

Partial Cardiac Assistance Begun Immediately after Latissimus Dorsi Muscle Mobilization and Cardiomyoplasty

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Abstract

Cardiomyoplasty, in which the latissimus dorsi muscle (LDM) assists the heart, is a relatively new surgical treatment for heart failure. Currently, the LDM is unstimulated for the first two weeks postoperatively, then stimulated for the next four weeks with one or two impulses every other heart beat. We wanted to determine if the LDM could provide assistance earlier than six weeks postoperatively. In four control sheep electrical stimulation (ES) was not applied to the LDM; in four sheep, ES was begun two hours after LDM mobilization; in another four sheep, ES mimicking cardiac assist (work-rest regimen) was added to the protocol twice daily for 30 minutes. In animals with the protocol mimicking cardiac assist, contractile force (CF) changed minimally on days 6, 11, and 16 following two successive 30 minute fatigue tests ($98 \pm 3\%$; $102 \pm 3\%$, and $104 \pm 2\%$ respectively). The typical ischemic state of the mobilized LDM was not aggravated with this protocol. The percent area occupied by capillaries increased to $5.04 \pm 0.33\%$ (compared with $3.02 \pm 0.6\%$ in control muscle). In the control series, CF decreased to $85 \pm 4\%$, $78 \pm 3\%$, and $77 \pm 3\%$ on days 6, 11, and 16, respectively. We concluded that a cautious stimulation regimen using a work-rest protocol and a slow rate of contraction does allow for earlier partial cardiac assist.

Key words: Cardiomyoplasty, cardiac assist, latissimus dorsi muscle, electrical stimulation, contractile force.

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Since the introduction of Cardiomyoplasty into clinical practice 12 years ago [2, 15] there seems to be more questions than answers. A successful Cardiomyoplasty should resolve several areas of concern: minimize the risk of ischemia-reperfusion damage to the newly mobilized latissimus dorsi muscle (LDM); preserve existing vascular network and accelerate angiogenesis of LDM and myocardium; successfully convert skeletal muscle from its fatigable state to a fatigue-resistant state using electrical stimulation training; allow cardiac assistance using the wrapped LDM to begin early in the postoperative course.

Notwithstanding these questions, Cardiomyoplasty has spread quickly worldwide, making it imperative to resolve the problems as soon as possible. Several problems concern the period two months after the Cardiomyoplasty procedure, when the LDM has been wrapped around the heart but is inactive and unable to augment cardiac contractions. The most challenging problem is muscle fatigue, since it impedes and delays effective cardiac assist. The prevailing theory is that the LDM must be allowed to recover for two weeks after subtotal mobilization before it can be subjected to an electrical stimulation training protocol (over a period of eight weeks). This theory stems

from apprehension about exacerbating ischemic injury to the distal portion of the LDM after mobilization by not observing the classical two-week healing period, or by applying bursts of impulses to the muscle shortly after the wrap. Electrical stimulation training may indeed aggravate muscle ischemia and be hazardous if begun immediately after Cardiomyoplasty. Thus, the vigorous muscle contractions needed for cardiac assistance must be deferred for the first eight weeks after Cardiomyoplasty, despite the LDM's high plasticity. It is necessary to follow this time course if the LDM is to obtain the adaptive transformation needed to enhance its fatigue resistance through a progressive, sequential stimulation protocol.

Results of our clinical [5] and experimental investigations [8] have persuaded us to challenge this prevailing theory. One of the authors' clinical experience with Cardiomyoplasty began in 1988 in Russia. The design of the Russian-built cardiomyostimulators allows the electrical stimulation protocol to be begun immediately after completion of the Cardiomyoplasty surgery, employing initial impulses consisting of four impulses per burst at a ratio of 1:8 (Stiminak 805) or 1:16 (ECS 445) [5]. This stimulation pattern did not exacerbate ischemia in the

LDM and it produced clinical results that showed a cautious approach to electrical stimulation training does not harm newly mobilized LDM. In a recent experimental investigation [8], we showed that the contractile force (CF) of newly mobilized LDM (one hour after mobilization) does not decrease when tested under a work-rest regimen consisting of alternating one minute work (15 contractions per minute, CPM), then one minute rest. This investigation broached the idea of beginning partial cardiac assist immediately after cardiomyoplasty. However, in order to provide more cardiac assistance and to introduce assistance much earlier postoperatively, a stimulation protocol needed to be developed based on a better understanding of the requirements for conditioning and activating the muscle.

The investigation reported here was designed to study the suitability of newly mobilized LDM for work in a cardiac assist regimen for a short duration. It was also intended as the first step in investigating whether starting electrical stimulation immediately after LDM mobilization can decrease the time needed for muscle training substantially below the 10 weeks used in the prevailing regimen.

Methods

Animal preparation

Adult St. Croix sheep (n = 12) weighing 30 ± 8 kg were used in this study. The sheep were maintained under a controlled environment of constant temperature (22°C) and light (12 hours per day). They were fed a commercial sheep diet and provided water *ad libitum* in our American Association of Laboratory Animal Care-approved research center. The protocol followed was approved by our Institutional Animal Care and Use Committee, and adheres to NIH and American Physiological Society "Guide for the Care and Use of Laboratory Animals."

Surgical preparation

Food was withheld for 24 hours prior to anesthesia induction. Strict sterile technique was followed at all times to reduce the potential for infection. Antibiotic therapy (amoxicillin, 15 mg/kg intramuscularly, once daily) continued for seven days postoperatively. All surgical procedures and force tests were conducted while the animal was under general anesthesia. Anesthesia was induced with Valium (5 mg/kg intravenously) and sodium Pentothal (10-15 mg/kg intravenously). *j* animals were intubated, placed on a Drager ventilator, and maintained on Halothane gas (1-2% with 4.0 L O₂). Oxygen saturation levels and heart rate were monitored via a pulse oximeter placed on the animal's tongue.

A 25 cm longitudinal skin incision was made in the right mid-axillary line to expose the right LDM. The muscle was then subtotally mobilized by severing all muscle attachments (except the proximal pedicle and the distal attachment to the ribs), and all vessels supplying the LDM (except the thoracodorsalis artery) leaving the muscle *in*

situ. Two intramuscular electrodes were then inserted into the proximal portion of the LDM perpendicular to the neurovascular pedicle and connected to a Myostim 7220 pacing system (Teletronics Pacing System, Inc., Denver, CO, USA) which was then implanted in a subcutaneous pocket and the wound closed.

Electrical stimulation protocol

The myostimulator was programmed by an external myoprogrammer (model 7100, Teletronics Pacing Systems, Inc.). The animals were randomly assigned to three groups, different only in the type of electrical stimulation training protocol that was begun after LDM mobilization. The electrical stimulation protocol was conducted for sixteen days, after which the study was terminated. Series I: As a control group, four animals received no electrical stimulation after LDM mobilization. Implanted electrodes and the myostimulator were utilized for fatigue testing. Series II: In four animals an electrical stimulation protocol was started two hours after subtotal LDM mobilization using single impulses with an amplitude of 5 volts (V) and at a rate of 15 CPM. Series III: In four animals the same electrical stimulation protocol as in series II was started two hours after subtotal LDM mobilization. Additionally, electrical stimulation that mimicked cardiac assist (six impulses per burst, amplitude of 10 V, frequency of 30 Hz, and a rate of 15 CPM) was added twice daily for 30 minutes under a work-rest regimen (one minute work, then one minute rest.). Four hours elapsed between the two additional stimulation sets mimicking cardiac assist.

Contractile force measurements Day 1

Baseline CF measurements of the LDM were obtained pre- and post-mobilization in all animals at preloads of 20 g/kg and a pulse amplitude of 10 V. A force transducer (ACCU Force III, Ametec, FL) attached to the foreleg of the animal was used to acquire all measurements which were recorded using a Gould ES 1000 recording system (Gould Instrument Systems, Inc, Valley View, OH). One hour after LDM mobilization a 30 minute fatigue test (10 V, 30 Hz, 20 g/kg preload, 6 impulses per burst) was performed in all 12 animals using a work-rest regimen of paced contractions (one minute of work followed by one minute of rest). Upon completion of the fatigue test, CF was measured at five minute intervals until it returned to baseline levels.

Days 6, 11, and 16

Two fatigue tests were performed using the same parameters as on day 1. The first fatigue test lasted 30 minutes; the LDM was then allowed to rest. CF was measured at five minute intervals until it returned to baseline levels. After two hours of complete rest, a second 30 minute fatigue test was performed (days 6 and 11 only); on day 16, the fatigue test was extended to 60 minutes. After the completion of the second fatigue test, CF was again measured at five minute intervals until returning to baseline.

Biopsies

Light microscopy

On day 1, biopsy samples (3x4 mm) for light microscopy were excised from the LDM *in situ* (control), immediately after mobilization, and after completion of the fatigue test. On day 16, samples were excised before and after the first 30 minute fatigue test. Samples were placed in 10% formalin and taken to the hospital's pathology department for embedding and staining. Transverse sections were made for conventional histological (Hematoxylin & Eosin) staining and for subsequent evaluation. Multiple slides were made of each biopsy sample. Particular attention was paid to evidence of muscle degeneration, margination of leukocytes, and the presence or absence of muscle cell swelling.

Immunohistochemistry

To assess angiogenesis, conventional indirect immunoperoxidase en-face staining procedures were followed after fixation and proteolytic predigestion of formalin-fixed tissue, after which samples were incubated with von Willebrand Factors (vWF) to serve as angiogenic markers. Polyclonal rabbit antibodies, commercially available from DAKO were studied immunohistochemically by the manufacturers and our Cell Biology laboratory. It was determined that DAKO rabbit anti-human vWF cross-reacted with vWF from sheep. This analysis was highly important since it yielded information as to overall angiogenesis and vascularization. The degree of vascularization was assessed by counting the number of vessels per unit area.

Results

Contractile force measurements

Initial findings

At baseline before LDM mobilization, CF was 1723 ± 141 g. After subtotal LDM mobilization, when the muscle was in severe ischemic shock, CF decreased in all animals to a mean of 1374 ± 109 g (an average decrease of 21% from baseline CF of the nonmobilized LDM). After mobilization CF for each animal was calculated as 100%; therefore CF before mobilization averaged 121%.

One hour after mobilization

In a previous investigation [8], we showed that LDM performance was poor at 30 and 60 CPM when either a continuous or a work-rest regimen of contraction was applied for 30 minutes to newly mobilized muscle: CF decreased to $50 \pm 4\%$ (continuous contraction, 60 CPM), $61 \pm 3\%$ (continuous contraction, 30 CPM), $64 \pm 3\%$ (work-rest regimen, 60 CPM), and $75 \pm 2\%$ (work-rest regimen, 30 CPM). We noted the visible cyanosis in the distal portion of the LDM after 30 minutes of testing with 30 or 60 CPM. In this study, a slower contraction rate of 15 CPM was applied for 30 minutes to the newly mobilized LDM in all animals. This rate appeared harmless to the LDM - CF decreased overall to $92 \pm 3\%$ and dropped minimally ($98 \pm 1\%$) during the first 10 minutes of testing.

There was no cyanosis in the distal portion of the mobilized LDM. After completion of the fatigue test, CF was measured every five minutes until returning to baseline. Previous data [8] showed that when using 30 CPM even under a work-rest regimen, CF returned to baseline after 80 minutes of rest. At 15 CPM, CF returned to baseline after 25 minutes of rest (figure 1).

Six days after mobilization

On day 6 post mobilization several investigations were performed. First, a 30 minute fatigue test was conducted using a work-rest regimen and 15 CPM (identical to that used one hour after mobilization). CF was measured every five minutes after testing until CF returned to baseline. The muscle was then allowed to rest completely with no testing for two hours. A second fatigue test (same parameters as above) was then performed, again followed by a rest period until CF returned to baseline.

Control group animals in series I (no electrical stimulation) sustained a marginal decrease in CF ($88 \pm 3\%$) after the first fatigue test using the work-rest regimen, and CF returned to baseline after 45 minutes of rest. After two hours of complete rest, during the second fatigue test, CF decreased to $85 \pm 4\%$, and returned to baseline levels after 65 minutes (figure 2).

Series II animals (electrical stimulation for six days after mobilization) lost only 5% CF ($95 \pm 2\%$) during the first fatigue test, and CF returned to baseline after 25 minutes. CF decreased to $93 \pm 1\%$ after the second test, and returned to baseline after 40 minutes (figure 2).

In series III animals (electrical stimulation and cardiac assist stimulation twice daily), CF remained basically unchanged ($99 \pm 2\%$) after 30 minutes of fatigue testing, and returned to baseline after five minutes of rest. The second test after two hours of rest showed similar results: CF decreased to $98 \pm 3\%$ and returned to baseline levels after 10 minutes (figure 2).

Eleven days after mobilization

On day 11 post mobilization the same investigations as performed on day 6 were repeated. As expected no significant changes in CF were recorded during either fatigue test

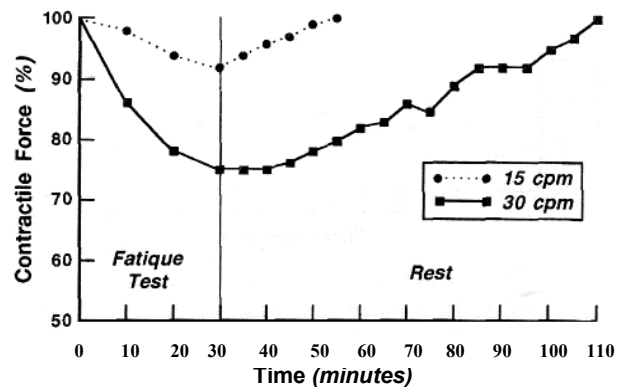


Figure 1. Results of a fatigue test using a work-rest regimen one hour after LDM mobilization. CPM, contractions per minute.

Immediate stimulation after cardiomyoplasty

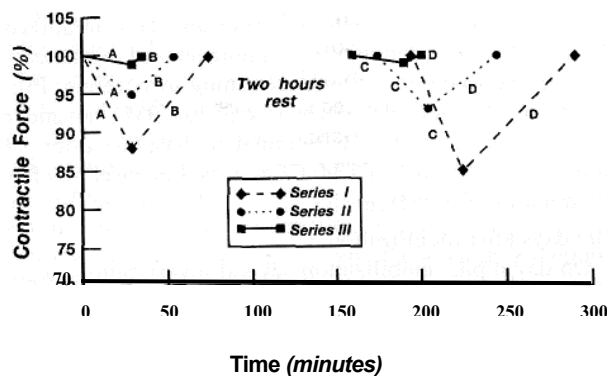


Figure 2. Results of fatigue tests using a work-rest regimen and restoration of contractile force (CF) 6 days after LDM mobilization. A) first fatigue test (30 min); B) restoration of CF after first fatigue test; C) second fatigue test (30 min) starting 2 hours after CF restoration; D) restoration of CF after second fatigue test.

on day 11 as compared to day 6.

Series I animals (control) showed a greater decrease in CF ($85 \pm 4\%$ in the first test and $78 \pm 3\%$ in the repeat test), but these results did not vary with any statistical significance when compared to results of day 6. Further, more time was needed for CF to return to baseline levels (50 minutes after the first test, 85 minutes after the repeat test) than on day 6 (figure 3).

Series II animals had a minimal change in CF after the fatigue tests ($95 \pm 3\%$ after the first test and $93 \pm 4\%$ after the second test), again similar to results of day 6, and CF returned to baseline in approximately the same amount of time as on day 6 (20 minutes after the first test, 45 minutes after the repeat test) (figure 3).

Series III animals showed no change in CF after testing on day 11. CF actually increased to $102 \pm 3\%$ after the first

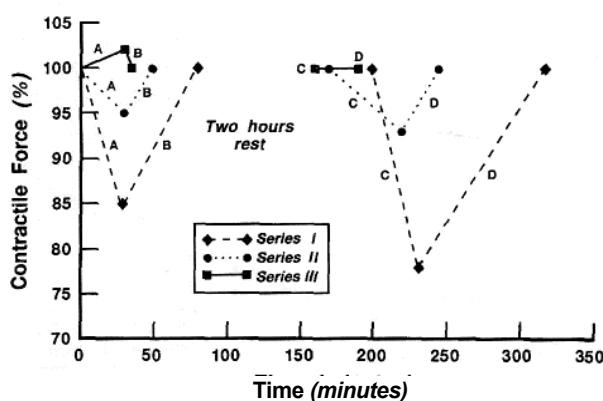


Figure 3. Results of fatigue tests using a work-rest regimen and restoration of contractile force (CF) 11 days after LDM mobilization. A) first fatigue test (30 min); B) restoration of CF after first fatigue test; C) second fatigue test (30 min) starting 2 hours after CF restoration; D) restoration of CF after second fatigue test.

test, and no rest period was needed. Further, CF also did not change during the repeat test ($100 \pm 2\%$) (figure 3).

Sixteen days after mobilization

In series I animals, CF decreased to $85 \pm 4\%$ after the first test and took 50 minutes to return to baseline. In series II animals CF decreased to $94 \pm 3\%$ after the first test, and it required 20 minutes to return to baseline. Series III animals showed results similar to those recorded on day 11: CF did not decrease after the first fatigue test, but rather increased to $104 \pm 2\%$ (figure 4).

On day 16, the second fatigue test was extended to one hour (after the two hour rest period). In series I animals, CF decreased to $72 \pm 9\%$ and returned to baseline after 100 minutes. In series II animals, CF decreased to $85 \pm 6\%$ and returned to baseline after 45 minutes. In series III animals, results were again intriguing; CF did not decrease until 50 minutes of testing, then dropped only to $92 \pm 4\%$ by the end of the one hour test, returning fully to baseline after 20 minutes (figure 4).

Light microscopy

One hour after LDM mobilization but before fatigue testing, granulocytic pavements were present in biopsy samples from all groups. Scattered fibers showed swelling. After the 30 minute fatigue test using 15 CPM, there were no significant changes compared with pre-test samples. In animals tested at 30 CPM (previous data), the degenerative process was more evident. A high proportion of swollen degenerative cells was seen; margination of leukocytes was more significant than at 15 CPM, and there were many shrunken, wavy fibers.

On day 16, histological examination of series III samples after the first fatigue test showed no changes which could be interpreted as degenerative or necrotic. Leukocyte margination was considerably less than in samples one hour after mobilization. Some samples from the peripheral LDM showed occasional fiber degeneration and fibrosis. Morphological changes in the control animals (series I)

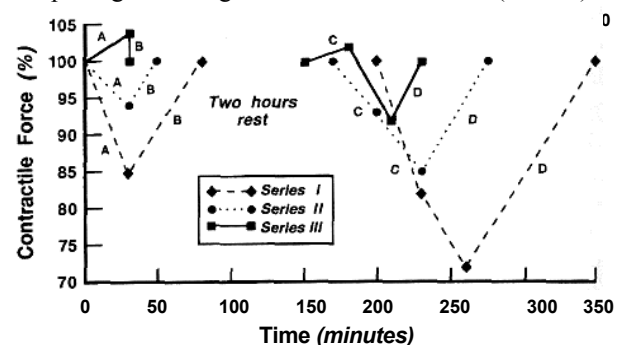


Figure 4. Results of fatigue tests using a work-rest regimen and restoration of contractile force (CF) 16 days after LDM mobilization. A) first fatigue test (30 min); B) restoration of CF after first fatigue test; C) second fatigue test (60 min) starting 2 hours after CF restoration; D) restoration of CF after second fatigue test.

were worse: the muscle appeared damaged and edematous, some muscle fibers had a wrinkled appearance, and zones of calcified necrosis were noted in the peripheral LDM.

Capillary ingrowth

In a previous investigation [21], our immunohistochemistry results showed that capillaries occupied $3.99 \pm 0.24\%$ of the area in nonmobilized nonischemic LDM. On day 16 using 30 CPM, capillaries occupied only $2.63 \pm 0.39\%$ of the area. On day 16 in the current study, series I animals had capillaries occupying $3.02 \pm 0.60\%$ of the area. There were few capillaries in the ischemic LDM between muscle fibers and in the partially degenerated areas. On day 16 in series II animals (electrical stimulation training at 15 CPM), there were numerous small capillary structures occupying $4.49 \pm 0.44\%$ of the area. In series III animals (electrical stimulation training and cardiac assist), capillaries occupied $5.04 \pm 0.33\%$ of the area (figure 5).

Discussion

In light of a shortage of donor hearts, cardiomyoplasty promises to be an indispensable surgical method for treating patients with congestive heart disease who are in need of a heart transplant [17]. However, cardiomyoplasty was

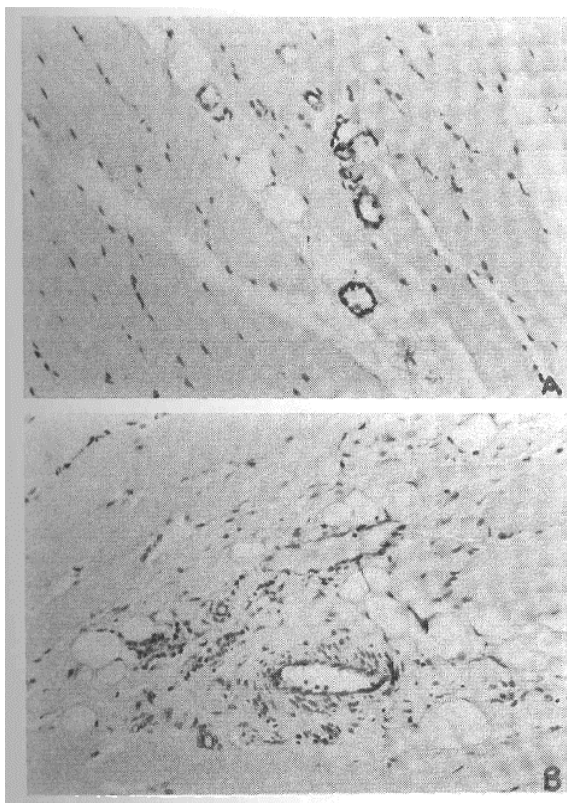


Figure 5. Immunostaining. A) A decrease in the numbers of capillaries in the control series (series I) without electrical stimulation, as compared with B) the intense neovascularization seen 16 days after LDM mobilization when electrical stimulation and stimulation mimicking cardiac assistance were applied to the LDM (series III).

introduced clinically before questions about its benefits and shortcomings were resolved [10, 13, 16, 25]. Despite numerous experimental investigations and more than 600 cardiomyoplasties performed clinically worldwide, many problems still remain regarding this procedure. These problems are often termed the "weak points" of cardiomyoplasty. Our investigation focused on resolving several of these weak points: preventing ischemia-reperfusion damage using an autologous biological glue with an added pharmacological component [6]; accelerating the angiogenic process using biological glue with added autologous endothelial cells and a cautious electrical stimulation protocol [22]; and pinpointing a suitable electrical stimulation protocol for transforming skeletal muscle so that it may sustain contractions in a cardiac regimen. In this paper we are presenting only the results regarding the use of a cautious stimulation protocol so that partial cardiac assist may be begun immediately after cardiomyoplasty.

Electrical stimulation protocol

The "immutable" principle

One immutable principle has constrained the electrical stimulation protocol: one must wait two weeks after cardiomyoplasty for the LDM to stabilize before beginning the eight weeks of required stimulation training. Therefore, cardiac assistance can only begin eight weeks after cardiomyoplasty when the electrical stimulation training protocol has proceeded to four impulses per burst [3]. This principle rests on early discoveries that 1) skeletal muscle becomes fatigue resistant only after several weeks of cautious electrical stimulation training [24], and 2) the muscle may become necrotic if training begins right after mobilization [19]. While good for the muscle, this delay is of no help to the patient in need of cardiac assistance shortly after surgery.

Long term results uncovered another "weak point" of cardiomyoplasty: no hemodynamic benefit was seen in many patients even with significant clinical improvement [11, 9]. It is thought that the LDM failed to produce effective systolic augmentation because the rate of contraction was sub optimal for the muscle. With these two "weak points" in mind - the unavoidable delay in cardiac assistance during prolonged training and the failure to achieve peak systolic augmentation - we directed our investigation to answer two questions: 1) is it possible to prevent ischemic-reperfusion damage and increase the angiogenic potential of the LDM, and 2) is it possible to modify the electrical stimulation training protocol in order to perform partial cardiac assist immediately after cardiomyoplasty if needed.

Protocol variations

Investigators who have examined the question of varying the training protocol include Salmons and Jarvis [23], who explained cardiomyoplasty's lack of consistent long term direct hemodynamic benefits on the sub optimal loading of the mobilized muscle. Magovern [18] noted that stimu-

lation for 24 hours a day at a ratio of 1:2 may overtax skeletal muscle. Chekanov et al. [5] showed that a 1:1 or 1:2 cardiosynchronization stimulation regimen yielded poorer long-term results than a 1:4 regimen (because the LDM rests more than it works using a 1:4 regimen). Moreira et al. [20] suggested another version of a work-rest regimen: cease LDM stimulation completely for a set period of time each day. In a preliminary investigation [7], we found in a chronic heart failure model that such cessation of continuous stimulation for as much as 12 hours each day did not impair hemodynamic results after cardiomyoplasty. Bailey's [1] findings confirmed that skeletal muscle did not need to be stimulated 24 hours each day, and he pointed out that stimulation cessation each day was especially desirable if myocardial revascularization was the primary goal of the cardiomyoplasty procedure.

Gealow et al. [12] directed their attention to the differences in how skeletal muscle is transformed under different regimens of stimulation: they increased the number of impulses per burst while using the same rate of contraction (1:2), and increased the rate of contraction (from 1:16 to 1:2) while using the same number of impulses per burst (six). They applied these stimulation regimens to LDM two weeks after mobilization. This "progressive pulse train rate" stimulation regimen mirrored the stimulation regimen used in Russia since 1988 [5].

Early Russian protocol

From 1988-1997, 96 patients were implanted with Russian stimulators using the original electrical stimulation protocol [4]. There have been no reports of damage to the LDM from stimulation started immediately after surgery. As previously described [4, 5], the Russian stimulation regimen differs from the conventionally used protocol in a number of ways. The most important difference is that the Russian electrical stimulation training begins immediately after the heart wrap, even before the chest is closed. Since a burst of impulses is utilized, the heart receives assistance every sixteenth (EKS 445, Tochmash, Moscow) or every eighth beat (Stiminak 805, Moscow) during the first week after cardiomyoplasty. During week 2 assistance comes every seventh beat (in the both stimulators); during week 3 assistance is every fifth beat, etc. In week 4 (when the rate has changed to 1:4), the number of impulses in the burst increases to seven for the duration of training. By contrast, the conventional protocol begins electrical stimulation training two weeks after cardiomyoplasty, using only single impulses. Thus, contraction is insufficient for systolic assist. Based on the demonstrated efficacy of the Russian protocol, we directed our investigation to study the morphological status, angiogenic response, contractile force, and fatigue resistance of newly mobilized LDM under a cautious electrical stimulation protocol, which would include short periods of work in a cardiac regimen.

Contractile force and fatigue resistance

In a previous investigation [8], we found that 30 minutes of fatigue testing using continuous contractions decreased

the CF of newly mobilized LDM to $50 \pm 4\%$ at 60 CPM and to $61 \pm 4\%$ at 30 CPM. Even at 15 CPM using continuous contractions, CF decreased to $77 \pm 2\%$. These early results raised questions about subjecting newly mobilized LDM to a cardiac assist regimen. We then repeated this study with a work-rest regimen of 30 and 60 CPM [8]. After 30 minutes of fatigue testing, results were marginally better ($64 \pm 3\%$ at 60 CPM and $75 \pm 2\%$ at 30 CPM), however we did not feel that this was acceptable.

In the present study, we applied 15 CPM in a work-rest regimen to the newly mobilized LDM. The decrease in CF to $92 \pm 3\%$ after 30 minutes of work showed us that this variation of muscle contraction for cardiac assist could be used immediately after cardiomyoplasty, if needed. When the LDM was left *in situ* (series I) without electrical stimulation (a situation similar to the "healing period" in the traditional protocol), fatigue was pronounced: CF decreased to $88 \pm 3\%$ on day 6, $85 \pm 4\%$ on day 11, and $85 \pm 4\%$ on day 16; and the LDM needed 45-50 minutes of rest to return to baseline. Fatigue was even greater when the 30 minute test was repeated two hours later ($85 \pm 3\%$ on day 6; $78 \pm 3\%$ on day 11, and $78 \pm 3\%$ after 30 additional minutes of testing on day 16). The LDM needed 65-85 minutes of rest to restore CF on days 6 and 11. When the second test was prolonged to one hour on day 16, CF decreased to $72 \pm 9\%$, and the LDM showed evidence of further morphological damage.

A cautious electrical stimulation protocol in series II animals (single impulses at 15 CPM, equivalent to a 1:5-1:6 cardiosynchronization rate) seemed to enhance the LDM's fatigue resistance, while sparing it from further damage. During the first fatigue test, CF decreased to $95 \pm 2\%$ on day 6, $95 \pm 3\%$ on day 11, and $94 \pm 3\%$ on day 16; the LDM needed only 20-25 minutes for CF to fully return to baseline levels. More importantly, when the 30 minute test was repeated after two hours of complete rest, the LDM again showed no fatigue: CF decreased to $93 \pm 1\%$ on day 6, $93 \pm 4\%$ on day 11, and $93 \pm 3\%$ on day 16. When the test was continued for an additional 30 minutes on day 16, CF dropped only to $85 \pm 6\%$, and restored to baseline levels after 45 minutes. These findings showed that the LDM, submitted to a cautious electrical stimulation training protocol during the initial "healing period" (the first two weeks after cardiomyoplasty), can be used for short periods of cardiac assist several times per day without considerable fatigue.

Very impressive results were seen in series III animals, where a stimulation protocol mimicking cardiac assistance was added to the cautious electrical stimulation protocol twice daily. During fatigue testing on day 6, CF changed minimally ($98 \pm 3\%$), and was restored completely after 5-10 minutes of rest. On day 11 we received inexplicable results: after 30 minutes of fatigue testing, CF was greater than initial CF ($102 \pm 3\%$), and therefore no rest was needed in order to continue work. On day 16, CF increased to $104 \pm 2\%$. After the two hour rest period the LDM continued to contract without fatiguing ($98 \pm 3\%$ on day

6, $100 \pm 2\%$ on day 11, and $102 \pm 3\%$ on day 16 after the first 30 minutes of testing). However, on day 16 after the fall 60 minute fatigue test, CF dropped to $92 \pm 4\%$, which is still sufficient for cardiac assist. CF was restored to initial levels after 20 minutes of rest, showing this to be a useful variation that should benefit the cardiomyoplasty patient.

Light microscopy

One hour after LDM subtotal mobilization, evidence of ischemia-reperfusion damage was seen: margination of leukocytes, swelling, eosinophilia. It seemed logical that additional muscle loading would lead to severe damage. In previous investigations we showed that after a 30 minute fatigue test using 60 CPM there were dramatic changes in muscle morphology: margination of leukocytes and granulocytic pavements were noted in all areas; all muscle fibers looked swollen and shrunken. When 30 CPM was utilized the degenerative process was less evident, however there was still a high proportion of swollen degenerative cells with some progression to basophilic degeneration. There were also many shrunken wavy fibers. In our current study, we applied two fatigue tests using 15 CPM to the ischemic LDM. After the rest periods there were no significant morphological changes when comparing these samples to those taken one hour after mobilization and to control samples taken before testing. The cautious electrical stimulation protocol and short term muscle contraction in a cardiac assist regimen did not aggravate the LDM's morphological status during the 16 day study. Although there was some occasional fiber degeneration and fibrosis in the peripheral regions of the LDM in series II and III animals, the morphological changes in the control animals (series I) were worse: wrinkled fibers, edema, and sometimes calcified necrosis. Without treatment, the LDM after mobilization recovered from the ischemia-reperfusion damage very slowly. We hypothesize that a cautious electrical stimulation protocol stimulates the muscle to recover more quickly, mobilizes its internal potential, and serves to help it heal.

Capillary ingrowth

Confirmation of our hypothesis that a cautious protocol aids repair in mobilized LDM came through a study of muscle angiogenesis. In a preliminary investigation, when 30 CPM was applied to newly mobilized LDM, the number of capillaries decreased to $2.63 \pm 0.39\%$, which also correlated with an aggravation of the LDM's morphological status. In this investigation, in control muscle (series I), the number of capillaries per area decreased to $3.02 \pm 0.6\%$ (as compared to $3.99 \pm 0.24\%$ in normal muscle). Electrical stimulation seemed to increase the muscle's angiogenic response, on day 16 using 15 CPM (series II) the number of capillaries per area was $4.49 \pm 0.44\%$. When we utilized electrical stimulation at 15 CPM plus a regimen mimicking cardiac assistance (series III), the percent area occupied by capillaries increased to $5.04 \pm 0.33\%$. This data proved to us that we not only preserved the angiogenic potential of

the muscle, but enhanced it using a cautious electrical stimulation regimen. These findings correlate with the results of an investigation by Hudlicka et al. [14]. They showed an increase in capillarization after just seven days of chronic electrical stimulation and demonstrated that chronic continuous electrical stimulation of muscle with a compromised blood supply resulted in muscle atrophy. We also concluded that continuous electrical stimulation with a fast rate of contraction applied to newly mobilized LDM may aggravate its ischemic state, depress its angiogenic potential, and lead to muscle fibrosis and degeneration. However, as reported here, under a cautious stimulation regimen, mobilized muscle may be stimulated towards faster recovery and repair: increased angiogenesis, capillary ingrowth, and healing of ischemia.

Conclusion

Many proponents of cardiomyoplasty, fearing further injury to the mobilized LDM, adhere to an "immutable" principle of cardiomyoplasty: a two week recovery period is necessary after cardiomyoplasty, after which, the electrical stimulation training protocol may be begun, thus deferring the use of the LDM for cardiac assist by at least six weeks postoperatively - precluding any hemodynamic benefit to the patient. Our investigation showed that a slow rate of contraction (single impulses, 15 CPM) applied to the LDM immediately after mobilization does not further damage the ischemic LDM, but rather accelerates the angiogenic response and vascular remodeling. Moreover, we showed that vigorous contractions (10 V, 30 Hz, 6 impulses per burst) will not damage newly mobilized LDM if used for a short duration (30 minutes) with at least two hours between intervals, and if a work-rest regimen with a slow rate of contraction (15 CPM) is utilized. When newly mobilized LDM is contracted in this manner, mimicking cardiac assist, no fatigue or morphological damage is seen. This investigation brings cardiomyoplasty closer to a long-sought goal: electrical stimulation training beginning immediately after LDM mobilization and cardiomyoplasty, thus providing partial cardiac assistance immediately postoperatively, if needed.

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