Intermittent stimulation enhances function of conditioned muscle

CHANGPING DUAN, DENNIS R. TRUMBLE, DEBORAH SCALISE, AND JAMES A. MAGOVERN
Cardiothoracic Surgery Research, Allegheny University of the Health Sciences, Department of Surgery, Allegheny University Hospitals, Allegheny General, Pittsburgh, Pennsylvania 15212

Duan, Changping, Dennis R. Trumble, Deborah Scalise, and James A. Magovern. Intermittent stimulation enhances function of conditioned muscle. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1534–R1540, 1999.—Skeletal muscle is highly adaptable in that its metabolic and contractile characteristics are largely regulated by its pattern of use. It is known that muscle phenotype can be manipulated via chronic electrical stimulation to enhance fatigue resistance at the expense of contractile power. Type 2A fibers are fatigue-resistant, powerful, and considered most desirable for cardiac assist purposes. We have found that 12-wk of intermittent-burst stimulation produces a high percentage of 2A fibers and increases fatigue resistance and power in rabbit latissimus dorsi muscle. Fixed-load endurance tests were used to quantify fatigue resistance among normal and trained muscle groups. Control muscles were found to fatigue completely within 10–20 min. Muscles stimulated continuously for 6 wk retained 35% (71.5 ± 19.5 g·cm) of their initial stroke work at 40 min. Muscles stimulated 12 h/day for 12 wk had the highest initial stroke work (449.7 ± 92.4 g·cm) and the highest remaining stroke work (234.7 ± 50.1 g·cm) at 40 min. Results suggest that employing regular resting periods during conditioning preserves strength in fatigue-resistant muscle.

The hypothesis that intermittent stimulation of the latissimus dorsi (LD) muscle will improve muscle function and limit the conversion of type 2 fibers to type 1 fibers for an extended period.

MATERIALS AND METHODS
Animals and Animal Care
New Zealand White rabbits (2–4 kg, female) were used in these experiments. All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (DHEW Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205).

Surgical Procedure
Sterile techniques were used for all operative procedures. Medtronic I tre3 neuromuscular stimulators (model 7425) and custom paraneural leads (US Patent no. 5,158,097) were used to condition the muscles. Animals were preanesthetized with xylazine and ketamine, intubated, and placed on a ventilator. Anesthesia was maintained with a mixture of 50% oxygen, 48–49% nitrous oxide, and 1–2% isoflurane. Acepromazine and atropine were given to control secretions. Electrocardiogram monitoring was performed in each case. A lateral incision was made in the skin and subcutaneous muscle along the left hemithorax. Hemostasis was achieved without the use of electrocautery. The proximal medial border of the LD was dissected free from adjacent structures and elevated from the chest wall by gentle retraction. The thoracodorsal nerve branch entering the insertion of the LD was identified. The cathode of the paraneural electrode was sutured to the muscle fibers adjacent to the neural bundle. The LD was secured in its native location in preparation for in situ stimulation. The stimulator was tunneled into the abdominal subcutaneous tissue, and the incision was closed in the standard fashion. Antibiotics were given preoperatively and for three days postoperatively.

Stimulation Parameters
The left and right LD from each animal were chosen for stimulation and control studies, respectively. Muscles were conditioned using the following stimulation parameters.

Continuous-burst stimulation. Continuous-burst stimulation was at 2.0 V, 250-ms burst duration, 880-ms interburst interval, 210-µs pulse width with a 10-Hz (week 1), 16 Hz, (week 2), 20 Hz (week 3), and 25 Hz (week 4 and above) burst frequency for 24 h/day.

Intermittent-burst stimulation. Intermittent-burst stimulation was at 2.0 V, 250-ms burst duration, 880-ms interburst interval, 210-µs pulse width with a 10-Hz (week 1), 16 Hz (week 2), 20 Hz (week 3), and 25 Hz (week 4 and above) burst frequency for 12 h/day.

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Protocol I. This experiment was designed to investigate the effects of long-term continuous-burst stimulation on LD muscle function and morphology. Eight rabbits were assigned to one of two groups (6 wk and 12 wk stimulation). The entire left LD muscle from each animal was stimulated, and the right LD served as a paired control. Peak isometric force was measured over five contractions with the same stimulation pattern used for muscle conditioning. Contractile energetics and fatigue resistance were determined under the same stimulation conditions for 40 min. Immunohistochemical analysis of the muscle was used to determine muscle fiber types and measure fiber CSA.

Protocol II. This experiment was designed to test the effects of continuous low-frequency stimulation on muscle fiber transformation. Continuous single-pulse stimulation delivered at 10 pulses/s is known to induce complete conversion of skeletal muscle fibers to the type 1 phenotype. It was hypothesized that single-pulse stimulation delivered at lower frequencies would reduce the degree of phenotype transformation and yield a stable population of powerful type 2A muscle fibers. Six rabbits were divided into two groups (6 wk and 12 wk) with continuous single-pulse stimulation. Immunohistochemical analysis of the muscle was used to identify muscle fiber types and measure fiber CSA.

Protocol III. This experiment was designed to determine the effect of long-term intermittent-burst stimulation on muscle fiber phenotype expression, contractile function, fiber CSA, and metabolism. Ten rabbits were randomly assigned to two groups of five each: 1) 6 wk of continuous-burst stimulation (6-wk continuous, 24 h/day) and 2) 12 wk of intermittent-burst stimulation (12-wk intermittent, 12 h/day). Burst-stimulation parameters were identical to protocol I. Because LD muscles after intermittent-burst stimulation training were so powerful and resistant to fatigue, muscle fatigue resistance was determined beyond 40 min (up to 8 h). Immunohistochemical analysis of the muscle was used for muscle fiber typing and CSA measurement. Electrophoretic analysis was used to identify the various myosin heavy chains (MHC) present. Biochemical analysis techniques were used to measure muscle metabolic enzymes.

Analytic Procedures

Immunohistochemical analysis for muscle fiber types and CSA. Fiber type identification was performed as described by Schiaffino et al. (18). Cryosections of muscle samples were fixed with 50 ml of 37–40% formaldehyde + 370 ml 95% ethanol + 25 ml glycerol (acetic acid) for 5 min. Slides were then washed twice with 1X PBS. A 5% dry milk block in 1X PBS was then applied to the slides and they were incubated in a humid chamber at room temperature for 1 h. Excess milk block was then removed, the primary antibodies to the myosin heavy chains, type I (BA-DS), type 2A (SC-71), type 2B (BF-F3), and type 2X (BF-35), were added to the appropriate sections, and the slides were incubated at 4°C overnight. The slides were then washed two times with 1X PBS, and a secondary antibody (Biogenex-biotinylated anti-mouse IgG) was applied to the sections that were incubated in a humid chamber at room temperature for 30 min. Slides were washed two times as above, and a streptavidin alkaline phosphatase conjugate (Biogenex) was added. The slides were incubated for another 30 min at room temperature. The streptavidin conjugate was removed by washing (as in prior steps), and the substrate, comprising a naphthol substrate, was added. Slides were observed under the microscope and the development was stopped when the desired degree of staining was achieved. Muscle cross sections were divided into four or five evenly spaced regions, depending on the size of the sample. Representative sections were photographed with a digital camera and the images were analyzed with an image analysis program. The system consists of a microscope with an attached camera coupled to a Compaq personal computer, optical mouse, and an image processor.

Analysis of MHC. Myosin extraction was performed as described by La Framboise et al. (10). Muscles were minced finely, and myosin was extracted on ice for 40 min in four volumes of buffer (pH 6.5) as previously described by Butler-Browne and Whalen (5). The extracted myosin was diluted in.
Muscle fiber type and CSA of rabbit LD muscle in control, 6-wk, and 12-wk continuous-burst stimulation

Table 1. Muscle fiber type and CSA of rabbit LD muscle in control, 6-wk, and 12-wk continuous-burst stimulation

<table>
<thead>
<tr>
<th>Muscle CSA Occupied, %</th>
<th>Control</th>
<th>6 wk</th>
<th>12 wk</th>
</tr>
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<tbody>
<tr>
<td>Type 1</td>
<td>17.9 ± 2.4</td>
<td>59.0 ± 5.8*</td>
<td>62.3 ± 6.7*</td>
</tr>
<tr>
<td>Type 2A</td>
<td>19.9 ± 2.2</td>
<td>28.3 ± 2.4</td>
<td>28.5 ± 7.0</td>
</tr>
<tr>
<td>Type 2X</td>
<td>62.3 ± 4.3</td>
<td>12.8 ± 4.4*</td>
<td>9.3 ± 2.2*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean CSA of Fibers, µm²</th>
<th>Control</th>
<th>6 wk</th>
<th>12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>2,724 ± 241</td>
<td>2,233 ± 140</td>
<td>2,321 ± 193</td>
</tr>
<tr>
<td>Type 2A</td>
<td>3,068 ± 140</td>
<td>2,376 ± 138*</td>
<td>2,465 ± 57</td>
</tr>
<tr>
<td>Type 2X</td>
<td>3,904 ± 102</td>
<td>2,526 ± 179*</td>
<td>2,294 ± 150*</td>
</tr>
</tbody>
</table>

Data are means ± SD. CSA, cross-sectional area; LD, latissimus dorsi. *Statistically significant change relative to control (P < 0.05).
their initial work capacity. Conditioned muscles were fatigued by 17% in the 6-wk group and 18% in the 12-wk group after the first 5 min of testing and retained 47% (6 wk) and 57% (12 wk) of their initial stroke work at 40 min.

Fiber composition and CSA data for control and conditioned groups are shown in Table 1. The percentage of type 1 fibers in both 6-wk and 12-wk groups was higher than control. The percentage of type 2X fibers in both groups was lower than control. Type 2A differences were not significant between stimulated and control groups. There were no differences in the CSA of type 1 fibers. However, the CSA of type 2A fibers was decreased in the 6-wk group and the CSA of type 2X was reduced in both 6-wk and 12-wk groups.

Protocol II

This experiment was designed to measure the effects of continuous low-frequency stimulation on muscle fiber transformation. Histochemical examination of the muscles confirmed that there was a higher percentage in type 2A fibers after 6 wk of continuous single-pulse stimulation (Table 2). However, type 2A fibers were only provisional. When muscles were trained 6 more weeks, nearly half of the type 2A fibers were transformed into type 1 (Table 2). These data suggest that continuous single-pulse stimulation will, in the long term, lead to complete conversion to type 1 fibers and hence cannot be used to regulate phenotype expression to produce stronger fatigue-resistant muscle.

Protocol III

Protocol III was designed to determine the effect of long-term intermittent-burst stimulation on muscle fiber phenotype expression, contractile function, fiber CSA, and metabolism. Results indicate that intermittent-burst stimulation not only prevents power loss in fatigue-resistant muscle, but 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%) (Fig. 2A). On completion of the 3- or 8-h fatigue tests, peak isometric force was measured in the intermittent stimulation group after a 5-min rest period and was found to be higher than forces generated by the 6-wk continuous group before fatigue testing (392.3 ± 109.7 vs. 175.8 ± 24.4 g). Intermittent-burst stimulation also significantly improved chronic work capacity relative to continuous-burst stimulation (Fig. 2B). At the conclusion of the 40-min fatigue test, control LD muscles retained <1% of their initial work capacity. Stimulated muscles in the 6-wk continuous group fatigued by 61% (from 205.0 ± 22.4 to 124.3 ± 8.2 g·cm) after the first 10 min of testing and retained 35% (71.5 ± 19.5 g·cm) of their initial stroke work at 40 min. Muscles stimulated intermittently had the highest initial stroke work (449.7 ± 92.4 g·cm) and the highest remaining stroke work (234.7 ± 50.1 g·cm) at 40 min. Fatigue tests for the 12-wk intermittent group were continued to 3 or 8 h and yielded the following temporal stroke-work profile (in g·cm): 239.3 ± 54.7 at 1 h, 198.3 ± 39.0 at 2 h, 213.0 ± 61.0 at 3 h, 228 at 4 h, 258 at 5 h, 225 at 6 h, 181 at 7 h, and 165 at 8 h.

Table 2. Muscle fiber type and CSA of rabbit LD muscle in control, 6-wk, and 12-wk continuous single-pulse stimulation

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Control CSA Occupied, %</th>
<th>Type 1</th>
<th>Type 2A</th>
<th>Type 2X</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean CSA of Fibers, µm²</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>20.7 ± 1.8</td>
<td>35.3 ± 2.9*</td>
<td>62.3 ± 2.6*</td>
<td>2,807 ± 60</td>
</tr>
<tr>
<td>Type 2A</td>
<td>11.7 ± 1.1</td>
<td>32.1 ± 6.6*</td>
<td>19.3 ± 4.8</td>
<td>3,091 ± 64</td>
</tr>
<tr>
<td>Type 2X</td>
<td>67.6 ± 4.8</td>
<td>32.6 ± 13.5</td>
<td>17.5 ± 3.9*</td>
<td>4,071 ± 24</td>
</tr>
</tbody>
</table>

Data are means ± SD. *Statistically significant change relative to control (P < 0.05). †Statistically significant change relative to 6-wk single-pulse stimulation (P < 0.05).
In addition to changes in muscle function, concurrent changes in muscular structure were also measured. Intermittent-burst stimulation significantly increased the percentage and CSA of type 2A fibers. Muscle fiber CSAs and relative distribution are shown in Figs. 3 and 4. The percentage of type 1 fibers in both the 6-wk continuous group (52.1%) and the 12-wk intermittent group (22.4%) was higher than that in control (15.3%). Muscles in the 6-wk continuous group had a higher percentage of type 1 fibers than that in the 12-wk intermittent group. The percentages of type 2X fibers in the 6-wk continuous (12.0%) and the 12-wk intermittent (19.5%) groups were lower than that in control (62.8%). Type 2A differences were significant between stimulated and control groups. The highest proportion of type 2A fibers (58.1%) was in the 12-wk intermittent group. There were no differences in the CSA of type 1 and 2X fibers between control and 12-wk intermittent groups. However, the CSA of type 2A fibers was increased in the intermittent-stimulated muscles. The CSA of all type fibers was reduced in the 6-wk continuous group.

The electrophoretic mobility of the MHCs found in rabbit muscle fibers was nearly equivalent to that of rat MHC2X, MHC2A, and MHCb/slow as determined by analysis of rat mixed vastus lateralis muscle myosin containing all four rodent MHC isoforms (2B, 2X, 2A, b/slow) (Fig. 5). Control LD muscles exhibited an overwhelming majority in the MHC2X band, which matched with the distribution of type 2X fibers in Fig. 3. Muscles in the 6-wk continuous group had majority bands in MHCb/slow and MHC2A. However, intermittent-burst stimulation consistently altered MHC composition of LD muscles, resulting in conversion to MHC2A.

Enzyme activities measured in these muscles are presented in Table 3. PFK and LDH levels were reduced only by 6-wk continuous stimulation, whereas MDH was not enhanced in the stimulated groups.

![Fig. 3. Fiber typing (A) and cross-sectional area (CSA) of fiber types (B) in rabbit LD muscles in control, 6-wk continuous-burst stimulation, and 12-wk intermittent-burst stimulation groups. *Statistically significant change relative to control (P < 0.05); **statistically significant relative to 12-wk intermittent-stimulation group (P < 0.05).](image)

![Fig. 4. Immunohistochemical analysis for muscle fiber types. A and B: serial sections obtained from control LD muscle. C and D: serial sections from 6-wk continuous-burst stimulated muscle. E and F: serial sections from 12-wk intermittent-burst stimulated muscle. A, C, and E are reacted with monoclonal antibody BA-D5 specific for myosine heavy chain (MHC) b/slow, whereas B, D, and F indicate reactivity with antibody SC-71 specific for MHC2A.](image)
Significant increases in CS activity were seen in both stimulated groups.

**DISCUSSION**

Observations from these three experiments provide insight into how phenotype expression may be controlled to improve function in chronically stimulated skeletal muscle. Traditional training techniques produce skeletal muscle comprising 100% type 1 fibers, which are very durable but slow and relatively weak. Thus the capacity of such “fully trained” muscles to move blood is limited. The ideal muscle for long-term cardiac assist would, in principle, comprise mostly type 2A fibers, which combine fatigue resistance, contractile power, and rapid relaxation rates. These fibers contain the fast MHC2A isoforms, display a high aerobic-oxidative potential, and contain large amounts of the fast isoformal of the sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase (15). The purpose of this study was to determine whether a significant population of type 2A muscle fibers can be maintained under stimulation conditions typically used for direct cardiac assistance.

Results from the first set of experiments (protocol I) indicate that continuous-burst stimulation effectively transforms muscle fibers from fast (type 2) to slow (type 1) phenotype, with a concomitant increase in fatigue resistance and a marked decrease in muscle strength. Data from protocol II suggest that continuous single-pulse stimulation will eventually lead to complete conversion to type 1 fibers, even if delivered at very low frequencies (i.e., 1 pulse/s). Taken together, these studies suggest that long-term continuous stimulation, either burst or single pulse, cannot be used to regulate phenotype expression to produce stronger fatigue-resistant muscle.

![Image](http://www.jpah.org/.

**Table 3.** Muscle enzyme activities of rabbit LD muscle in control, 6-wk continuous burst stimulation, and 12-wk interval burst stimulation.

<table>
<thead>
<tr>
<th></th>
<th>PFK</th>
<th>LDH</th>
<th>MDH</th>
<th>CS</th>
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<tbody>
<tr>
<td>Control</td>
<td>31.0 ± 3.5</td>
<td>545.3 ± 61.2</td>
<td>130.8 ± 32.9</td>
<td>7.9 ± 1.0</td>
</tr>
<tr>
<td>6-wk Continuous</td>
<td>11.0 ± 1.5</td>
<td>124.7 ± 28.6</td>
<td>204.1 ± 25.4</td>
<td>21.5 ± 3.7*</td>
</tr>
<tr>
<td>12-wk Interval</td>
<td>28.6 ± 5.5</td>
<td>412.8 ± 56.8</td>
<td>194.7 ± 20.1</td>
<td>16.9 ± 12*</td>
</tr>
</tbody>
</table>

Data are means ± SD. Muscle enzyme activities are expressed as μmol·g<sup>-1</sup>·min<sup>-1</sup>. PFK, phosphofructokinase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; CS, citrate synthase. *Statistically significant change relative to control (P < 0.05). †Statistically significant change relative to 6-wk continuous stimulation (P < 0.05).

A third set of experiments (protocol III) was conducted to determine whether chronic burst stimulation combined with regular rest periods would produce a more powerful fatigue-resistant muscle. Intermittent-burst stimulation significantly increased maximum isometric force generation and chronic work capacity (at clinically relevant stimulation frequencies and burst duration) compared with both the continuous stimulation group and control muscles. In addition to changes in muscle function, concurrent changes in muscular structure were also observed. Intermittent-burst stimulation significantly increased the percentage of type 2A fibers relative to both control and 6-wk continuous groups. The percentage of type 1 fibers in both 6-wk continuous and 12-wk intermittent groups was also higher than control. There were no differences in the CSA of type 1 and 2X fibers between control and 12-wk intermittent groups. The CSA of type 2A fibers, however, was increased in muscles stimulated intermittently. The CSA of all fiber types was reduced in the 6-wk continuous group. The activity of enzymes involved in glycolysis (PFK and LDH) was reduced only by 6-wk continuous stimulation, whereas the oxidative enzyme MDH was not enhanced in either stimulated group. However, significant increases in the activity of a second enzyme involved in terminal oxidation (CS) was seen in both stimulated groups. Results suggest that a stable population of type 2A muscle fibers can be generated and maintained via intermittent-burst stimulation and that improvements in muscle strength and fatigue resistance can be achieved when fiber transformation to the type 1 phenotype is controlled.

Although fiber type distribution is critical to long-term contractile function, it is also important that the CSA of muscle fibers not decrease with stimulation. Tables 1 and 2 and Fig. 3 show that type 2X fibers occupy the most space in control LD muscle because they have the biggest CSA and the greatest number of fibers. This is why normal LD muscle is powerful but prone to fatigue. Both continuous-burst stimulation and continuous single-pulse stimulation produced muscles composed mostly of type 1 fibers with <30% CSA occupied by type 2A fibers. These changes were accompanied by a reduction in muscle strength and improvements in fatigue resistance. Muscles with the largest proportion of type 2A fibers (56% of total CSA) were conditioned via intermittent stimulation. With this fiber distribution, muscle strength and steady-state work capacity were seen to improve significantly.

**Fig. 5.** Electrophoretic analysis of MHCs in rabbit LD muscle (lanes 1–3) and rat mixed vastus lateralis muscle (lane 4) that contains 4 MHC types common to rodent appendicular muscle. Control muscle exhibited an overwhelming majority in the MHC2X band. Muscle in 6-wk continuous group had majority bands in MHC<sub>b</sub>slow and MHC2A. Twelve-week intermittent-burst stimulation consistently altered MHC composition of LD muscle, resulting in conversion to MHC2A.
(Fig. 2). The mechanism by which muscles composed mainly of type 2A fibers generate higher isometric force than those with mainly type 2X fibers (control muscle) is not known. One hypothesis is that the function of the neuromuscular junction, such as the maintenance of the acetylcholine receptor, is improved by intermittent stimulation.

It is important to note that measurements of peak isometric force reported here do not equate to the maximum force-generating capability of these muscles but rather indicate their capacity to perform under stimulation conditions generally accepted for clinical use. In practical terms, preserving a given fiber type distribution over the long term requires that skeletal muscle be activated with the same pulse pattern used during training. We therefore chose to limit testing to a single stimulation regimen (25 Hz, 250-ms burst duration, 53 contractions/min) that was used to train the muscle and was proven safe for clinical use. Slightly higher stimulation frequencies (on the order of 35–40 Hz) might also prove effective for cardiac assist purposes.

Regarding the practical application of these results it should be stressed that, to maintain favorable LD fiber composition and contractile characteristics, cardiac assistance must be provided with the same on/off intervals used to train the muscle. With the use of the training protocol tested here for example, circulatory support could be provided for 12 h each day by harnessing energy from a single conditioned LD muscle. In this instance, cardiac assist would be provided during waking hours when the patient is most active and circulatory demands are at their peak. Continuous cardiac support could be provided by harvesting a second muscle to operate during the 12-h rest period of the first. Further studies are needed to determine whether similar results can be achieved using rest periods of shorter duration.

Perspectives

These findings suggest that the insertion of rest periods during chronic electrical conditioning preserves myofiber CSA and yields fatigue-resistant fiber distributions that are stronger than those achieved via conventional training techniques. 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. Our findings indicate that future clinical applications of stimulated skeletal muscle for cardiac assist should use intermittent stimulation protocols that produce more powerful fatigue-resistant muscles.

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REFERENCES


