Effect of Stimulation Frequency on Metabolism of Skeletal Muscles

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We studied the effects of stimulation frequency on the energy metabolism of rat skeletal muscles during electrical low-frequency stimulation, using in vivo phosphorus-31 magnetic resonance spectroscopy. The sciatic nerve of Wistar-Kyoto rats (n=16) was stimulated electrically using low frequency stimulation (LFS) at 30 Hz and high frequency stimulation (HFS) at 100 Hz as tetanizing frequencies. The initial tension during LFS was significantly different from that during HFS, 200.49±19.47 vs. 516.67±5.38 dynes (mean±S.E.; n=8) (p<0.01). In the first 2 min of stimulation, the ratio of phosphocreatine (PCr) to inorganic phosphate (Pi) + sugar phosphate (SP) during LFS and HFS were 0.28±0.04 and 0.53±0.06 (mean±S.E.; n=8), respectively. Subsequently, the ratio during LFS remained lower than that during HFS. The intracellular pH during LFS and HFS in the first 2 min of stimulation was 6.27±0.02 and 6.75±0.03 (mean±S.E.; n=8), respectively. From 2 min to 12 min of stimulation, the intracellular pH during LFS remained lower than that during HFS. The results showed that lower levels of both energy and intracellular pH of skeletal muscles were maintained during LFS than during HFS, facilitating aerobic glycolysis to produce ATP during stimulation.

Key words: ³¹P-MRS, Skeletal muscle, Electrical low frequency stimulation, Muscle fatigue

Electric stimulation of skeletal muscles is an effective physical therapy for muscle atrophy and weakness (Eriksson et al., 1979; Gould et al., 1983; Johnson et al., 1977; Williams et al., 1976). It has also been proposed for improving the muscles of athletes (Webster, 1975).

Previous studies using phosphorus-31 magnetic resonance spectroscopy (³¹P-MRS) showed that the energy levels in skeletal muscle remain low during both twitch-producing (Challiss et al., 1987) and tetanus-producing stimulation (Shenton et al., 1986; Dawson, 1986), and suggested that these low energy levels might contribute to decreased contraction force, or muscle fatigue. However, we found that during tetanus-producing stimulation the energy level recovered with time, although the muscle tension did not recover (Takata et al., 1988). The purpose of this study was to evaluate the differences between low frequency stimulation (LFS) of skeletal muscles at 30 Hz and high frequency stimulation (HFS) at 100 Hz, using ³¹P-MRS. The importance of these energy metabolism differences in increasing muscle volume and function, and in treatment of muscle atrophy and weakness, is discussed.

MATERIALS AND METHODS

Animals. Male WKY (n=16) of 25 weeks old, weighing 300-380g were used. They were obtained from Charles River Japan, and were housed in a room maintained at 25°C with a 10:14 hour light-
dark cycle. The rats were allowed free access to food and water until used for experiments. Studies were made on hindlimb muscles that contain 84% fast twitch muscles (Armstrong et al., 1983).

Operational procedures. General anesthesia was induced with sodium pentobarbital (60mg/kg, intraperitoneally). The right sciatic nerve was exposed and a small bipolar electrode was placed in contact with it. The tendons of the right gastrocnemius, plantaris and soleus muscles were isolated at the ankle, tied together, cut free from the heel and finally connected to a strain gauge.

Stimulation. The sciatic nerve was stimulated electrically to induce tetanus contractions for 20 minutes (min). The stimulation voltage was 4 V, and the frequency was 30 Hz for LFS, or 100 Hz for HFS. For both tetanizing stimulations, the sciatic nerves were stimulated electrically at intervals of 2 seconds (s) to induce intermittent tetanic contractions. As a result, the gastrocnemius and soleus muscles were induced to maintain a 1.5 s tetanus contraction every 2 s. For $^{31}$P-MRS, this stimulation was continued for 20 min.

Tension (dyne) development. Tension was measured every 2 min for 20 min. Tension development induced by LFS or HFS was recorded with a strain gauge (TB-611T, NIHON KOKKI) connected to a polygraph system (RM-6000, NIHON KOKKI). The strain gauge output was displayed on a pen recorder, and the time course of change in tension was expressed in dynes.

Magnetic Resonance Spectroscopy. $^{31}$P-MRS spectra were recorded with a BEM 250/80 spectrometer (OTSUKA ELECTRONICS U.S.A. INC.) operated at 32 MHz. The spectrometer was operated in the Fourier transform mode with a pulse duration of 15 μs, and a pulse interval of 2 s. The acquisition time was 2 min. Consequently, each spectrum was the sum of 60 free induction decays (FIDS). Each 2 min MRS spectrum corresponded to a 2 min period of stimulation. The hindlimb was placed in a 2 cm diameter solenoid coil. The peak of phosphocreatine (PCr) was assigned a chemical shift of zero. The tissue levels of PCr, inorganic phosphate (Pi), and sugar phosphate (SP) were estimated from the areas under individual peaks. The energy level was expressed as the PCr/(Pi+SP) ratio. Quantitative values for these parameters were in good agreement with the results of chemical analyses. The chemical shift of Pi is pH-sensitive and so is a good measure of the intracellular pH. The latter was estimated from the chemical shift (d) of Pi peaks and calculated by the following equation:

$$\text{Intracellular pH} = 6.90 - \log((d-5.805)/(3.290-d))$$ (Flaherty et al, 1982)

Statistical analysis. Results are expressed as the means±standard error. Paired measurements were compared using Student's t test.

**RESULTS**

Tension development (Fig. 1). The initial tension on LFS was 200.49±19.47 dynes, and that on HFS was 516.67±5.38 dynes (p < 0.01). In the first 2 min of stimulation, the tension decreased rapidly to 166.11±14.80 dynes during LFS, and to 180.64±24.11 dynes during HFS. Thus, the decrease in tension was greater during HFS than during LFS. During

![Tension (dyne) vs Time course (min)](image-url)

**Fig. 1** Changes in tension (dynes) during LFS and HFS. The initial tension during LFS was abot 40% of that during HFS (**p<0.01 by the t-test**). Values are means ± S.E.; n = 8.
the remainder of the periods of LFS and HFS, the tension decreased gradually, but similarly in the two groups, and remained low; after LFS and HFS for 20 min, the tensions were 144.68±17.51 dynes and 90.64±17.55 dynes, respectively.

Typical stacking spectra of $^{31}$P-MRS (Fig. 2). Spectra were obtained at 32 MHz. The acquisition time was 2 min, and each 2 min MRS spectrum corresponded to a 2 min period of stimulation. During both LFS and HFS for 2 min, the PCr peak rapidly decreased from the control level, whereas the Pi and SP peaks both increased and became broader. During further HFS and LFS, the PCr peaks decreased and then increased again, but the peak during LFS, was consistently lower than that during HFS. During recovery for 16 min, the PCr peak returned to the control level more rapidly during HFS than during LFS.

![Fig. 2 Typical stacking spectra of $^{31}$P-MRS during LFS and HFS. SP: sugar phosphate; Pi: inorganic phosphate; PCr: phosphocreatine; ATP: adenosine triphosphate.](image)

The PCr/(Pi+SP) ratio (Fig. 3). The PCr/(Pi+SP) ratio is an indicator of the energy level. This ratio rapidly decreased to a minimum in the first 4 min during LFS, and in the first 2 min during HFS. In the first 2 min, the ratios during LFS and HFS were 0.28±0.04 and 0.53±0.06, respectively (p<0.05). The minimum in the first 4 min of LFS was 0.25±0.05, which was lower than that during HFS. During further LFS and HFS, the ratios increased again. The recovery was greater during HFS than during LFS, and consequently, the ratio was significantly higher during HFS than during LFS from 2 min to the end of the stimulation period (p<0.05 or p<0.01). In both cases, the ratios increased rapidly in the first 2 min of recovery. The ratio during HFS returned to the control value in the first 4 min of recovery, whereas the ratio during LFS was significantly lower (p<0.05 or p<0.01).

![Fig. 3 PCr/(Pi+SP) ratios before, during and after stimulation. Values are means ± S.E.; n = 8.](image)

Intracellular pH (Fig. 4). The intracellular pH after 2 min of LFS and HFS were 6.27±0.02 and 6.75±0.03, respectively (p<0.01). After stimulation for 4 min, the values were 6.27±0.02, and 6.79±0.02, respectively (p<0.01). The intracellular pH reached a minimum after LFS and HFS for 2 min. In both cases, the intracellular pH returned to control values with time; however, the rate of recovery was greater during LFS than during HFS. From 2 min to 12 min of stimulation, the intracellular pH was significantly lower during LFS than during HFS (p<0.05 or p<0.01). During recovery for 16 min, the intracellular pH in both groups increased gradually to the control values with little difference between groups.
INTRACELLULAR pH

![Graph showing Intracellular pH values before, during, and after stimulation. Values are means ± S.E.; n = 8.]

**DISCUSSION**

To elucidate the therapeutic effects of electric stimulation on muscle, we have been studying the relationship between muscle work and energy metabolism. Muscle contraction induced by LFS decreases the gross energy reserve of the muscle, whereas HFS induces muscle fatigue, presumably because of decreased hydrolysis of ATP during stimulation. During both LFS and HFS in this study, the energy level of skeletal muscle, indicated by the PCr/(Pi+SP) ratio, decreased to a minimum and then gradually increased. This ratio during LFS remained low, whereas that during HFS initially decreased to an even lower level, and then increased. After the first 2 min of stimulation, the energy level was lower during LFS than during HFS, although the tensions were not significantly different. The rate of energy recovery during HFS was greater than that during LFS, suggesting that skeletal muscles may continue to consume energy by ATP hydrolysis to a greater extent during LFS than during HFS. Therefore, the exercise induced by LFS may eventually decrease the gross energy reserve of skeletal muscle, whereas HFS may result in less energetic loss than LFS due to decreased ATP hydrolysis.

Gordon et al. (1967) reported that highly repetitive low-resistance exercise induced synthesis of sarcoplasmic protein. In this study, LFS induced highly repetitive, low-resistance exercise. As evaluated by 31P-MRS, the energy level was lower during LFS than during HFS. The muscle exercise induced by LFS may promote synthesis of contractile protein, resulting in increased muscle volume and recovery from muscle atrophy.

In addition, SP peaks during LFS were higher than those during HFS. SP is formed by aerobic glycolysis during exercise, and hence the SP peak is an index of activity for aerobic glycolysis during exercise; that is, it is directly proportional to mitochondrial respiration and is a measure of aerobic muscular work. Its increase during LFS may indicate an increase in the ratio of aerobic glycolysis. Therefore, our results suggest that LFS prevented exhaustion of the energy reserve of muscle fibers by increasing the enzyme activity for aerobic glycolysis.

HFS induces insufficient ATP hydrolysis during exercise, possibly because of a raised threshold of excitation of sarcolemma (muscle fiber membranes) in excitation-contraction coupling. This may be due to an accumulation of Na+ in the sarcolemma, which raises this threshold during exercise (Bigland-Ritchie et al., 1979; Jones, 1981; Jones et al., 1979; Moritani et al., 1985). Na+ accumulation in the sarcolemma may cause a decrease in intensity of the action potential from nerve fibers, and this may result in a decrease in Ca2+ release from the sarcoplasmic reticulum. Ca2+ is thought to be very important in controlling excitation-contraction coupling (Ebashı et al., 1967). Hence, a raised threshold intensity for sarcolemma excitation may be a determinant factor causing insufficient ATP hydrolysis in fatigue induced by HFS. Prolonged HFS probably increases muscle endurance and decreases muscle weakness by increasing resistance to Na+ accumulation in the sarcolemma.
The intracellular pH decreased more during LFS than during HFS. Tesch (1980) reported muscle fatigue with lactate accumulation in humans during exercise. The intracellular pH depends mainly on the quantity of lactic acid accumulation. Dawson et al. (1978) observed linear relationships between changes in muscle tension with time and the adenosine diphosphate (ADP) level and intracellular pH. Thus, increased ADP and/or hydrogen ions may be involved in muscle fatigue. Hydrolysis of ATP and production of lactic acid is greater during LFS than during HFS, and the removal of lactic acid from the muscle fiber and blood is more rapid during HFS than during LFS. As a consequence of this higher level of lactic acid, the intracellular pH remained lower during LFS than during HFS, suggesting the function induced by LFS is highly dependent on the lactic acid system. Therefore, prolonged LFS training may be more effective than HFS for increasing the resistance of muscle fibers to decreased intracellular pH during exercise.

Our results suggest that the low energy level during contraction induced by LFS may stimulate synthesis of muscle protein and increase muscle volume. Thus, LFS may be more effective than HFS for treatment of muscle atrophy. In contrast, HFS may raise the threshold for sarcolemma excitation during exercise and contribute to increased resistance to muscle fatigue. Therefore, HFS may be more effective in increasing muscular endurance.

REFERENCES
Jones, D.A., Bigland-Ritchie, B. and Edward, H.T., 1979 : Excitation frequency and muscle fatigue : mechanical responses during voluntary and
stimulated contractions. Exp. Neurol., 64: 401-413.


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