducer that was traditionally used for apex-cardiography. The LD wrap mechanography has been validated by fluoroscopic contraction analysis [54]. Comparison of the contraction/relaxation curves with both methods revealed a significant identity [53].

Tetanic Fusion Frequency of the LD wrap is 43 Hz be-

*Table 6. Light-demand dynamic cardiomyoplasty. Functional analyses in light-demand dynamic cardiomyoplasty: VO2 Max.*

| | Pre-Op | Stimulation | |
|---|---|---|---|
| Subjects | | Light Continual | Demand |
| Pd01 | 14.0 (96.01) | 16.6 [96.08] | 20.1 [97.10]; 20.1 [98.05] |
| Pd02 | 10.7 (95.05); 10.8 (96.01); 12.0 (96.02) | 13.1 [96.08]; 15.0 [97.03]; | 15.5 [97.10]; 15.0 [98.05] |
| Pd03 | 12.7 (96.09); 13.0 (97.02) | 13.2 [98.05]; 12.0 [98.09]; | 14.1 [99.01] 14.8 [99.10] |
| Pd04 | 12.5 (95.06); 12.4 (96.01); 9.5 (97.03); 9.8 (97.06); | 10.6 (a)[98.05] | |
| Mean | 12.3 (4 subjects) | 13.4 (4 subjects) | 16.6* (3 subjects) |
| SEM | ±0.7 | ±1.3 | ±1.7 |
| Increase (Δ%) | | +9 | +35 |

Values refer to analyses performed at the indicated times (before operation) or [after demand stimulation]; (a), test interrupted due to diabetic artheriopathy *claudicatio*; * p = 0.05 v.s. Pre-op, and p = 0.21 v.s. Light continual stimulation.

fore conditioning, and in between 43 and 32 Hz at the end of the conditioning period by the light-stimulation protocols (4-5 weeks post-operation). The value slows down to around 25 Hz after several months of daily light stimulation. When the wraps are stimulated long-term according to the FDA Protocol they are even slower, i.e., in between 5 and 20 Hz. The FDA 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, two years, and eight years after Dynamic Cardiomyoplasty histochemical and gel electrophoretic isomyosin analyses revealed only type 1 (slow-type) myofibers in the LD flap stimulated every cardiac cycle with 32 Hz bursts lasting 155 msec [11, 21, 38].

Measurements of LD muscle shortening from X-ray films [54] and tissue Doppler analysis [24] demonstrated that the contraction-relaxation cycle of the LD wrap might last up to 600 msec after long-term clinical stimulation according to the stimulation Protocol of FDA trials. The observations are in full agreement with results of chronic low-frequency electrical stimulation experiments in rodents, rabbit, goat, sheep and man [9, 43, 44, 46].

After six-twelve months of continuous light stimulation the fast-to-slow transformation of LD wrap is reversed by activity-rest stimulation. The demand stimulation, based on cardiac frequency, allows resting every day the LD flap during patient's low-activity periods both during day and night. Previous studies that demonstrated some LD wrap systolic assist on the basis of short periods (hours or days) of discontinued electrical stimulation, also shown that continuous LD support is not mandatory for short-term patients' health [25, 33, 51]. Patients enjoy the activity-rest regime, and, due to lighter LD stimulation, they have any sleeping problems. We also introduced Demand Dynamic Cardiomyoplasty after years of clinical stimulation according the FDA trials. As expected, in both cases the LD wrap shifts back to faster characteristics after a few months of the activity-rest regime, and stays for years at a value intermediate between fast and slow human muscles. When standard FDA trial stimulation is compared to demand stimulation the difference in tetanic fusion frequency of the LD wrap is highly significant.

These observations are in full agreement with results of long-term training-detraining experiments in rodents, rabbit, goat, sheep and man [43, 44, 46]. These effects of intermittent stimulation are now corroborated by additional evidence, using the pattern of stimulation mandatory for Dynamic Cardiomyoplasty [17, 27, 35, 48]. In animal experiments, increased speed of wrap contraction is accompanied by significant increase of muscle power. It is important to note that measurements of peak isometric force reported in [18], do not equate to the maximum force-generating capability of the muscles but rather indicate their capacity to perform work under stimulation conditions generally accepted for clinical use [14, 22, 39].

The muscle fully transformed by continuous electrical stimulation (100% type 1 myofibres, i.e. slow myofibres) displays significant losses in power [30, 36, 38, 57], which is generally attributed to fiber-type change or loss of type 2 myofibres (that is, fast contracting myofibers). The reduction in muscle mass that results from the transformation process when continuous stimulation is applied also decreases power delivered by the LD wrap [29, 34, 39, 45, 52].

Several studies have demonstrated that voluntary exercise training in humans (of course limited to a few hours per day of exercises) does not increase type 1 fibers content, but rather produces shift from type 2X or 2B to type 2A [43, 46].

Several weeks of intermittent-burst stimulation produces a high percentage of 2A fibers and increases fatigue resistance and power in rabbit latissimus dorsi muscle. Muscles stimulated 12 h/day for 12 wk had the highest initial stroke work and the highest remaining stroke work at 40 min: control muscles fatigue completely within 10-20 min, while muscles stimulated continuously for 6 wk retain 35% of their initial stroke work at 40 min of fixed-load endurance test. Intermittent-burst stimulation not only prevents power loss in fatigue-resistant muscle, but also can actually increase contractile function beyond baseline values. Intermittent-burst stimulation significantly increased isometric force generation under clinically relevant stimulation conditions compared with both the continuous-stimulation group (391%) and control muscles (175%). On completion of 3-h or 8-h fatigue tests, peak isometric force measured after a 5-min rest period was found to be higher in intermittent-burst group than forces generated by the 6-wk continuous-burst before fatigue testing. These findings suggest that the insertion of rest periods during chronic electrical conditioning preserves myofiber cross section area and yields fatigue resistant fiber distributions that are stronger than those achieved via conventional training techniques [18].

The implication is that skeletal muscle phenotype can be controlled by manipulating stimulation patterns to produce fatigue-resistant muscle capable of providing clinically significant levels of work production. Previous efforts to adapt skeletal muscle for cardiac assistance have used continuous-burst stimulation protocols, which result in fatigue resistance at the expense of reduced muscle power. These findings indicate that clinical applications of stimulated skeletal muscle for cardiac assist should use intermittent (activity-rest) stimulation protocols that produce more powerful fatigue-resistant muscles. This conclusion is substantiated by results of recent

animal studies. The effects of nine weeks of intermittent stimulation (10 hours on/14 hours off per day) vs. standard daily stimulation of LD wrap were compared in a canine model of Dynamic Cardiomyoplasty after inducing left ventricular dysfunction by intracoronary microsphere injection. Significantly larger percent increases in peak aortic pressure (+18%), left ventricular pressure (+22%), peak positive LV dP/dt (+25%), stroke volume (+38%), stroke work (+71%), and aortic flow (+64%) were observed in the intermittent stimulated LD wraps (Santamore WP, personal communication).

These increased performances are considered the result of the maintained intermediate transformation of the LD wrap, which produces faster, more powerful contractions.

The biological basis of all these results is that "intermediate" myofibers do exist in nature. Several different types of myofibers with intermediate characteristics between very fast- and very slow-contracting fibers exist in skeletal muscles of mammals, including Homo Sapiens. Their characteristics are induced and maintained by different level of activity against load [9, 43, 43, 46]. It is well established that in animals cessation of stimulation has pronounced effects on the mRNA pattern leading to a rapid reversal (hours) of the stimulation-induced changes [45]. In the case of MHC isoform transitions, a conspicuous delay exists between the changes at the mRNA level, protein synthesis, and protein accumulation. In rat muscle the apparent half-life of mRNA encoding MHCIIb isoform is about 60 h, whereas that of the protein product is 11 days. During the transformation process the mRNA of MHC2a is upregulated soon after the onset of electrical stimulation, as is also the rate of MHC2a protein synthesis. On the other hand, accumulation of the new products is detectable only after more than a week of continuous electrical stimulation. This suggests that degradation of the existing MHC (which is no longer synthesized) have to precede the insertion of the newly synthesized MHC isoforms into the thick filament. That protein degradation is the limiting step in the remodeling of such a complex structures seems to be reasonable if one takes into account the mechanical stress they have to generate.

These observations clearly show that the adaptive responses of myofibers to changed activity include transcriptional, translational, and posttranslational regulation. The changes in mRNA levels show that the muscle fiber responds to altered demand much more rapidly than reflected by the changes in its protein composition. The quick reversibility of the changes at mRNA level, when electrical stimulation is discontinued, may be taken as an additional illustration of the rapid responses of the system to contractile activity both imposed by the different motoneurones and by superimposed functional electrical stimulation [26, 31, 37].

Therefore it is not surprising that in the patients we are studying, conversion from burst-continual to demand stimulation (i.e., to a daily activity-rest regime) reverses fast-to-slow transformation of dynamic characteristics of the LD wrap. When standard FDA trial stimulation is compared to demand stimulation the difference in tetanic fusion frequency of LD wrap is dramatic and highly significant. All these studies strongly support our hypothesis that activity-rest stimulation (demand stimulation in the present study) is superior to daily continuous stimulation, not only because it minimize incremental activity-induced muscle damage [2, 16, 19, 28, 41], but also because it maintains an intermediate state of fast-to-slow fiber type change.

All the subjects of our series submitted to demand stimulation are alive, and we find unethical to perform biopsies of the LD wrap, even using the Menghini needle approach [55, 56]. Thus, we have not direct information by morphological and molecular analyses on the extent of LD wrap transformation in Demand Dynamic Cardiomyoplasty. In a group of patients, in which main result is a better quality of life, our choice is to reduce to a minimum invasive analyses (i.e., biopsies and PV loops), though this decreases the chance to collect small, significant evidence of increased systolic performance on a non-assisted/assisted beats basis. We have not yet direct evidence that the muscle wrap's power has improved to the level to provide a systolic assist measurable with echocardiography, but we stress that the faster contraction-relaxation cycle of the demand-stimulated LD wrap is "per se" an evidence of increased muscle power [30, 47].

On the other hand, in the subset of patients in which light stimulation started with muscle conditioning and Demand Stimulation was introduced earlier than one-year after surgery, exercise capacity stays increased in comparison to pre operation value at two-year follow-up. Furthermore, after Demand Dynamic Cardiomyoplasty there are no deaths and quality of life is substantially improved with significant reduction of heart failure symptoms.

In conclusion, the regime we introduced into management of Dynamic Cardiomyoplasty (demand stimulation to avoid full transformation of LD characteristics, and LD wrap mechanography) is safe and seems to improve the results of the procedure. There is hope to further increase muscle performance by: i) reducing mobilization-related muscle damage by mini-invasive surgery, and a two-stage operation, i.e., a true "vascular delay approach"; ii) testing nerve vs. intramuscular electrostimulation; iii) using different work-rest stimulation regimens pre- and post-Cardiomyoplasty; and iv) administrating local anabolic and angiogenic agents to the LD flap. Whatever the future, we hope that all together the results here reported attract attention of cardiologists

and, reinforcing the concept of light activity-rest stimulation, contribute to a larger acceptance of Dynamic Cardiomyoplasty.

We are confident that these data will be substantiated by long-term results in a larger patient population, and that Demand Stimulation could offer long-term the benefits of Dynamic Cardiomyoplasty to patients prone to pharmacologically-intractable heart failure.

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