Fatigue of Paralyzed and Control Thenar Muscles Induced by Variable or Constant Frequency Stimulation

Christine K. Thomas, Lisa Griffin, Sharlene Godfrey, Edith Ribot-Ciscar, and Jane E. Butler. Fatigue of paralyzed and control thenar muscles induced by variable or constant frequency stimulation. J Neurophysiol 89: 2055–2064, 2003. First published December 11, 2002; 10.1152/jn.01002.2002. Muscles paralyzed by chronic (>1 yr) spinal cord injury fatigue readily. Our aim was to evaluate whether the fatigability of paralyzed thenar muscles (n = 10) could be reduced by the repeated delivery of variable versus constant frequency pulse trains. Fatigue was induced in four ways. Intermittent supramaximal median nerve stimulation (300-ms-duration trains) was delivered at 1) constant high frequency (13 pulses at 40 Hz each second for 2 min); 2) variable high frequency (each second for 2 min). The first two intervals of each variable frequency train were 5 and 20 ms. The remaining pulses were evenly distributed in time across 275 ms. The number of pulses varied for each subject such that the force time integral in the unfatigued state matched that evoked by a constant 40-Hz train; 3) constant low frequency (7 pulses at 20 Hz each second for 4 min); and 4) variable low frequency (each second for 4 min). The pulse pattern was the same as that for variable high frequency except that the force-time integral was matched to that produced by the constant low-frequency stimulation. These same experiments were performed on the thenar muscles of five able-bodied control subjects. The variable high-frequency trains used to fatigue paralyzed and control muscles had an average (± SE) of 12 ± 2 and 10 ± 1 pulses, respectively. Variable low-frequency trains had 7 ± 1 and 6 ± 1 pulses, respectively. Significant mean force declines of comparable magnitude (to 20–25% initial fatigue force or to 13–21% initial 50 Hz force) were seen in paralyzed muscles with all four stimulation protocols. The force reductions in paralyzed muscles were always accompanied by significant increases in half-relaxation time and decreases in force-time integral, irrespective of the stimulation protocol. Significant force decreases also occurred in control muscles during each fatigue test. Again, these force declines were similar whether constant or variable pulse patterns were used at high or low frequencies (to 40–60% initial fatigue force or to 29–36% initial 50 Hz force). The force reductions in control muscles were significantly less than those seen in paralyzed muscles, except when constant high-frequency stimulation was used. The variations in stimulation frequency, pulse pattern, and pulse number used in this study therefore had little influence on thenar muscle fatigue in control subjects or in spinal cord–injured subjects with chronic paralysis.

INTRODUCTION

Muscles paralyzed by spinal cord injury are not necessarily weak and atrophied, but they always fatigue much more quickly than control muscles (Lenman et al. 1989; Shields 1995; Thomas 1997a,b). This rapid and exaggerated force decline with repetitive stimulation may relate to the chronic changes that occur in muscle use, metabolism, vascularization, muscle perfusion pressure, and/or fiber type composition (Butler et al. 2001; Castro et al. 1999; Grimby et al. 1976; Martin et al. 1992; Mohr et al. 1997; Stein et al. 1992). In the short-term, both the pattern and the frequency of stimulation used to excite these muscles will also influence the amount of force that they generate and their rate of fatigue (Jones et al. 1979). To restore functional movements to paralyzed muscles with electrical stimulation effectively, it is therefore important to optimize and conserve their capacity to produce force.

Various studies have explored the pulse patterns that maximize contraction strength. It is clear that two closely spaced electrical stimuli markedly enhance the force and force-time integral evoked from single motor units and whole muscles compared with that generated by two single stimuli that elicit twitches (Cooper and Eccles 1930; Duchateau and Hainaut 1986a; Karu et al. 1995; Macafeield et al. 1996). The “doublet-to-twitch” force increase is even greater than usual in muscles that have been paralyzed chronically by spinal cord injury (Griffin et al. 2002). This force difference between paralyzed and control thenar muscles (relative to maximum) remains when more pulses are added to the stimulus train. However, similar pulse patterns, a short interval followed by longer interpulse intervals, always evoke peak force in both kinds of muscle (Griffin et al. 2002). Comparable pulse patterns have been shown to maximize the force and force-time integral from single motor units and various whole muscles across species (Burke et al. 1976; Parmigiani and Stein 1981; Thomas et al. 1999; Zajac and Young 1980; cf. Mela et al. 2001). Thus trains of stimuli that include an initial doublet followed by longer intervals are an effective way to evoke strong forces in many muscles, particularly after chronic paralysis.

The obvious practical use of these data would be to test whether stimulus patterns that maximize force or force-time integral also reduce the excessive fatigue that occurs during the electrical stimulation of paralyzed muscles. In whole control thenar muscles (Bigland-Ritchie et al. 2000) and fatigable cat tibialis posterior motor units (Bevan et al. 1992), less force decline (fatigue) occurred when pulse trains were delivered at

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supramaximal intensity and included an initial doublet (with subsequent pulses delivered at variable interpulse intervals) compared with stimuli at a constant frequency. However, submaximal stimulation of control human quadriceps muscles caused the same, less, or greater force reduction when the pulse trains were repeatedly delivered with variable versus constant interpulse intervals (Binder-Macleod and Barker 1991; Binder-Macleod and Scott 2001). All of these studies have been performed on muscles that are under voluntary control. But, it is in paralyzed muscles in which functional movements need to be restored. Given the known differences in contractile properties of control and chronically paralyzed muscles (and possibly unknown differences), findings from control muscles may not be applicable to paralyzed muscles. Thus it is important to find ways to reduce the rapid and unacceptable force reductions that occur in paralyzed muscles when they are activated by trains of electrical stimuli to generate functional movements. One possibility yet to be tried in paralyzed muscles is to begin the stimulus trains with a doublet.

The main aim of the present study was to compare the fatigue induced in paralyzed thenar muscles by intermittent, supramaximal median nerve stimulation at constant frequencies (40 or 20 Hz) versus variable frequencies (each train included an initial doublet followed by longer interpulse intervals). The force-time integrals produced by the respective high and low constant frequency pulse trains were used to determine the longer interpulse intervals employed in the corresponding variable frequency pulse trains. Pulses at 40 Hz were chosen because this is the frequency most commonly used to test muscle fatigue (Burke et al. 1973). This frequency also evokes close to maximal force in both paralyzed and control thenar muscles (Thomas 1997a,b). In comparison, 20 Hz generates a fairly fused, but weaker contraction in many human muscles. It is also the stimulation frequency, or close to the stimulation frequency, that is commonly used to restore some function in paralyzed muscles (Westling et al. 1997a,b). SCI subjects remained in their wheelchairs. Each able-bodied subject sat in a chair. The test forearm and hand rested on a tray beside the subject. The forearm was stabilized in a vacuum cast while the hand was embedded in Thera-Putty (North Coast Medical, San Jose, CA). A metal plate was strapped across the hand and fingers to immobilize them. The thumb was extended and positioned against a force transducer that registered the isometric abduction and flexion forces at right angles to each other. Each force was amplified (model 2310, Measurements Group, Raleigh, NC), filtered (DC-100 Hz), and sampled on-line (400 Hz) to a computer using a SC/Zoom system (Department of Physiology, University of Umeå, Umeå, Sweden).

Three electrodes made from braided strands of silver-coated, copper wire were used to record electromyographic activity (EMG) from the distal and proximal surfaces of the thenar muscles (Westling et al. 1990). One electrode was positioned over the metacarpal–phalangeal joint of the thumb (distal). Another electrode was placed along the base of the thumb (proximal). Both of these electrodes were referred to a third electrode, which was positioned between them across the eminence of the thenar muscles (common). A ground wire was placed over the wrist crease. The proximal and distal surface EMG signals were amplified (Grass PS11 Amplifiers, Astro-Med, West Warwick, RI), filtered (30–1,000 Hz), and sampled on-line (3,200 Hz).

**Stimulation**

The median nerve was stimulated just proximal to the wrist using a bipolar electrode (cathode distal; TECA 6030–1, Oxford Instruments, Hawthorne, NY) and a constant current stimulator (DSTH, Digitimer Ltd., Hertfordshire, UK) controlled by Fystat software (Dataid, Sweden). The best site for nerve stimulation was determined by delivering low-intensity pulses (50 µs duration) every 2 s at different locations. The position at which the largest thenar muscle EMG was evoked was where the stimulating electrode was taped and held in place by an experimenter for the delivery of all subsequent stimuli. The stimulus intensity was then increased in 1-mA increments until there were no further increases in the amplitude of the compound muscle action potential (M wave) or twitch force. All subsequent stimuli were delivered at an intensity approximately 25% greater than that which evoked a maximal M wave. This stimulus intensity was 14% higher than that which evoked a maximal twitch. On average, the maximal twitch force was reached when the current was 11% higher than that used to evoke a maximal M wave, possibly due to the staircase potentiation in force that occurs when skeletal muscle is stimulated repeatedly at low frequencies (Desmedt and Hainaut 1968). To ensure that these stimuli remained supramaximal, all EMG signals were continuously monitored on an oscilloscope (Tektronix, Wilsonville, OR).

**Experimental protocol**

Four experiments were performed on each subject. There was at least 1 day between each experiment to allow for any long-term fatigue effects to recover. To check the consistency of the setup between experiments, a train of supramaximal stimuli was delivered to the median nerve at 50 Hz for 1 s. Maximal muscle force was measured. The setup for all four experiments was only acceptable if the maximal tetanic forces were within ±10% of each other. When the evoked forces were out of this range, the discrepancy was corrected by making small adjustments in the position of the thumb on the transducer.

Supramaximal stimuli were then delivered in the following order: 1) 5 pulses at 1 Hz to evoke twitches; 2) 2 pulses 5 ms apart, repeated three times at 1-s intervals to assess the force evoked by doublets; 3) 50 Hz for 1 s to evoke maximal force; 4) 13 trains of stimuli, each 300
ms in duration. One train was at 40 Hz, another at 20 Hz, while the other 11 trains contained variable interstimulus intervals and between 4 to 14 pulses (see Matching force time integral); 5) after a 10-min rest, the thenar muscles were fatigued using trains of stimuli (300 ms duration) applied once per second. The pattern of pulses delivered (high or low constant frequencies versus high or low variable frequencies) and the length of the test were dependent on which of the four experimental protocols was used (see Matching force time integral). After fatigue, stimuli included 6) 50 Hz for 1 s to evoke maximal force; 7) 5 pulses at 1 Hz to evoke twitches; and 8) 2 pulses 5 ms apart, repeated three times at 1-s intervals to evoke doublets.

Matching force time integral

The only differences in the fatigue protocol between the four experiments were the pulse train patterns and the duration of stimulation. In the “constant high-frequency” experiment, the thenar muscles were fatigued using trains of 13 pulses at 40 Hz (300-ms train duration) each second for 120 s (Burke et al. 1973). In the “variable high-frequency” experiment, the thenar muscles were fatigued using trains of pulses with variable interstimulus intervals that were unique to each subject (300-ms duration trains each second for 120 s), as described by Bigland-Ritchie et al. (2000). That is, the experimenters selected a variable pulse pattern that would elicit a force-time integral that matched the force-time integral evoked by a 300-ms train at 40 Hz (13 pulses). This was done by delivering a series of 11 different trains of pulses (300-ms duration) of variable interstimulus intervals. The total number of pulses in the different trains ranged from 4 to 14. The trains always started with a doublet (2 pulses, 5 ms apart). The second interpulse interval was always 20 ms. Remaining pulses were evenly distributed in time across the last 275 ms. These pulse patterns were chosen because previous studies have shown that they maximize the force produced by control and paralyzed thenar muscles, as well as by control thenar motor units (Griffin et al. 2002; Thomas et al. 1999).

Figure 1A shows an example of the force records obtained from the paralyzed thenar muscles of one SCI subject. The force from a constant high-frequency train of stimuli (13 pulses at 40 Hz) is overlaid with the force produced by the variable pulse pattern using 12 pulses. This 12-pulse train was chosen to fatigue the thenar muscles of this subject because the force-time integral best matched that produced by the constant high-frequency stimulation. We chose to match the force-time integral so that the initial metabolic work done by the muscles was the same for constant and variable frequency trains. The variable pulse pattern that evoked a force-time integral that matched the force-time integral elicited by the constant high-frequency train of stimuli (300 ms) was determined for each subject in the same way. It was then used in the respective variable high-frequency fatigue test.

The other two experiments, termed “constant low-frequency” and “variable low-frequency,” were conducted in the same manner. In the constant low-frequency experiments, the muscles were fatigued using trains of 7 pulses at 20 Hz (300-ms duration) each second for 240 s. In the variable low-frequency experiments, the variable pulse pattern used in the fatigue protocol was determined by matching the force-time integral produced by the constant low-frequency stimulation. Figure 1B shows an example from the thenar muscles of the same SCI subject. The force-time integral for 7 pulses at 20 Hz was matched by delivering 7 pulses at variable interpulse intervals.

The order of the fatigue protocols performed on each subject was rotated. In variable high-frequency experiments, the mean (± SE) number of pulses used to fatigue the paralyzed muscles was 12 ± 2 (range: 8–14 pulses). For the control group, 10 ± 1 pulses (range: 9–11 pulses) were used. The number of pulses chosen for variable low-frequency experiments was 7 ± 1 pulses (range: 6–10 pulses) for SCI subjects and 6 ± 1 pulses (range: 6–7 pulses) for controls. In paralyzed muscles, the force-time integrals corresponding to the constant high-frequency, variable high-frequency, constant low-frequency, and variable low-frequency pulse trains were 7.2 ± 3.6, 7.2 ± 3.6, 5.3 ± 2.6, and 5.4 ± 2.7 Ns, respectively. The respective data for control muscles were 6.2 ± 3.1, 6.1 ± 3.0, 4.9 ± 2.5, and 5.1 ± 2.6 Ns. There were no significant differences in the force-time integrals of the constant and variable frequency trains (high or low) chosen to fatigue paralyzed and control muscles.

Data analysis

Data analyses were performed off-line using Zoom software. Resultant force (√(abduction force)² + (flexion force)²) measurements included force peak, half-relaxation time (time for the force to fall to 50% of its peak value), and force-time integral (from force onset until the force returned to the baseline, determined from the increase in the differential of the force just after the start of the pulse train and the return of the differential to the baseline at the end of the force relaxation, respectively). When force fusion occurred between successive trains of stimuli, due to the slowing of relaxation that occurred with fatigue, force-time integrals were measured from the force increase due to a train of pulses until the first pulse of the next train. The first five responses and then every fifth response from the fatigue test were measured.

To evaluate the importance of the number of pulses on fatigue, the final values from variable frequency protocols were compared with those measured from the last complete contractions in the corresponding constant frequency protocols that resulted in the delivery of the same numbers of pulses. The forces (relative to the initial force evoked by 50 Hz stimulation) evoked by constant high- or constant low-frequency stimulation were also compared initially and after the delivery of 320, 590, 910, 1,230, and 1,500 pulses; places where the number of pulses that were delivered were very similar.

Force peak, half-relaxation time, and force-time integral were also measured for the twitches, doublets, and 50-Hz responses that were evoked before and after the fatigue protocol. EMG measurements from the proximal portion of the muscles included onset latency, peak-to-peak amplitude, duration of the potential, and the integral of the first two phases defined by isoelectric crossings. EMG was only evaluated for twitch responses before and after the fatigue protocol to evaluate the maximality of the stimulation.

Statistics

Data are expressed as means (±SE). Statistical significance was set at P < 0.05. Force, half-relaxation time, and force-time integral changes during fatigue for both constant and variable, high- and low-frequency stimulation were assessed using two-way ANOVA. Comparisons were made within each subject group over time for each protocol, between the two subject groups over time for each protocol.
and across all protocols over time within and between groups. These same tests were conducted again using the number of pulses delivered rather than time. Nonparametric data were analyzed using two-way ANOVA on ranks. Any differences were evaluated using Dunn’s post hoc tests. Data resulting from the force-time integral matching and the number of pulses chosen for the experiments involving variable frequency stimulation were each analyzed using one-way ANOVA. The pre- and postfatigue maximal force responses evoked by 1 s of 50 Hz stimulation are also shown.

**Results**

Figure 2 shows the typical force decline seen in paralyzed muscles with each of the four fatigue protocols. Notice that more force was produced by the high-frequency stimulation than the low-frequency stimulation at the beginning of the tests. In contrast, the final forces were similar for all four protocols. This was the case with each subject. Thus force declined to a similar level in each test, despite the relative intensity of the initial contractions. The results from the four different fatigue protocols for both the SCI and control groups are summarized in Table 1. Table 2 compares the 50-Hz, doublet, and twitch responses evoked before and after fatigue in both groups.

**SCI group**

In SCI subjects there were significant decreases in force during all four fatigue protocols. This was the case whether force was expressed relative to the force evoked by the first train of stimuli or to the maximal force evoked by 50 Hz

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### Table 1. Fatigue parameters

<table>
<thead>
<tr>
<th></th>
<th>Constant high</th>
<th>Variable high</th>
<th>Constant low</th>
<th>Variable low</th>
<th>Constant high</th>
<th>Variable high</th>
<th>Constant low</th>
<th>Variable low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Force (N) I</td>
<td>20.7 ± 4.5</td>
<td>19.7 ± 4.3</td>
<td>14.8 ± 2.7</td>
<td>12.6 ± 2.4</td>
<td>18.5 ± 2.8</td>
<td>16.9 ± 2.3</td>
<td>13.5 ± 2.9</td>
<td>13.7 ± 3.0</td>
</tr>
<tr>
<td>F</td>
<td>3.9 ± 0.9</td>
<td>3.6 ± 0.7</td>
<td>2.4 ± 0.4</td>
<td>2.7 ± 0.5</td>
<td>6.9 ± 0.7</td>
<td>8.4 ± 0.7</td>
<td>7.7 ± 1.5</td>
<td>7.1 ± 1.2</td>
</tr>
<tr>
<td>%I</td>
<td>24.1 ± 5.5*</td>
<td>22.7 ± 4.0*</td>
<td>20.7 ± 3.9*</td>
<td>24.8 ± 4.3*</td>
<td>40.2 ± 6.8*</td>
<td>52.2 ± 5.7*</td>
<td>60.3 ± 9.5*</td>
<td>54.6 ± 7.4*</td>
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<tr>
<td>Force (% 50 Hz) I</td>
<td>88.8 ± 4.6</td>
<td>82.5 ± 3.7</td>
<td>67.3 ± 5.1</td>
<td>55.7 ± 5.7</td>
<td>77.6 ± 2.4</td>
<td>68.4 ± 6.0</td>
<td>53.6 ± 3.3</td>
<td>53.7 ± 2.8</td>
</tr>
<tr>
<td>F</td>
<td>21.4 ± 4.9*</td>
<td>18.3 ± 3.1*</td>
<td>14.5 ± 3.7*</td>
<td>13.1 ± 2.5*</td>
<td>30.6 ± 4.2*</td>
<td>36.0 ± 5.3*</td>
<td>31.3 ± 3.8*</td>
<td>28.9 ± 3.1*</td>
</tr>
<tr>
<td>HRT (ms) I</td>
<td>107.2 ± 16.3</td>
<td>85.0 ± 10.1</td>
<td>87.9 ± 16.8</td>
<td>89.5 ± 16.5</td>
<td>91.3 ± 9.2</td>
<td>92.3 ± 9.1</td>
<td>99.3 ± 17.7</td>
<td>81.5 ± 4.8</td>
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<tr>
<td>F</td>
<td>493.1 ± 64.8</td>
<td>536.4 ± 61.1</td>
<td>313.0 ± 41.4</td>
<td>378.0 ± 68.9</td>
<td>307.5 ± 23.0</td>
<td>216.0 ± 33.4</td>
<td>163.0 ± 28.8</td>
<td>135.7 ± 7.8</td>
</tr>
<tr>
<td>%I</td>
<td>585.1 ± 113.6*</td>
<td>730.9 ± 110.3*</td>
<td>386.6 ± 33.4*</td>
<td>557.3 ± 115.5*</td>
<td>349.8 ± 44.0</td>
<td>240.5 ± 38.3</td>
<td>165.9 ± 11.6</td>
<td>169.0 ± 14.2</td>
</tr>
<tr>
<td>FTI (Ns) I</td>
<td>6.9 ± 1.7</td>
<td>6.5 ± 1.5</td>
<td>4.4 ± 0.8</td>
<td>4.8 ± 0.9</td>
<td>5.9 ± 0.9</td>
<td>5.4 ± 0.9</td>
<td>4.1 ± 0.8</td>
<td>4.7 ± 1.1</td>
</tr>
<tr>
<td>F</td>
<td>3.2 ± 0.7</td>
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<td>1.5 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>4.5 ± 0.7</td>
<td>4.1 ± 0.4</td>
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<td>3.1 ± 0.6</td>
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<tr>
<td>%I</td>
<td>57.9 ± 9.3*</td>
<td>55.9 ± 8.5*</td>
<td>42.8 ± 7.0*</td>
<td>43.3 ± 4.8*</td>
<td>81.8 ± 15.2</td>
<td>79.2 ± 9.9</td>
<td>77.5 ± 11.3</td>
<td>68.4 ± 8.5</td>
</tr>
</tbody>
</table>

Values are mean ± SE for force peak (absolute and % 50 Hz force), time to half relaxation, and force-time integral. HRT, time to half relaxation; FTI, force-time integral; I, initial; F, final. * Significant change from initial to final values for each fatigue protocol, also expressed as a percentage of initial. † Significantly different from control at t s; Note there were no differences for any of these data between constant and variable frequency stimulation protocols, P < 0.05.
TABLE 2. Pre- and postfatigue parameters

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<tr>
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<th>Control</th>
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<tr>
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<td>Variable high</td>
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<tr>
<td></td>
<td>Force (N)</td>
<td>Force (N)</td>
</tr>
<tr>
<td>I</td>
<td>24.0 ± 5.1</td>
<td>24.2 ± 5.1</td>
</tr>
<tr>
<td>F</td>
<td>7.6 ± 2.6</td>
<td>5.5 ± 1.3</td>
</tr>
<tr>
<td>%I</td>
<td>34.0 ± 6.7*</td>
<td>25.5 ± 3.6*</td>
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<tr>
<td>HRT (ms)</td>
<td>115.9 ± 8.4</td>
<td>112.1 ± 7.9</td>
</tr>
<tr>
<td>F</td>
<td>356.8 ± 37.1</td>
<td>378.1 ± 40.2</td>
</tr>
<tr>
<td>%I</td>
<td>319.7 ± 36.9*</td>
<td>362.4 ± 50.1*</td>
</tr>
<tr>
<td>FTI (Ns)</td>
<td>23.3 ± 4.9</td>
<td>24.1 ± 5.5</td>
</tr>
<tr>
<td>F</td>
<td>7.6 ± 2.0</td>
<td>6.4 ± 1.2</td>
</tr>
<tr>
<td>%I</td>
<td>38.8 ± 7.4*</td>
<td>31.3 ± 3.6*</td>
</tr>
<tr>
<td>Doublet Force (N)</td>
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<td></td>
</tr>
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<td>I</td>
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<td>8.0 ± 1.4</td>
</tr>
<tr>
<td>F</td>
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<td>2.3 ± 0.7</td>
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<tr>
<td>%I</td>
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<td>29.6 ± 5.8*</td>
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<td>HRT (ms)</td>
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<td>69.7 ± 9.4</td>
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<td>198.1 ± 31.5</td>
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<tr>
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<td>320.3 ± 65.5*</td>
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<tr>
<td>%I</td>
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<td>57.0 ± 9.8*</td>
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<td>2.9 ± 0.5</td>
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<tr>
<td>F</td>
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<td>1.0 ± 0.2</td>
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<tr>
<td>%I</td>
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<td>60.4 ± 6.0</td>
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<td>F</td>
<td>225.9 ± 39.9</td>
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<tr>
<td>%I</td>
<td>394.5 ± 65.7*</td>
<td>419.6 ± 73.7*</td>
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<tr>
<td>FTI (Ns)</td>
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<tr>
<td>%I</td>
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<tr>
<td>I</td>
<td>5.2 ± 0.8</td>
<td>6.0 ± 1.3</td>
</tr>
<tr>
<td>F</td>
<td>5.7 ± 0.9</td>
<td>6.8 ± 1.7</td>
</tr>
<tr>
<td>%I</td>
<td>112.0 ± 10.6</td>
<td>112.9 ± 9.4</td>
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<tr>
<td>EMG area (µV · s)</td>
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<td></td>
</tr>
<tr>
<td>I</td>
<td>15.3 ± 2.8</td>
<td>16.7 ± 4.1</td>
</tr>
<tr>
<td>F</td>
<td>22.4 ± 4.1</td>
<td>22.7 ± 6.2</td>
</tr>
</tbody>
</table>

Values are mean ± SE of force peak (absolute and % 50 Hz force), time to half relaxation, and force-time integral for 50 Hz, doublet, and twitch data. EMG amplitude and area are shown for twitch responses. HRT, time to half relaxation; FTI, force-time integral; EMG, electromyographic; amp, amplitude; I, initial; F, final. * Significant change from initial prefatigue values to final postfatigue values, also expressed as a percentage of initial prefatigue values; † Significantly different from control postfatigue data. P < 0.05.

stimulation (Figs. 3 and 4, Table 1). In addition, the force decline was similar whether a constant or variable pulse pattern was delivered at either a high or low frequency. Each fatigue protocol also induced significant decreases in the mean 50-Hz, doublet, and twitch forces, which again were similar irrespective of the pattern or frequency used to induce fatigue (Table 2).

This muscle fatigue was always accompanied by significant increases in half-relaxation time (Fig. 5, Table 1). The half-relaxation time of the postfatigue 50-Hz, Twitches, and doublets also increased significantly after all four protocols (Table 2). Force-time integrals decreased significantly during each fatigability test for paralyzed muscles (Fig. 6). Postfatigue 50-Hz and doublet force-time integrals also decreased with all four protocols. There was no significant change in the force-time integral of the postfatigue Twitches.

Control group

In control muscles there were significant decreases in force during each fatigue test, both when expressed relative to the force produced by the first train of stimuli and to the maximal
force evoked by 50 Hz stimulation (Figs. 3 and 4). As for SCI subjects, the amount of force loss was similar whether constant or variable pulse patterns were used at either high or low frequencies. However, the relative reductions in force were significantly different between the two groups. The muscles of control subjects fatigued significantly less in three of the four protocols. Only when the muscles were stimulated with pulses at a constant high frequency were comparable force declines seen in paralyzed and control muscles.

When the postfatigue responses were examined in the control group, significant decreases in force occurred only in certain cases. For example, there was a significant decline in 50-Hz force in each protocol except after delivery of variable low-frequency stimulation (Table 2). With doubles, force loss was significant after both high-frequency experiments, whereas, with twitches, the force decline was only significant after the constant high-frequency stimulation. But again, control muscles fatigued significantly less than paralyzed muscles when 50-Hz, doublet, and twitch forces were compared, except for constant high-frequency stimulation (Table 2).

There were no significant changes in half-relaxation time during any of the fatigue protocols for control muscles (Fig. 5). However, there was significant slowing of 50-Hz, doublet, and twitch half-relaxation time after each protocol except for the twitches after variable high-frequency stimulation (Table 2).

The final force-time integral produced by control muscles was not significantly different in any of the fatigue protocols (Fig. 6). Any changes in the force-time integrals due to force decline were presumably counteracted by the slowing of the force relaxation. The only significant changes in force-time integrals after fatigue occurred after constant high-frequency stimulation for the 50-Hz and twitch responses. All the other 50-Hz, doublet, and twitch force-time integrals after fatigue were not significant. Thus, in contrast to paralyzed muscles, slowing of relaxation and changes in force-time integral were less marked in control thenar muscles.

Across protocols

Low-frequency stimulation was delivered for 4 min whereas high-frequency stimulation was stopped after 2 min. Fatigue at the same point in time was therefore compared for constant

![Fig. 3](image-url) Mean (±SE) force data across the 4 fatigue tests as a percentage of the initial force response. Data are shown for the first 5 s and then every fifth second throughout the tests involving constant high-frequency stimulation (A), variable high-frequency stimulation (B), constant low-frequency stimulation (C), and variable low-frequency stimulation (D) of paralyzed (●) and control (○) muscles. Dashed lines show the initial force.

![Fig. 4](image-url) Mean (±SE) force data across the 4 fatigue tests expressed relative to the initial maximal force evoked by 1 s of 50 Hz stimulation. Data represent the first 5 s and then every fifth second throughout the tests involving constant high-frequency stimulation (A), variable high-frequency stimulation (B), constant low-frequency stimulation (C), and variable low-frequency stimulation (D) of paralyzed (●) and control (○) muscles.
high- and low-frequency stimulation. After 2 min of constant low-frequency stimulation there was significantly less force decline (% initial) compared with the force decline at 2 min of constant high-frequency stimulation for control, but not paralyzed muscles. When we compared the force during fatigue relative to the maximal force (% 50 Hz) at the 2-min time point, the differences were not significant even though the peak forces evoked were somewhat higher at this time with constant low-frequency stimulation than with high-frequency stimulation for both paralyzed (24.1 ± 4.4 vs. 21.4 ± 4.9% 50 Hz) and control muscles (37.7 ± 3.5 vs. 30.6 ± 4.2% 50 Hz). This is despite the fact that initial forces were higher in constant high-versus low-frequency stimulation for both paralyzed (88.8 ± 14.6 vs. 67.3 ± 16.1% 50 Hz) and control muscles (77.6 ± 5.4 vs. 53.6 ± 7.4% 50 Hz). In addition, peak force produced by 50 Hz and doublet stimulation in control muscles was actually less after 2 min of constant high-frequency stimulation than after 4 min of low-frequency stimulation (Table 2). Variable frequency protocols were not compared in this way because the numbers of pulses that were delivered varied across subjects.

**Force expressed according to number of pulses delivered**

Compared with the constant high-frequency protocol, the variable high-frequency stimulation protocol delivered 1 less pulse per train for SCI subjects and 3 fewer pulses per train for control subjects (on average, 120 and 360 less pulses delivered over 2 min, respectively). For the low-frequency stimulation, the constant and variable frequency trains delivered the same number of pulses for SCI subjects, on average. One less pulse per train was delivered with the variable versus constant frequency stimulation for control subjects (i.e., 240 less pulses delivered over 4 min in control muscles). When the force (% initial and % 50 Hz), half-relaxation time and force-time integral data were compared after the delivery of the same number of pulses, there were no significant differences in any of these parameters for either high- or low-frequency stimulation of control and paralyzed muscles. Thus inclusion of a doublet in the train did not change the fatigue effects.

Figure 7 compares the force decline (% initial 50-Hz force) that occurred with constant high- or low-frequency stimulation in relation to the number of pulses that were delivered to

![Figure 5](image_url)  
*(Mean ± SE) half-relaxation time data expressed relative to the initial response for constant high-frequency stimulation (A), variable high-frequency stimulation (B), constant low-frequency stimulation (C), and variable low-frequency stimulation (D) of paralyzed (●) and control (○) muscles.*

![Figure 6](image_url)  
*(Mean ± SE) force-time integral data as a percentage of the initial response evoked by constant high-frequency stimulation (A), variable high-frequency stimulation (B), constant low-frequency stimulation (C), and variable low-frequency stimulation (D) of paralyzed (●) and control (○) muscles.*
paralyzed (Fig. 7A) and control muscles (Fig. 7B). As expected, constant high-frequency stimulation initially evoked significantly more force than constant low-frequency stimulation in both types of muscle. This force difference remained significant after the delivery of 590 pulses to paralyzed muscles (after 320 pulses, \( P = 0.06 \)), as well as after 320 and 590 pulses had been applied to control muscles. After 910, 1,235, and 1,500 pulses had been delivered to each kind of muscle, similar forces were evoked with both 40 and 20 Hz stimulation.

**EMG**

After fatigue of both paralyzed and control muscles, the amplitude and the area of the EMG potentials were maintained at prefatigue values or increased. EMG amplitude increased significantly only after variable low-frequency stimulation in the SCI group and constant high-frequency stimulation in the control group. The areas of the EMG potentials increased significantly in paralyzed muscles with each of the four fatigue protocols. In contrast, the EMG area in control muscles was well maintained. All these data indicate that the stimuli were still supramaximal postfatigue and thus continued to evoke contractions of all the thenar muscles. These results also suggest that the fatigue was within the muscle and not at the neuromuscular junction.

**DISCUSSION**

These data show that fatigue of paralyzed and control thenar muscles was not reduced when contractions were induced with trains of supramaximal stimuli at variable versus constant frequency. This was true for both high- and low-frequency stimulation and for all force parameters measured. Thus beginning the pulse trains with a doublet had little influence on thenar muscle fatigability either in control subjects or in SCI subjects with chronic muscle paralysis.

**Variable versus constant frequency stimulation**

To maximize evoked muscle force many studies have utilized the “catch-like” property of muscles. This is when the maximal isometric force of an unfatigued muscle is produced by a pair of closely spaced stimuli (approximately 5-ms apart, termed a doublet) followed by lower subtetanic frequency stimulation (e.g., Burke et al. 1976; Parmiggiani and Stein 1981; Thomas et al. 1999; Zajac and Young 1980). An initial doublet is particularly beneficial after chronic muscle paralysis (Griffin et al. 2002). It is also effective during dynamic contractions (Lee and Binder-Macleod 2000; Sandercoc and Heckman 1997). One reason suggested for this doublet-force enhancement is increased calcium release from the sarcoplasmic reticulum (Duchateau and Hainaut 1986a,b). Another explanation relates to the delivery of a second pulse to a stiffer muscle because the “slack” in the muscle has been taken up as a result of the contraction evoked by the first pulse (Hill 1949, 1953).

Use of the catch-like property of muscle has also been applied to studies of fatigue (Bevan et al. 1992; Bigland-Ritchie et al. 2000; Binder-Macleod and Barker 1991; Binder-Macleod and Scott 2001). In some studies, trains of stimuli with or without doublets have only been delivered pre- and postfatigue. In general, the inclusion of a doublet at the start of a train of stimuli also enhanced the force produced in fatigued muscles compared with that produced by constant frequency trains of stimuli. Other studies have questioned whether repetitive activation of muscles with variable frequency stimulation alters muscle fatigue. Some conflict in the results exists. Reports suggest that variable frequency trains cause similar, more, or less fatigue in control human muscles or cat motor units (Bevan et al. 1992; Bigland-Ritchie et al. 2000; Binder-Macleod and Barker 1991; Binder-Macleod and Scott 2001). The present results from control thenar muscles are similar to those from Bigland-Ritchie et al. (2000) in that variable high-frequency stimulation tended to induce less fatigue than constant high-frequency stimulation. However, the differences in the current study were not significant, possibly because fewer control subjects were studied. Furthermore, constant high-frequency stimulation induced more force decline than variable high-frequency stimulation in all but one control subject in the present study. In contrast, constant high-frequency stimulation always caused more fatigue than variable high-frequency stimulation in every subject in our previous study (Bigland-Ritchie et al. 2000).

Most of these earlier studies have been performed in muscles that are under voluntary control (cf. Karu et al. 1995). In the current study we tested both chronically paralyzed and control thenar muscles because their contractile properties differ (Thomas 1997a,b). In the present study, control thenar muscles showed significantly more force decline after 2 min of constant high- versus low-frequency stimulation. This was not the case in paralyzed thenar muscles. Such differences highlight the potential difficulties in transferring information obtained from stimulating control muscles to functional electrical stimulation of paralyzed muscles. The present data also confirmed that muscles paralyzed by SCI are much more fatigable than control muscles (Lenman et al. 1989; Shields 1995; Stein et al. 1992; Thomas 1997b). Our data also show that the exaggerated fatigue of paralyzed thenar muscles was not altered by includ-
ing a doublet at the beginning of pulse trains delivered at high or low frequencies. Moreover, the stimulation was always supramaximal. Therefore the fatigue cannot be explained by nerve stimulation activating the more fatigable motor units. The results from the present study thus support other evidence that suggests the excessive fatigability of paralyzed muscles relates to changes in fiber type composition, muscle metabolism, blood flow, use, or some combination of these factors (Butler et al. 2001; Castro et al. 1999; Gerrits et al. 2000; Grimby et al. 1976; Martin et al. 1992; Mohr et al. 1997).

Another problem with many earlier studies on control muscles has been the use of submaximal stimulation intensities (cf. Bigland-Ritchie et al. 2000). It is difficult to compare forces both within and across protocols with submaximal muscle activation because there are activity-dependent changes in axonal excitability that are frequency dependent. The stimulus current required to activate any axon will increase with activity, regardless of the duration used. Thus it is almost impossible to be certain that submaximal stimuli continue to excite the same portion of the muscle, particularly when the stimulation frequency is varied (see Kiernan et al. 2000; Vagg et al. 1998). Muscle sarcolemmal changes during fatigue also affect the size of the compound muscle action potential (Cupido et al. 1996; Rabischong et al. 1995). Unless there is concurrent evaluation of the submaximal and supramaximal EMG potentials during a fatigue protocol, the stability of muscle activation is questionable. In our study, we have used supramaximal stimulation of the median nerve in both paralyzed and control muscles to avoid these issues. The compound muscle action potentials were monitored and maintained throughout the stimulation protocols. As a result, it is very likely that all of the thenar motor units were activated during each stimulus train. The changes in muscle force production were therefore due entirely to processes within the muscle.

Matching force-time integral

In this experiment we chose the variable frequency pulse patterns by matching their force-time integrals to the force-time integrals produced by the constant high- or low-frequency stimulation trains. We attempted to subject the muscles to the same initial work and thus place the same metabolic demands on the muscles. This resulted in lower absolute forces (%50 Hz) for the variable frequency trains compared with the constant frequency trains (Fig. 4, Table 1). While this process may have underestimated the fatigue produced by variable frequency stimulation, even greater fatigue may have been seen using these trains of stimuli at variable frequencies if we had matched peak force.

Fatigue in relation to the number of pulses delivered

The number of pulses delivered to a muscle to activate the contractile machinery may be a limiting factor in terms of muscle force production (Garland et al. 1988; Marsden et al. 1983). On average, the variable frequency trains required larger numbers of pulses per train for the SCI subjects than for the control subjects. This may relate to the faster muscle twitch properties of paralyzed muscles, resulting in less fusion for a given frequency of stimulation than seen in control muscles. However, there were fewer pulses in the variable versus constant frequency trains for each subject group. Despite these differences, our data suggest that the number of pulses delivered is not critical to the induced fatigue. After the same number of pulses had been delivered at low or high, constant or variable frequencies, there were no differences in any of the force parameters measured from either paralyzed or control muscles. Rather, fatigue seems more related to the magnitude of the force produced during the contractions.

When the force decline resulting from high and low constant frequency was compared (Fig. 7), fatigue also depended on the strength of the contractions. For constant frequency stimulation (but not variable frequency stimulation), contraction strength also relates to the frequency at which the stimulus pulses are delivered to the muscle.

High- versus low-frequency stimulation

As expected, stronger forces were produced initially with high- versus low-frequency stimulation. But the force declined to a similar level in every fatigue test even though the initial relative contraction intensity, and thus probably metabolic demand, was greater with high-frequency stimulation at constant or variable rates. Moreover, after the initial decline in force seen in the high-frequency protocols, the same force could be achieved whether high or low, constant or variable stimulation frequencies were used. This probably related to the greater slowing of muscle contractile properties that occurred with high-frequency stimulation and, hence, changes in force fusion with fatigue. As a result, there may be little benefit in driving paralyzed muscles at high frequencies. If a muscle is strong enough, many tasks may be able to be performed using reasonably low stimulation frequencies.

Functional implications

Although doublets are clearly useful for enhancing muscle force, particularly in paralyzed muscles (Griffin et al. 2002), the inclusion of a doublet at the beginning of the pulse trains used in this study did not reduce the fatigability of either paralyzed or control thaner muscles. Hence, the longstanding assumption that this kind of variation in pulse pattern will markedly reduce fatigue of paralyzed muscles may be unfounded. If we are to improve the function of paralyzed muscles, future studies must evaluate other factors that may contribute to muscle fatigue, such as alterations in muscle use, metabolism, and blood flow.

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REFERENCES


Optimal stimulation of paralyzed muscle


