Improved Function in Muscles Trained Via Interval Stimulation

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Abstract

Latissimus dorsi (LD) muscles of eight rabbits were studied to test the hypothesis that periodic intervals of rest can improve the functional capacity of fatigue-resistant muscle. Animals were divided into two groups of four: one group stimulated continuously (24 hours/day) for six weeks and the other intermittently (12 hours/day) for 12 weeks. Contralateral LD were used as unstimulated control. Muscle strength and endurance were tested using a custom skeletal muscle ergometer and biopsies were collected for histochemical analysis. Isometric force measurements revealed a 105% increase in muscle strength due to interval stimulation while training via continuous stimulation reduced force generation by 47%. Isotonic stroke work in the 12-wk interval group was 115% higher than the 6-wk continuous group and 32% higher than control levels. After 40 minutes of uninterrupted work, the control group was completely fatigued while the 12-wk interval group continued to generate 222.0 g-cm of external work. Histologic data show increases in the number and size of fast oxidative fibers in the 12-wk interval group while 6-wk continuous training yielded smaller fibers dominated by the slow oxidative type. These findings suggest that intermittent rest periods may be important in maintaining contractile function in chronically stimulated skeletal muscle.

Key words: rest periods, stimulation, skeletal muscle, rabbits, contractile power.

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The use of latissimus dorsi (LD) muscle as an internal provider of mechanical energy for circulatory assist is considered a plausible alternative to extracorporeal drive units. Because skeletal muscle does not require energy transmission from the outside, its use eliminates the need for percutaneous tethers and or external battery packs. Initially, the principal prerequisite for using skeletal muscle to assist cardiac function was that the muscle be fatigue-resistant through continuous electrical stimulation [9], LD muscle, which is composed primarily of fast-twitch glycolytic (FG) fibers, is prone to fatigue [7]. Chronic continuous stimulation gradually causes sequential transitions of muscle fibers to a slow-twitch oxidative (SO) type which is resistant to fatigue [4, 7, 11]. However, muscle strength and speed are significantly reduced in muscles conditioned in this manner [11, 12].

To avoid overstimulation of LD muscle, continuous stimulation may be replaced by 'interval' stimulation programs which incorporate daily rest periods [5]. Regular resting periods during interval stimulation may allow muscles to repair stress-induced myocyte damage and thereby prevent degeneration and atrophy often seen in muscles trained via continuous stimulation. It has also become clear that fast-to-slow fiber transformation can be affected by interval stimulation at a higher frequency [10]. Little is known about the effects of interval stimulation on isometric force, stroke work, and fatigue resistance in LD muscle. This project was designed to determine the effect of longterm interval stimulation on these indicators and on muscle fiber cross-sectional area for different fiber types. The aim of this study focuses on the relative effects of interval and continuous stimulation regimens on preservation of fiber transformation, cross-sectional area, and contractile strength in rabbit LD muscle.

Materials and Methods

Animal model and surgical procedure

Experiments were performed on eight female New Zealand white rabbits (2.5-3.5 kg). They were randomly assigned to two groups of four each: 1)6 weeks of continuous stimulation (6 wk - continuous), and 2) 12 weeks of interval stimulation (12 wk - interval). 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" prepared by the Institution of

Laboratory Animal Resources and published by the National Institutes of Health (NIH publication 85-23, revised 1985). Sterile techniques were used for all operative procedures. Medtronic Itrel and Itrel3 neuromuscular stimulators (Models 7420 and 7425) and custom paraneural leads (U.S. Pat. No. 5,158,097) were used to condition the muscles. Animals were pre-anesthetized 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. EKG 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 muscle 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 muscle was identified. The cathode of the paraneural electrode was sutured to the muscle fibers adjacent to the neural bundle. The LD muscle was secured in its native location in preparation for *in situ* stimulation. The stimulator was tunneled into the abdominal subcutaneous tissue and the incision closed in the standard fashion. Antibiotics were given preoperatively and for three days postopera-tively.

Stimulation parameters

The entire left LD muscle from each animal was stimulated while the right LD muscle served as control for testing and sampling. Muscles were conditioned using burst stimuli (210 |asec pulse width, 250 msec on, 880 msec off, 2.0 volts) with stimulation frequencies increased from 10 to 25 Hz over the course of four weeks. One group was stimulated continuously (24 hours/day) for six weeks while the other was stimulated intermittently (12 hours/day) for 12 weeks. The total number of pulses delivered to the 6-wk and 12-wk were roughly equivalent (about 10% more pulses were delivered to the 12-wk group over the course of training).

Muscle mechanics test

Upon completion of the stimulation protocol, the animals received premedication similar to that used for surgery, without endotracheal intubation. Muscle peak isometric force was tested over five contractions with the same stimulation pattern used for muscle conditioning. Muscle contractile energetics and fatigue resistance were determined under the same stimulation conditions from 40 minutes to 3 hours. Each study employed a custom skeletal muscle ergometer designed to impose isometric or isotonic loading conditions on the muscle (Figure 1). This apparatus enables the measurement of linear motion under conditions of active shortening and passive extension. The muscle load comprised a stack of weights attached to the LD muscle by a thin cord traversing a stationary pulley (total weight =145 grams). Motion was measured with a pair of sonomicrometry crystals (sonomicrometer model

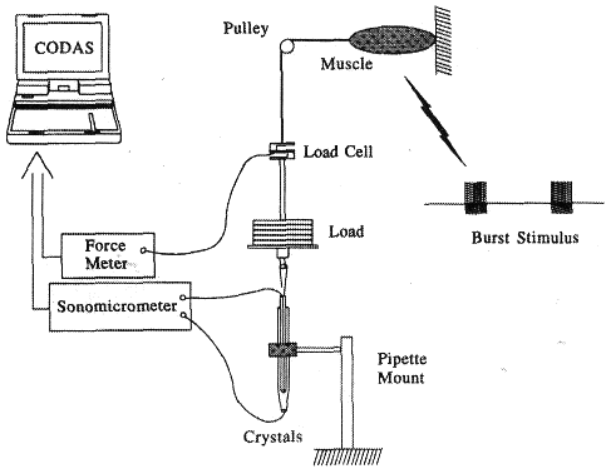

Figure 1. Skeletal muscle ergometer designed to measure LD force generation and contractile motion. This apparatus enables measurement of LD motion under conditions of active shortening and passive extension. The muscle afterload consists of weights attached to the LD muscle by a thin cord traversing a stationary pulley. LD motion is measured with a pair of sonomicrometry crystals mounted on telescoping, fluid-filled pipettes. Forces are measured through a strain gauge mounted between the load and the muscle. Force and displacement waveforms are digitized, recorded, and processed using a commercial data acquisition system (CODAS, Dataq Instruments, Dayton, OH).

120, Triton Technology Inc.) mounted beneath the rack on telescoping, fluid-filled pipettes. Forces were measured through a strain gauge (model LCL-816G, Omega Engineering Inc.) mounted between the load and the muscle. To ensure that this load was transmitted directly to the LD, the lead cable of the ergometer was secured to the LD origin via Teflon felt sutured to the cable. Each animal was held in position to minimize body displacement in response to muscle contraction against the load.

Tissue preparation

Muscles harvested from both sides of all animals underwent the same set of studies. Two biopsies (0.5-1.0 cm^2) were taken from the distal, proximal, anterolateral and posterolateral areas of LD muscle. These samples were used for histochemistry, immunofluorescence and biochemistry determinations. In this preliminary study, we only report on the histochemistry analysis pending completion of the other determinations.

Histochemical analysis for muscle fiber types and cross sectional area

Samples were frozen in 2-methylbutane cooled with liquid nitrogen. Serial 8-|um cross sections were cut on a cryostat microtome at *-21°C,* mounted on a coverslip, and air dried. Cross sections were subsequently stained for ATP-ase activity (preincubation at pH 4.3, 4.55 and 10.4)

for fiber type identification and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) activity as an indicator of oxidative potential. Fibers were identified as fast-twitch glycolytic (FG), fast-twitch oxidative glycolytic (FOG), and slow-twitch oxidative (SO) [6]. This classification appeared more relevant to intermediate fibers produced by electric stimulation. Identification of 300-500 fibers from each sample were made and presented as percentages. The fiber cross sectional area was measured using myosin ATP-ase stained with preincubation pH 4.55. Muscle cross sections were divided into 4-5 evenly spaced regions, depending on the size of the sample. Representative fascicles with fibers cut perpendicular to their long axes were measured with the use of an OPTIMAS 6 image processing system [2]. This system consists of a microscope with an attached camera coupled to a Compaq PC computer, optical mouse, and an image processor.

Data collection and statistical analysis

Force and displacement waveforms were digitized at a rate of 100 samples/sec and stored in an IBM PS/2 personal computer (data acquisition package: CODAS, Dataq Instruments). Data collection was initiated immediately prior to muscle activation and continued uninterrupted during the first fourty minutes of fatigue testing. Beyond this time, discrete datasets (30 seconds in length) were collected every 10 minutes. Results are expressed as means ± SE. Paired data sets were analyzed using two-way analysis of variance (Student's t-test) with nonrepeating measures and Duncan's multiple range test. Differences were considered significant at the $p < 0.05$ level.

Results

Isometric contraction and fatigue resistance

Muscles stimulated in the 6 wk - continuous group produced a marked reduction in maximum isometric force (Figure 2). Comparison of maximum isometric force generation with that of control muscles shows a 47.6% overall decrease in the 6 wk - continuous group (334.2 ± 47.8 g vs 175.0 ± 34.5 g). However, the strength in the 12wk-interval group (687.5 + 95.2 g) was significantly higher than that in control muscles ($p < 0.02$).

Stimulated muscle groups displayed significantly improved endurance capacity relative to control, as illustrated in Figure 3. Stroke work in the control group was reduced by 86% (from 342.0 ± 44.3 g-cm to 48.3 ± 20.5 g-cm) after the first ten minutes of testing and then dropped almost all power by the twenty-minute mark. At the conclusion of the 40-minute test, the control LD muscles retained only 2% of their initial work capacity. Stimulated muscles in the 6wk-continuous group fatigued by 44% (from 210.0 ± 30.9 g-cm to 117.0 ± 5.5 g-cm) after the first ten minutes of testing and retained 25% (52.7 ± 7.2 g-cm) of their initial stroke work at 40 minutes. Interval stimulated muscles had the highest initial stroke work (452.0 ± 160.0 g-cm) and the highest remaining stroke work (222.0 ± 84.0 g-cm) at 40 minutes. Fatigue tests for the 12 wk - interval group

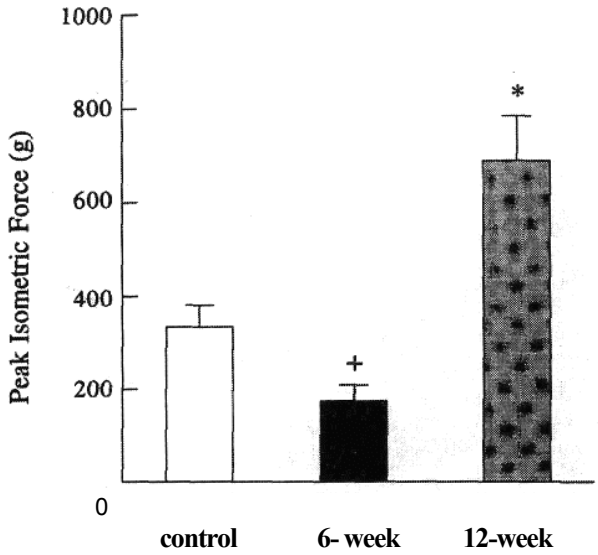

*Figure 2. Peak isometric force generated by control and stimulated muscles. Six-week continuous stimulation decreased force generation by 47.6% relative to control. Strength in the 12-week interval stimulation group was 105% higher than control. * Statistically significant change relative to control ($p < 0.05$); + statistically significant relative to 12-wk interval stimulation group ($p < 0.05$).*

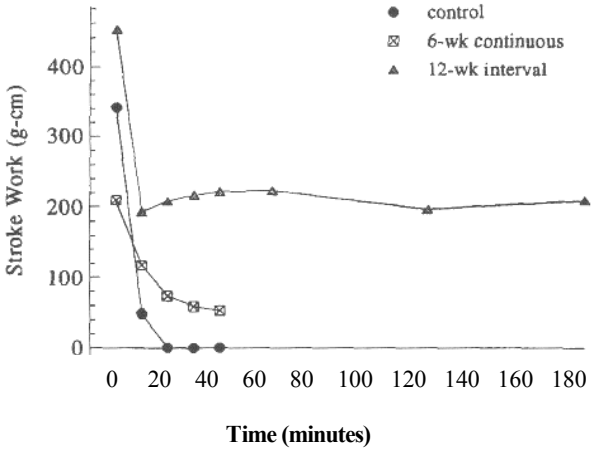

Figure 3. Endurance characteristics of control and conditioned LD muscles. Stroke work is measured and charted over 40-minutes in control and 6-wk continuous stimulation groups and over 180 minutes in 12-wk interval stimulation group. The two conditioned muscle groups displayed improved endurance capacities relative to control. Following an initial decrease in the first 10 minutes of testing, contractile function was maintained for 170 minutes without fatigue in the 12-wk interval stimulation group.

were continued to 3 hours and yielded the following temporal stroke-work profile: 224.0 ±*91.0* g-cm at 1 hr; 196.5 ± 67.5 g-cm at 2 hr and 209.5 ± 105.5 g-cm at 3 hr. Upon completion of the 3 hour fatigue test, peak isometric force

was measured following a 5 minute rest period and was found to be higher than control muscles measured prior to fatigue testing (471.5 ± 131.5 g vs. 334.2 ± 47.8 g).

Muscle fiber types

Muscle fiber composition and cross-sectional area data are shown in Figures 4 and 5 respectively. The percentage of type SO fibers in both the 6 wk - continuous group (48.4%) and the 12 wk - interval group (26.8%) was higher than control (17.1%). The muscles in the 6 wk - continuous group had higher percentage of type SO fibers (48.4%) than those in the 12 wk - interval group (26.8%). The percentages of type FG fibers in the 6 wk - continuous (15.0%) and the 12 wk - interval (18.6%) groups were lower than that in control (61.3%). Type FOG differences were significant between stimulated and control groups. The highest proportion of type FOG fibers (55%) was in the 12 wk - interval group. There were no differences in the CSA of type SO and FG fibers between control and 12 wk - interval groups. However, the CSA of type FOG fibers was increased in the interval stimulated muscles. The CSA of all type fibers was reduced in the 6wk-continuous group.

Discussion

The feasibility of using skeletal muscle as a power source for circulatory assist is predicated upon two fundamental criteria: 1) that skeletal muscle can be made to contract continuously without fatigue; and 2) that these muscles retain enough power to provide significant cardiac support. The capacity to achieve this first criterion has been well-documented, starting with the work of Salmons and Sreter who in 1976 demonstrated that muscle phenotype expression is influenced by impulse activity [9]. Now, skeletal muscle can routinely be transformed to 100% slow oxidative fibers via a wide variety of conditioning protocols - all of which yield fatigue-resistant muscle suitable for chronic activation. The primary limitation however, has been the significant loss of strength and speed which accompanies this transformation [3, 8, 12]. As a result, current training regimens may not preserve enough muscle power to meet the second criterion for muscle-powered cardiac assist.

The principal goal of this work was to test the hypothesis that periodic intervals of rest can improve the functional capacity of fatigue-resistant muscle. This supposition was examined using two groups of rabbit LD muscles, one trained via conventional means (i.e., continuous burst stimulation, 6 weeks, 24 hrs/day) and the other using an identical stimulation pattern with 12-hour rest periods each day (for 12 weeks). Upon completion of the training protocol, each muscle was tested for isometric strength, chronic work capacity, and myofiber composition. Similar analyses were also performed on control (i.e., unstimulated) muscles for comparison.

Preliminary results indicate that interval stimulation not only prevents power loss in fatigue resistant muscle, but can actually increase contractile function beyond baseline values. Isometric force measurements revealed a 105% increase in muscle strength due to interval stimulation while training via continuous LD stimulation reduced force generation by 47%. Similarly, isotonic stroke work in the 12-wk interval group was 115% higher than the 6-wk continuous group and 32% higher than control levels. After 40 minutes of uninterrupted work, the control group was completely fatigued while the 12-wk interval group continued to generate 222 g-cm of external work (321% better than the 6-wk continuous group). This same level of contractile performance was maintained for 3 hours in the 12-wk interval group.

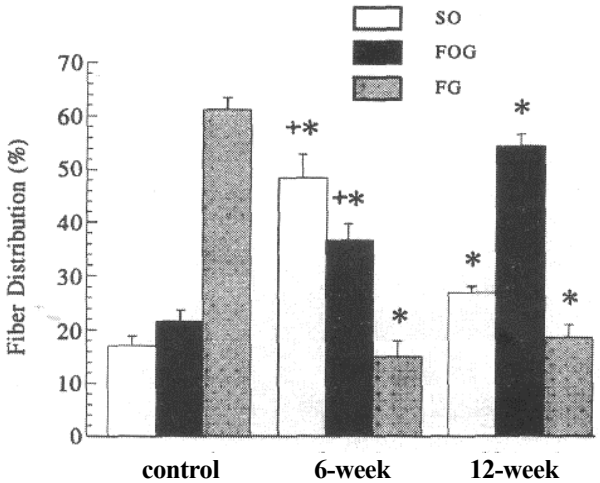

*Figure 4. Fiber typing of rabbit latissimus dorsi muscle in control, 6-wk continuous, and 12-wk interval groups according to histochemical staining for myosin ATPase. * Statistically significant change relative to control ($p < 0.05$); + statistically significant relative to 12-wk interval stimulation group ($p < 0.05$).*

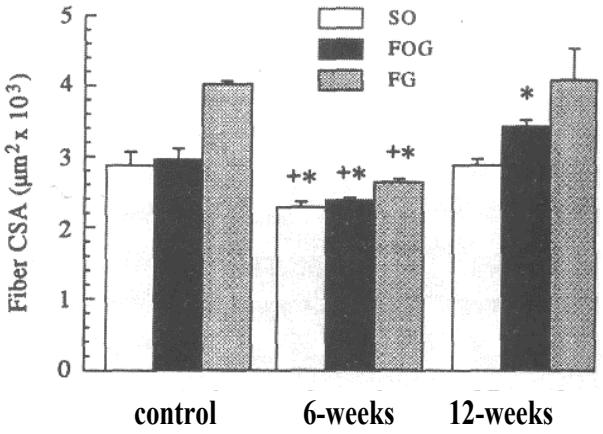

*Figure 5. Histogram showing the CSA of fiber types SO, FOG, andFG in the three muscle groups. Six-week continuous stimulation decreased fiber CSA in all fiber types. The CSA of type FOG was increased in the 12-wk stimulation group. * Statistically significant change relative to control ($p < 0.05$); + statistically significant relative to 12-wk interval stimulation group ($p < 0.05$).*

These profound differences in muscle performance can be explained, in part, by variations in fiber type distribution and fiber size induced by these two training regimens. Normal rabbit LD were found to comprise primarily FG fibers (61 %) which are prone to fatigue. The 6-wk continuous group contained 48% SO, 37% FOG, and 15% FG fibers, all of which were reduced in size relative to control. The 12-wk interval group however, comprised primarily FOG fibers (55%) which were significantly larger than control. These histologic findings are consistent with the contractile performance of these three groups (described above) and lends credence to the notion that ceaseless stimulation may be deleterious to skeletal muscle structure and function [1].

These findings suggest that regular rest periods help preserve myofiber cross-sectional area and yield fatigue-resistant fiber distributions which are stronger than those achieved via conventional training techniques. Hence, the insertion of rest periods into traditional training regimens may yield muscles capable of working at levels sufficient to provide significant cardiac support.

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