Alternative Training Protocols for Cardiomyoplasty
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Abstract
The following progressive training regimes, all twelve weeks in duration, were evaluated in dogs (n = 5 per group): 1) standard, as described by Carpentier and Chachques, 2) progressive pulse train rate (PPTR), and 3) progressive pulse number (PPN). Latissimus dorsi muscles (LD) were stimulated \textit{in situ} and biweekly monitors were performed to measure LD contractile force and shortening during afterloaded contractions. The stroke work (SW) and average power (P) were determined in response to the programmed parameters as specified for each week according to the three regimes as well as the response to pulse trains of frequencies varying between 15 and 50 Hz. All three regimes produced similar decreases in contractile velocity and fiber diameter associated with almost complete conversion to Type I fibers. The standard regime lead to the greatest amount of fibrosis (p < 0.0001). No significant muscle damage was observed. The PPTR regime produced markedly greater SW during the early weeks of training compared to the standard and PPN regimes (p < 0.001). The PPN regime, however, produced the greatest average power during the middle weeks of training (p = 0.05). These alternative training regimes may provide earlier benefit to the dynamic cardiomyoplasty patient.

Key words: electrical stimulation, cardiomyoplasty, fiber-type, latissimus dorsi, transformation.

The recognition of skeletal muscle plasticity made the concept of skeletal muscle powered cardiac assist a reality \cite{22}. Prior to the discovery of muscle fiber-type transformation through continuous, low-frequency, electrical stimulation \cite{23}, muscle fatigue precluded the success of muscle-powered assist \cite{13}. Transformation to Type I fibers produces a highly fatigue-resistant muscle, capable of doing continuous work similar to myocardium \cite{24}. As dynamic cardiomyoplasty entered the clinical realm, a training protocol that allowed for cardiac-synchronized, tetanic contraction was necessary. Intuitively, as taught in exercise physiology, a progressive training protocol was desirable. A twelve-week protocol was devised utilizing currently available technology \cite{5}. It delivers a low level of stimulation at the beginning of the protocol to elicit a non-strenuous muscle contraction. It then progressively applies a greater and greater demand to the muscle over the ensuing ten weeks. This protocol has now been used safely in nearly 600 patients.

The standard protocol initially delivers a single pulse with every other cardiac cycle. After two weeks, two pulses are delivered at a frequency of 10 Hz. This frequency is below the fusion frequency of the latissimus dorsi muscle (LD). Therefore, the LD response will be two, almost separate, twitches. At 4 weeks, three pulses are delivered at a frequency of 14 Hz. This frequency could still be below fusion and the full force capability of the LD will not be utilized. Eventually, 6 pulses are delivered at a frequency of 33 Hz which will result in a maximal contraction assuming the amplitude is programmed to a supramaximal value. However, the LD has now become a slow-twitch muscle so that the total shortening achieved during the six-pulse burst is limited.

This protocol provides a progressive demand with the intention of preventing excessive stimulation of the LD early on. However, it does not necessarily provide the best stimulation for either maximizing cardiac benefit or for producing the most desirable muscle properties. It was developed under the technical limitations of first generation cardiomyostimulators. These limitations have been overcome by current technology, allowing more freedom in varying stimulation parameters. Using new technology, it may be possible to take better advantage of the LD properties throughout the training period to provide more effective cardiac assist early after surgery. Since the muscle's fusion frequency is higher prior to transformation, it
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is hypothesized that a higher pulse train frequency should be used in the beginning rather than in the end. Another approach could be to begin using a full pulse train, rather than a single pulse, from the onset of training but at a very low synchronization ratio. This approach was adopted clinically by Akhmedov and colleagues [2].

In order to understand which protocols might be most useful in dynamic cardiomyoplasty, the effect of two modified training protocols on LD contractile properties and morphology were evaluated and compared to the standard protocol.

Methods

Three training protocols were evaluated in dogs (20 to 25 kg): 1) standard, 2) progressive pulse train rate (PPTR), and 3) progressive pulse number (PPN). The training protocols are illustrated in Figure 1. The PPN regime is similar to the standard regime except that when pulses are added the frequency is always 33 Hz. In this study, the LD was stimulated in situ so cardiac synchronization was not required. The pulse trains were delivered at a rate of 45 per minute corresponding to a muscle stimulation to cardiac rate synchronization ratio of 1:2 assuming a heart rate of 90 bpm.

The PPTR regime delivers a 6 pulse, 33 Hz burst from the beginning of the training period, starting at a very low synchronization ratio. The pulse train rate was progressively increased from 5 pulse trains per minute (ptpm) to 45 ptpm. This is equivalent to adjusting the synchronization ratio from 1:15 to 1:2 as listed in Figure 1, assuming a heart rate of 90 bpm.

In five dogs, the right LD was left unstimulated and the left LD was stimulated according to the PPTR regime. In an additional five dogs, the left and right LD were randomly assigned to either the standard or the PPN regimes.

Surgical procedures

Anesthesia was induced by thiopental sodium (12.5 mg/kg) and maintained with isoflurane. After standard surgical preparation, an axillary incision was made and two epimysial electrodes were implanted over the thoracodorsal nerve branches under standard sterile conditions. The electrodes were connected to an implanted pulse generator placed in a subcutaneous, abdominal pocket (Itrel, Model 7420 for standard regime, Itrel, Model 7424 for PPTR, and Transform_cardiomystimulator, Model 4710 for PPN, Medtronic, Inc., Minneapolis, MN). Incisions were closed in layers and analgésic treatment was given as needed. All animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication no. 85-23).

No stimulation was applied for the first two weeks after surgery as indicated in Figure 1. Beginning two weeks post-op, stimulation parameters were programmed according to each regime in the three groups described.

Monitoring

Biweekly monitors were performed beginning two weeks after surgery on the anesthetized animal. The forelimb on the side to be studied was attached to a contraction monitoring system incorporating a force transducer (Model LCF-25, Omega Engineering, Stamford, CT), a linear displacement transducer (Model LD600-25, Omega Engineering), and an adjustable afterload weight. Contractile force and shortening were measured in response to trains of six pulses delivered at frequencies varying between 15 and 50 Hz. The response to varying frequency indicates changes in contractile properties as the muscle converts to primarily slow-twitch fibers. All animals were followed for twelve weeks with no complications.

Morphometry

At termination, biopsies were sampled from proximal and distal regions of the LD. Samples were snap frozen in methyl butane or fixed in formalin. Fiber-type ratios were determined from myofibrillar ATP-ase staining of frozen sections. H & E and trichrome stains of formalin fixed, paraffin-embedded sections were used for determining cross-sectional fiber area and percentage area occupied by inter- and intrafascicular fibrosis and intrafascicular fat. Morphometric measurements were made using an image processing and analysis system (Quantimet 570, Leica, Cambridge, UK) under light microscopy. Six regions of each section were analyzed.

Data analysis

Force and shortening measurements were recorded on digital tape (TEAC, Montebello CA) and on a PC using CODAS data acquisition software (DATAQ Instruments, Akron, OH) at a minimum sampling rate of 500 samples per second. Data was processed using custom software.

Figure 1. The parameters of the three training regimes studied are listed and a typical response is represented by force tracings taken from one dog at each time point. The pulse number is indicated by the burst illustration; the pulse train frequency is listed in Hz and the synchronization ratio of muscle stimulation to heart rate is listed. In the present study, the in situ stimulation of the LD did not require cardiac synchronization. The stimulation rate was fixed at a rate that would correspond to a heart rate of 90 bpm.
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(Labview, National Instruments, Austin, TX) or Advanced CODAS analysis software (DATAQ Instruments). The data was analyzed using a statistical software package (Statview, Abacus Concepts, Berkeley, CA). The effect of the training protocol on contractile performance and morphological changes was determined by ANOVA. A p value less than 0.05 was considered statistically significant. All results are reported as the mean ± standard error.

Results

Contractile properties

Mean stroke work (SW) is shown in Figure 2 as a function of frequency at 2 weeks, that is before training, and at the end of the twelve-week protocol for all three groups. Prior to training, SW increased significantly with frequency up to 33 Hz for all groups (p < 0.0001). No further increase in SW occurred with further increases in frequency because the fusion frequency of the untrained muscle is approximately 33 Hz. A non-significant decrease in SW was observed at 50 Hz. This decrease is due to the shortened pulse intervals resulting in a shortened pulse train duration and slightly less time for muscle shortening.

SW did not vary significantly with frequency after training. The fusion frequency decreased to approximately 15 Hz or less over the ten weeks of stimulation. SW was therefore not increased by increasing the burst frequency at 12 weeks. Rather, a slight decrease in SW was observed with increases in frequency. This decrease is again the effect of a slightly shorter burst duration resulting in less time for muscle shortening.

Table I. After training, LD mass did not change significantly while the percentage of Type I fibers and the average fiber cross-sectional area significantly decreased compared to control. No statistical difference was found between groups. The greatest variability in fiber type and size was found in the PPTR group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Standard</th>
<th>PPTR</th>
<th>PPN</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD mass, grams</td>
<td>115± 4</td>
<td>110± 5</td>
<td>104±2</td>
<td>121±6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Type I fibers, %</td>
<td>31±4</td>
<td>96±4</td>
<td>79±13</td>
<td>93±6</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Fiber area, μm²</td>
<td>2158±80</td>
<td>891±45</td>
<td>1029±129</td>
<td>713±49</td>
<td>p &lt; 0.0001</td>
</tr>
</tbody>
</table>

Table II. The standard training protocol resulted in significantly greater fibrosis than either PPTR and PPN regimes. The greatest variability in intrafascicular fat was found in the PPTR group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Standard</th>
<th>PPTR</th>
<th>PPN</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Fibrosis, %</td>
<td>4.3±3.897</td>
<td>0.9±0.7</td>
<td>2.8±1.4</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Intrafascicular</td>
<td>4.1±3.3</td>
<td>0.9±0.7</td>
<td>2.8±1.4</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Intrafascicular Fat, %</td>
<td>0.3±0.8</td>
<td>0.02±0.02</td>
<td>0.04±0.03</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Intrafascicular Fat</td>
<td>0.8±1.7</td>
<td>1.4±3.5</td>
<td>0.5±0.9</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
with 15 Hz after training induces a stronger contraction than at two weeks, even though the overall strength of the muscle has decreased. The decrease in strength over time can be observed from the typical responses to a 33 Hz, 6 pulse burst (PPTR group) illustrated in Figure 1.

The SW at twelve weeks was highest for the PPTR group and lowest for the standard protocol, but these differences were not statistically significant. The greatest SW occurred at 25 Hz for the PPN group and 20 Hz for the standard and PPN groups. When all groups are combined, the frequency resulting in maximum stroke work was 33 Hz at two and four weeks, 25 Hz at six and eight weeks, and 20 Hz at ten and twelve weeks, assuming six pulses are used.

The rate of force development during isometric contractions is shown in Figure 3 as a function of training time. This rate decreased similarly for all three groups from week two to twelve (p < 0.0001). The greatest decrease occurred between weeks two and four followed by a slow, progressive decline. This observation can also be made from the representative force tracings shown in Figure 1.

**Morphometry**

Fibers were 31% Type I in control muscle and increased to 96%, 79% and 93% following the standard, PPTR and PPN regimes respectively (p = 0.0005). The LD mass did not change significantly from control. Average fiber area was significantly reduced from control (p = 0.0001) and was highest in the PPTR group (n.s.). These results are summarized in Table I.

The LD morphology was assessed for changes in interfascicular fibrosis and intrafascicular fat. Little change was expected as the muscles were stimulated in situ with no interruption of vascular supply. However, minor differences were found between groups after training (Table II). Muscle undergoing the standard training protocol had significantly greater fibrosis than the other two groups (p < 0.0001). This increase was greater in both interfascicular fibrosis and intrafascicular fibrosis. The PPTR group had the least total fibrosis (p = 0.003). Total fibrosis ranged from a minimum of less than 1% of the sample area to greater than 18%.

The PPTR group had the greatest amount of intrafascicular fat, however, the amount of fat as a percentage of fascicle area was small, on the order of 1%, and was not significantly greater than other groups. Intrafascicular fat ranged from less than 1% to as high as 22% in individual samples.

**LD performance according to regime**

The LD performance at each two week interval when stimulated according to the parameters defined by each training regime is shown in Figures 1, 4 and 5. Figure 1 shows a typical recording of force generation for all two week time points in response to the programmed settings defined by each regime. The changes in contractile properties are represented by the decreased slope of the force curve and the overall decrease in the magnitude of force development in the PPN group where a 6 pulse, 33 Hz burst is used for all weeks. The effect of changing the pulse number and pulse frequency is observed in the standard and PPN groups.

In Figure 4, the SW at each time point is shown for the three regimes. The results reflect both the change in programmed parameters and the change in contractile properties as the LD is undergoing fiber-type transformation. For example, for the PPTF regime, SW is initially high, significantly greater than the other two regimes (p < 0.001). It decreases over the first 4 weeks of stimulation even though the same 6 pulse, 33 Hz burst is delivered at all time points. This change reflects the decrease in contraction velocity and fiber diameter that has occurred.

On the other hand, the standard regime shows a steady increase in SW over the course of training. This is because very little work is performed early in the training period when only one or two pulses are used. The SW capacity of the untrained muscle is largely under-utilized. Over the next few weeks, pulses are added and the burst frequency is increased. Both changes are expected to produce a stronger contraction, and the SW is seen to increase. The SW is still less than the potential work available until the last four weeks when the PPTF and standard regimes become similar.

The SW for the PPN regime is similar to the standard regime in the beginning when only one or two pulses are used. During the middle weeks, when three and four pulses are used, the higher frequency of 33 Hz produces slightly greater SW than the lower frequencies used in the standard regime (p = 0.05). Again, at weeks ten and twelve, the SW performed with the PPN regime is similar to the other two regimes as the burst pattern becomes more similar.

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![Figure 3](image_url). The mean rate of force development (dF/dt) for all three groups is shown at each study week. dF/dt decreased significantly (p < 0.0001), primarily during the first four weeks of stimulation.
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Figure 4. The SW performed during each week of training is plotted when the stimulation is applied according to the three different regimes. During weeks two through six, the PPTR regime produces significantly greater SW ($p < 0.01$). From week six on, SW is similar.

Figure 5 shows the average power performed for the three regimes at each time point. Initially, the average power performed is greatest for the PPTR regime ($p < 0.001$). Even though the muscle is contracting only a few times per minute, a very strong contraction is induced so average power is higher than the other regimes. At week four, PPTR and PPN are similar. Though the SW for the PPN regime is low, the contraction rate is high so the average power is similar to the PPTR regime. The lowest power is performed with the standard regime. At weeks six and eight, the average power performed during the PPTR regime is lowest ($p = 0.03$). The SW has decreased with the effects of training, and the muscle is contracting at a much lower rate than in the other two regimes. The average power in the PPTR regime increases during the last weeks of training as the muscle is stimulated at a higher and higher rate. During weeks ten and twelve, the average power is not significantly different between the three regimes.

Average power with the standard regime progressively increased over the course of the training period. This increase is simply the effect of the progressive increase in SW as the pulse number and frequency were increased. The PPN regime results in somewhat greater power during the middle weeks because of the higher stroke work with the 33 Hz bursts compared to the standard regime ($p = 0.03$). Since the contraction rate for these two regimes is constant throughout, the change in average power reflects only the change in SW due to incrementing the pulse train pattern and the training effects on muscle properties.

Discussion

The final contractile performance was similar between all three groups. Fusion frequency, SW, and contractile velocity were not significantly different. Under the conditions studied, all three protocols were found to cause little or no muscle damage. Muscle fibers appeared normal with small increases in fibrosis and fat on the average. Greater occurrence of damaged fibers, fat, and fibrosis has been reported in experimental models which require full mobilization of the LD [6, 11, 18]. In these cases, damage is due primarily to surgical ischemia and not to the stimulation itself.

The cause for greater fibrosis associated with the standard regime could be that this regime may actually be more demanding than the other regimes during the early weeks. When two pulses are delivered at approximately 10 Hz from weeks four to six, two virtually separate twitches occur. Since greater metabolic demand is associated with tension development rather than sustaining tension [7], two separate twitches could conceivably be more demanding than a single fused contraction. A higher occurrence of damaged fibers has been observed with 10 Hz continuous stimulation than with 5, 2.5, or 1.25 Hz continuous stimulation in the rabbit, with 1.25 Hz causing the least damage [17]. When 10 Hz stimulation was applied in an intermittent pattern such that the aggregate frequency was equivalent to 5 Hz, less damage was observed than when 5 Hz continuous stimulation was applied. Therefore, the number of times the muscle is required to develop tension may be a greater determinate of demand and potential muscle damage than the duration of each contraction.

Since the endpoints of the three regimes were similar, the benefit of using one of the proposed regimes of PPN or PPTR is not achieving particularly better LD properties, but to deliver more effective cardiac assistance by having a more significant contraction beginning early in the stimulation protocol.
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The mechanisms of benefit in DCM have been shown to include both chronic dynamic girdling effects leading to reverse ventricular remodeling [14] and cyclic hemodynamic effects improving systolic function during assisted beats [25]. Both girdling and cyclic effects have been shown to reduce wall stress and myocardial oxygen demand experimentally [16].

DCM patients typically do not demonstrate clinical improvement for several months after surgery using the current training protocol. The standard protocol probably does not stimulate the LD in a way to have significant cyclic effects early on in the training. The dynamic girdling effect will require several weeks or months before the patient experiences clinical improvement. By stimulating the LD with a full burst early on, cyclic benefits may be achieved which could lead to earlier clinical benefit. Later, the stimulation demand could be reduced as the chronic effects begin to contribute.

The one-year clinical results in Sao Paulo showed more significant hemodynamic improvement than other centers' experience [19]. This difference may be because this center practiced 1:1 stimulation following the training protocol. The greater average power produced may have led to quicker, more significant myocardial recovery. Indeed, 1:1 stimulation has been shown to be more effective than 1:2 in improving load-independent indices of systolic function in a canine model of tachycardia-induced heart failure [20]. As a cautionary note, the 1:1 synchronization ratio when a short burst is used, but with the tradeoff of reduced SW. What is uncertain at this time is whether greater SW performed at a low rate or lower SW with a full burst early on, cyclic benefits may be achieved which could lead to earlier clinical benefit. Later, the stimulation demand could be reduced as the chronic effects begin to contribute.

Previous studies have shown that SW is maximized by a long burst duration which gives the muscle enough time to fully shorten [10]. However, with a long duration, the maximum contraction rate is limited. A higher contraction rate is possible when a short burst is used, but with the tradeoff of reduced SW. What is uncertain at this time is whether greater SW performed at a low rate or lower SW performed at a high rate is more effective in promoting myocardial recovery. Clinical studies are needed to determine which approach is most effective in short and long-term clinical outcome after dynamic cardiomyoplasty.

Many investigators have expressed interest in initiating the stimulation immediately after surgery. The two week healing period following surgery has been practiced in order to allow adhesions to develop between the myocardial surface and the LD, and to allow the LD to recover from surgical ischemia. Akhmedov has reported the use of a full burst beginning immediately after surgery with no complications [2]. Their patients typically experienced clinical improvement 1.5 to two months after surgery. The use of a full burst early after surgery may be even more important in aortomyoplasty where a forceful contraction is necessary to deliver effective counterpulsation assist [21].

Other modifications to stimulation regimes have recently been proposed which could effectively improve the LD contractile properties by maintaining higher force capabilities and faster contraction velocity. One proposal has been to stimulate the muscle intermittently during a 24-hour period [1, 4]. It is proposed that during resting hours a patient would require less cardiac assistance. Stopping the stimulation for several hours at night would allow the muscle time for metabolic recovery and may inhibit complete transformation to Type I fibers. This rest period may also prevent the risk of muscle damage. In animal studies, better preserved fiber area and a smaller increase in fibrosis has been demonstrated by turning off the stimulator at night compared to 24-hour per day stimulation [1]. This intermittent stimulation has also been shown to maintain a larger percentage of intermediate Type IIb fibers which have greater force capacity than fully transformed, Type I fibers [4].

All the regimes examined in the present study were composed of constant frequency bursts. The use of variable frequency bursts may be advantageous in reducing the number of pulses required to maximize and sustain contractile force. This strategy makes use of the "catch-like" property of muscle [3]. Pulse trains starting with a very short interval have been shown to augment force production and are more efficient than constant frequency trains. Optimization of these intervals can produce a maximal rate of tension development and minimize the rate of fatigue. The optimal intervals for maximum force production in the untrained goat LD have recently been identified [8]. Kwende et al., however, have shown that skeletal muscle which has undergone chronic, low-frequency electrical stimulation does not retain the catch-like property [15]. Variable frequency pulse trains no longer have an advantage. Training regimes that retain the catch property need to be developed. This could be achieved by training with an overall lower aggregate frequency [12] or by intermittent stimulation [4]. Both have been shown experimentally to lead to a final fiber type population consisting primarily of intermediate fibers.

The major limitation of the present study was that the muscle was trained in situ so that interruption of collateral circulation and change in resting tension did not occur as would be in the case of cardiomyoplasty. The in situ model was chosen because it allows direct measurement of LD contractile force and shortening. The goals of our study were to determine the effect of the training regimes on LD contractile performance. The relative differences in SW and power found between regimes are expected to be relevant in dynamic cardiomyoplasty. The hemodynamic effects of these differences require further study.

In the near future, experimental work will likely lead to the definition of new protocols that produce more desirable muscle properties for cardiac assist. In the meantime, clinical studies of modified regimes as described here are...
underway in an effort to deliver the most effective and earliest possible clinical benefit. Such studies will also provide a better understanding of the primary mechanisms in dynamic cardiomyoplasty which will facilitate further refinements in stimulation strategies.

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