Use of motor cortex stimulation to measure simultaneously the changes in dynamic muscle properties and voluntary activation in human muscles

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IN HUMANS, THE CONTRACTILE properties of muscle are usually assessed by measurement of the resting twitch evoked by a supramaximal stimulus to a muscle's motor nerve. The stimulus is delivered within seconds after a voluntary contraction when the muscle is still potentiated by prior activity (e.g., Refs. 5, 43). The characteristics of the resting twitch provide information about the speed of muscle contraction and relaxation and about the force output from the muscle. However, a major limitation of this technique is that it only reveals the state of the muscle at rest and not during voluntary contractions when muscle properties can be changing rapidly. For example, the potentiating and twitch dynamics in contracting muscle are likely to differ from relaxation conditioned by prior activity. To overcome this limitation, we recently proposed a new technique whereby changes in the contractile properties of muscle can be measured during voluntary contractions with the use of transcranial magnetic stimulation to the motor cortex (TMS) (53). The EMG and force responses to this stimulus provide information about motor cortical excitability (Refs. 13, 25, 56; for review, see Ref. 41), the strength of intracortical inhibition (30, 39), and voluntary activation of muscle (54). We have shown that the force output from muscle (54, 55) and the peak relaxation rate of muscle fibers (53) could also be measured at the same instant that we test the level of supraspinal drive to the muscle. This is particularly relevant for investigating the mechanisms underlying muscle fatigue when muscle properties and neural control change simultaneously.

The term muscle fatigue describes any exercise-induced reduction in the ability to produce force or power (20). It is accompanied by short- and long-lasting effects on the motor pathway, from supraspinal structures to the muscle itself. Much of the decline in force arises from processes occurring within the muscle (for review, see Ref. 16). During sustained high-intensity contractions, the concomitant elevation in intramuscular pressure can restrict blood flow and limit energy supply and clearance of metabolic by-products (4, 26, 27). These processes disrupt excitation-contraction coupling (Refs. 1, 49; for review, see Ref. 16) and reduce force output and short-twitch contractile properties. These changes occur in conjunction with a decline in motor unit firing rates (6, 8). It has been proposed that the decline in firing rates during sustained high-intensity contractions could protect against conduction failure and optimize force output by reducing firing frequency as contractile speed slows (known as the “muscle wisdom hypothesis”; for review, see Refs. 15, 33). However, motor unit firing rates drop too low to maintain full fusion of force (e.g., Ref. 17), thus demonstrating a central component of muscle fatigue.

In the present study, we aimed to show that measurement of the contractile properties of muscle using TMS during voluntary contractions is reproducible between days and that the technique can be used to demonstrate changes in contractile properties when muscle is fatigued or heated. Whereas fatigue slows twitch contractile properties, the relaxation rate of muscle is faster at higher muscle temperatures (e.g., Refs. 11, 12, 40, 57). The technique was then applied during a sustained hypothermia
fatiguing voluntary contraction to determine the time course of changes in muscle properties with the development of fatigue. It also allowed comparison with changes in voluntary activation and responses to cortical stimulation. Some of these data have been published previously in a different form (53). Our combined method furthers understanding of the relationship between muscle properties and voluntary drive during exercise.

METHODS

Two studies were performed to assess voluntary activation and the contractile properties of muscle in fresh, heated, and fatigued elbow flexor muscles. Eleven subjects (36 ± 10 yr; 4 men, 7 women) took part in one or both studies. In all experiments, subjects sat with the dominant arm held firmly at the wrist in an isometric myograph that measured elbow flexion torque. Subjects were positioned with the shoulder and elbow flexed at 90° with the forearm vertical and fully supinated. All experimental procedures were approved by the institutional ethics committee and conducted according to the Declaration of Helsinki. Written informed consent was obtained from the subjects.

Force and EMG Recordings

Isometric elbow flexion torque was measured using a myograph (2) built around a linear strain gauge (Xtran, Melbourne, Australia). EMG activity was recorded with surface electrodes (Ag-AgCl, 10-mm diameter) over the muscle belly and tendon of the biceps brachii and triceps brachii muscles. The interelectrode distance was ~5 cm. Surface EMG signals were amplified (×100–300), filtered (16–1,000 Hz), and sampled (2,000 Hz) for later analysis using a data acquisition system (CED 1401 interface with Signal and Spike software, Cambridge Electronic Design, Cambridge, UK).

Stimulation

Three forms of stimulation were used. These included stimulation of the brachial plexus, stimulation of intramuscular nerve fibers of the biceps and brachialis muscles (“motor nerve” stimulation), and TMS.

Stimulation of the brachial plexus. Single electrical stimuli of 100-μs duration were delivered to the brachial plexus via a cathode in the supraclavicular fossa (Erb’s point) and an anode on the acromion (model DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK). Ag-AgCl electrodes with a 10-mm diameter were used. Stimulation intensity (90–240 mA) was 50% above that required to produce a resting maximal compound muscle action potential (Mmax) in biceps and triceps muscles. On average, the peak-to-peak amplitude of the resting Mmax was 13.7 ± 7.5 mV for biceps and 8.8 ± 3.0 mV for triceps.

Stimulation of the biceps and brachialis motor nerve branches of the musculocutaneous nerve. Electrical stimuli of 100-μs duration were delivered (model DS7AH, Digitimer) to intramuscular nerve fibers innervating biceps and brachialis via a surface cathode located midway between the anterior edge of the deltoid and the elbow crease with the elbow flexed at 90° and an anode over the bicipital tendon (2–3 cm proximal to the elbow). Ag-AgCl electrodes with a 10-mm diameter were used. The stimulation intensity (110–418 mA) was set 10% above the level required to produce a resting twitch of maximum amplitude.

TMS of the motor cortex. A circular coil (13.5-cm outside diameter) positioned over the vertex elicited motor evoked potentials (MEPs) recorded from biceps and triceps (Magstim 200, Magstim, Dyfed, UK). The direction of current flow in the coil preferentially activated the left motor cortex. The stimulator output (50–80% of maximum) produced a large MEP in biceps (minimum amplitude 50–60% of Mmax) during brief maximal voluntary contractions (MVCs) of the elbow flexor muscles and only a small MEP in triceps (amplitudes <10–15% of Mmax).

Thermometry

Core body temperature was measured with a thermocouple inserted transnasally into the esophagus to a depth approximating the position of the right atrium (type T, Physitemp Instruments, Clifton, N.J.). Depth was estimated with the formula: depth (cm) = 0.479 × sitting height (cm) − 4.44 cm (35). Core temperature was recorded continuously at 10 Hz (Thermalert model TH-8, Physitemp Instruments) except when subjects were immersed in a water bath. During this time, core temperature was recorded every 2 min. In two subjects, the temperature of the biceps and brachialis muscle was measured with a thermocouple microprobe inserted into the muscle to a depth of ~3 cm (type IT-21, Physitemp Instruments).

Protocol

Muscle and central nervous system parameters were assessed during brief and sustained contractions of the elbow flexors in two studies (each containing 2 parts). The actual timing of contractions and stimulation are shown in separate panels in Fig. 1.

Study 1. The purpose of the first study was to demonstrate that muscle contractile properties can be measured during voluntary contractions and to validate the technique in two muscle states: fresh and fatigued. Two experiments were performed on separate days.

EXPERIMENT 1. Subjects (n = 6; 3 men, 3 women) performed three brief (~2-s duration) control MVCs. The peak torque of each contraction was measured, and two submaximal target torques of 50 and 75% of the mean of these peak torques was displayed on a visual feedback device. Subjects then performed 22 pairs of contractions in random order, with ≥2 min of rest between pairs to avoid fatigue. Each pair involved a brief MVC (~2 s) followed 8 s later by a brief submaximal contraction of the same duration (Fig. 1A). During each pair, stimulation of the motor cortex, brachial plexus, or motor nerve was delivered, and the torque and EMG responses were recorded. When motor nerve stimulation was delivered during contractions, an additional motor nerve stimulus was delivered with the muscle at rest between the two contractions (4 s after completing the MVC). Eight pairs of contractions were performed for motor cortex and motor nerve stimulation (4 pairs for each of the 2 submaximal target torques). In the remaining six pairs of contractions, brachial plexus stimulation was performed (3 pairs per submaximal target force).

EXPERIMENT 2. The same subjects performed pairs of contractions of the fatigued elbow flexor muscles. This experiment began in a similar way with subjects performing three brief control MVCs of the unfatigued elbow flexors. Subjects then performed 39 pairs of contractions of the fatigued elbow flexors, with minimal rest between pairs to maintain fatigue. Each pair was performed in pseudorandom order and consisted of a sustained MVC followed 8 s later by a brief maximal or submaximal test contraction (~2 s; Fig. 1B). The sustained MVC was used to fatigue the muscle, and it was maintained until torque had decreased to 60% of the maximal torque without fatigue. The submaximal target torques were set at 50 and 75% of the fatigued maximal torque (i.e., 30% and 45% of the MVC torque without fatigue). Motor cortex, brachial plexus, or motor nerve stimulation was delivered during each test contraction. Simulation of the motor nerve was also delivered at rest, 4 s after the sustained MVC. Fifteen pairs of contractions were performed for motor cortex and motor nerve stimulation with five pairs performed for each of the three sizes of brief test contractions. Nine pairs of contractions were performed with brachial plexus stimulation (3 pairs for each test contraction).

Study 2. The purpose of the second study was threefold. First, it assessed the reproducibility and variability of muscle contractile properties measured during voluntary contractions in fresh muscle. Second, it further validated the technique by measurement of muscle contractile properties in another muscle state: heated muscle. Third, it applied the technique to study alterations in the contractile properties of muscle and central nervous system parameters during a sustained
MVC. The last two objectives involved reanalysis of some data collected by Todd and colleagues (53). Two experiments were performed on separate days.

EXPERIMENT 1. Muscle contractile properties were assessed during brief contractions when core body temperature was normal and elevated to 38.5°C. Subjects (n = 7; 2 men, 5 women) performed six sets of brief contractions with superimposed brachial plexus or motor cortex stimulation. The contraction sets involved a MVC followed by a 50 and 75% MVC (Fig. 1C). The contractions were separated by 6 s rest, and the target contractions were calculated from the preceding MVC. Three MVCs were also performed with motor nerve stimulation delivered at rest, 4 s after the MVC. The contraction sets and three single MVCs were performed in a sequence and separated by at least 1 min to avoid fatigue. Subjects then entered a bath containing water of 39.5°C to passively elevate body temperature (Modena 1800, Decina Bathroomware, Carole Park, Australia). This procedure has been described in detail (53). Subjects remained in the bath until core body temperature reached 39.5°C. Once the target body temperature was reached, subjects returned to the testing apparatus. Maximality of the brachial plexus and motor nerve stimulation was checked at rest. Subjects then performed four sets of brief contractions and two single MVCs. Two subjects repeated this experiment for measurement of muscle temperature at intervals before and after immersion in the bath.

EXPERIMENT 2. This experiment began in a similar way to the first experiment with subjects performing six sets of brief control contractions. Subjects then performed a 2-min sustained MVC during which a series of stimuli were delivered (Fig. 1D). Motor cortex stimuli were delivered at 2, 25, 55, 85, and 110 s, and brachial plexus stimuli were delivered at 20, 50, 80, and 105 s. A supramaximal motor nerve stimulus was also delivered at rest, 5 s after completion of the sustained MVC.

Data Analysis

During contractions in which motor cortex or brachial plexus stimulation was delivered, the areas of MEPs and $M_{\text{max}}$ (Figs. 2A and 3, A and B) were measured between set cursors for the biceps and triceps muscles. The cursors were placed at the beginning and end of the evoked potential with the longest duration. To account for activity-dependent changes in the muscle fiber action potential, the area of each MEP was normalized to the area of $M_{\text{max}}$ elicited during nearby contractions of the same strength (e.g., Refs. 21, 52). The contractile properties of muscle were assessed by measurement of the torque responses evoked by motor nerve stimulation at rest and motor cortex stimulation during voluntary contractions (Figs. 2B and 3, C and D). The time to peak amplitude of twitch was taken as the interval from the onset of the twitch to the peak amplitude. The half relaxation time of the resting twitch was taken as the interval between the peak amplitude and the point at which torque was reduced by 50%. The relaxation rate of muscle was calculated during contractions by measurement of the steepest rate of decline of torque in the silent period following motor cortex stimulation (Figs. 2B and 3C). The peak rate of decline was then normalized to the total torque (voluntary plus evoked) before the silent period. This value reflects the relative peak relaxation rate of all muscles that contribute to the measured flexor force (voluntary plus evoked) and that are suppressed by the inhibitory effects of the magnetic stimulus. The duration of the silent period was measured by cursor and was taken as the interval from the stimulus to the return of continuous EMG. Voluntary torque was measured by calculation of the mean torque over a 100-ms period immediately before the
stimulus. For each subject, voluntary torque was normalized to the largest MVC recorded on the day during brief control contractions.

The amplitude of the resting twitch evoked by motor cortex stimulation was estimated rather than measured directly because motor cortical and spinal cord excitability increase with activity (e.g., Refs. 13, 25, 56; for review, see Ref. 41). For each subject, estimation involved linear regression between voluntary torque and the amplitude of the superimposed twitch evoked by motor cortex stimulation. Linear regression was performed on data collected during 50, 75, and 100% MVCs. One regression was performed for each muscle state for each subject. The y-intercept was taken as the estimated amplitude of the resting twitch evoked by motor cortex stimulation (54, 55). The reliability of this method has been established (55).

In the text and tables, group data are presented as the mean and SD, whereas in the figures, mean and SE are shown. Statistical analysis involved two-way repeated-measures ANOVA for comparisons between contraction strength and muscle state during brief contractions. Nonparametric data were transformed to ranks, and a two-way repeated-measures ANOVA on ranks was performed. One-way repeated-measures ANOVA or one-way repeated-measures ANOVA on ranks was used for comparison between brief control MVCs and time points during the sustained MVC. Student’s paired t-tests were performed to compare body temperature, amplitude of the resting twitch, half relaxation time of the resting twitch, average time to peak amplitude of the superimposed twitch, and the average peak relaxation rate of muscle between conditions. Post hoc discrimination between means was made with the Student-Newman-Keuls procedure. To assess variability, coefficients of variation (SD/mean × 100; in %) were calculated for each subject. The reproducibility between sessions was quantified with the intraclass correlation coefficient (ICC2,1) based on a repeated-measures ANOVA with session as the independent variable (45). Statistical significance was set at $P < 0.05$.

RESULTS

Torque and EMG responses to stimulation of the motor pathway were measured in two studies during brief and sustained contractions in fresh, heated, and fatigued elbow flexor muscles. We aimed to compare measures of contractile speed produced by nerve and cortical stimulation and to assess these measures in each of the three muscle conditions.

Study 1

Experiment 1: brief contractions in fresh muscle. Using the protocol shown in Fig. 1A, the average duration of the cortically evoked silent period ranged between 122 ± 57 and 149 ± 56 ms for contractions of 50 to 100% MVC. Raw traces from
one subject are shown in Fig. 2A. On average, the silent period was slightly longer during the 50% MVC compared with the stronger contractions ($F_{2,10} = 11.2; P = 0.003$; Fig. 4A; filled circles). During MVCs, the rate of torque decline during the silent period was initially slow but was followed by a rapid decline (for example see Figs. 2B and 3C). The decline in torque again slowed before the resumption of voluntary EMG. After cortical stimulation, the peak relaxation rate occurred at $96.7 \pm 6.7$, $92.7 \pm 5.8$, and $89.0 \pm 4.3$ ms after the cortical stimulus for the 50, 75, and 100% MVCs, respectively. When the peak relaxation rate was normalized to the total torque (voluntary plus evoked) before the silent period, the normalized peak relaxation rate was faster in the 75% MVC than in the 50 and 100% MVCs ($F_{2,10} = 6.3; P = 0.017$; Fig. 4C; filled circles). Between 50 and 100% of maximal torque, the average peak relaxation rate of muscle was $10.9 \pm 2.8$ s$^{-1}$ and was not significantly different from the peak relaxation rate of the resting twitch evoked by motor nerve stimulation ($12.7 \pm 3.8$ s$^{-1}$).

The time to the peak of the superimposed twitch decreased with increasing contraction strength ($F_{2,10} = 109.3; P < 0.001$; Fig. 5A).

The average MEP area in biceps was $89.0 \pm 10.4\%$ of $M_{\text{max}}$ during elbow flexion contractions of 50%–100% of maximal force, whereas in the antagonist triceps muscle, the average MEP area was only $10.2 \pm 3.8\%$ of $M_{\text{max}}$.

**Experiment 2: brief contractions in fatigued muscle.** Following a sustained MVC, fatigue was demonstrated by a reduction in the force-generating capacity of the muscle (protocol shown in Fig. 1B). The average size of the estimated resting twitch (see METHODS) decreased from $20.0 \pm 3.5\%$ MVC in fresh muscle to $12.2 \pm 2.5\%$ MVC with fatigue ($P = 0.013$). The estimated resting twitch was significantly larger than the motor nerve resting twitch ($P = 0.004$), which only showed a trend for a decline in size with fatigue (fresh: $9.1 \pm 2.3\%$ MVC; fatigue: $7.3 \pm 2.2\%$ MVC; $P = 0.12$). As in fresh muscle, the silent period after cortical stimulation was longer during the 50% MVC compared with the stronger contractions ($F_{2,10} = 11.2; P = 0.003$; Fig. 4A).

The normalized peak relaxation rate of fatigued muscle was faster in the 75% MVC than in the 50 and 100% MVCs ($F_{2,10} = 6.3; P = 0.017$; Fig. 4C). However, the peak relaxation rate was significantly slower in fatigued muscle overall.
In fatigued muscle, the average peak relaxation rate across this contraction range \((8.4 \pm 2.0 \text{ s}^{-1})\) was \(~20\%\) slower than in fresh muscle.

For the resting twitch evoked by motor nerve stimulation, there was only a trend for fatigue-induced slowing in the peak relaxation rate \((P = 0.14)\) and half relaxation time \((P = 0.09)\) (Table 1). The peak relaxation rate of muscle measured during a voluntary contraction appeared to be more sensitive to fatigue-induced changes than the characteristics of the resting twitch evoked by motor nerve stimulation just after the fatiguing contraction.

In fatigued muscle, the time to peak amplitude of the twitch produced by cortical stimulation also decreased with a decrease in superimposed twitch amplitude \(\left(F_{2,10} = 109.3; P < 0.001\right)\). The time to peak amplitude appears to be slightly longer with fatigue \(\left(F_{1,10} = 10.6; P = 0.023; \text{Fig. 5A}\right)\). However, this is difficult to interpret because the amplitude of the superimposed twitch

\[\left(F_{1,10} = 6.9; P = 0.047; \text{Fig. 4C}; \text{see also Fig. 3C}\right)\]. In fatigued muscle, the average peak relaxation rate across this contraction range \((8.4 \pm 2.0 \text{ s}^{-1})\) was \(~20\%\) slower than in fresh muscle.

### Figure 4

**A** Group data showing the average duration of the silent period after cortical stimulation and the peak relaxation rate of muscle during brief contractions of 50, 75, and 100% of maximal torque. 
**B** Group data showing the average duration of the silent period after cortical stimulation and the peak relaxation rate of muscle in fresh (■) and fatigued (○) muscle. 
**C** Group data showing the average duration of the silent period after cortical stimulation in fresh (■) and heated (○) muscle. 
**D** Group data showing the average duration of the silent period after cortical stimulation in fresh (■) and heated muscle (○).

### Figure 5

**A** Group data showing the average time to peak amplitude of the superimposed twitch produced by cortical stimulation during brief 50, 75, and 100% MVCs. The time to peak amplitude decreased as the size of the superimposed twitch decreased in each muscle state \((P < 0.001)\). The largest superimposed twitches occurred during the 50% and the smallest during the 100% maximal contractions. 
**B** Group data showing the average time to peak amplitude of the superimposed twitch produced by cortical stimulation in fresh (■) and fatigued (○) muscle. Dotted line is for comparison of the time to peak amplitude between muscle states at a given superimposed twitch size.
twitch changes with fatigue. For a given superimposed twitch amplitude, the time to peak amplitude was slower only for the large superimposed twitch evoked during contractions of 50% MVC (see vertical dotted line in Fig. 5A).

The average MEP area in biceps was 78.9 ± 17.9% of $M_{\text{max}}$ during elbow flexion contractions of 50%–100% of the fatigued maximal force. In the unfatigued triceps, the average MEP area was only 7.7 ± 4.0% of $M_{\text{max}}$.

### Study 2

**Experiment 1 vs. experiment 2:** reproducibility and variability during brief contractions in fresh muscle. Brief contractions in experiment 2 (Fig. 1D) were compared with brief contractions in the same subjects before heating in experiment one (Fig. 1C). During brief MVCs, the variability of the peak relaxation rate of muscle was minimal within and between sessions. Peak relaxation rate was also highly reproducible across sessions. The variability and reproducibility were similar to the peak relaxation rate and half relaxation time of the resting twitch evoked by motor nerve stimulation (Table 2).

**Experiment 1:** brief contractions in heated muscle. An elevation in core body temperature from 37.2 ± 0.3 to 38.5 ± 0.4°C increased muscle temperature from 36.9 and 36.2°C to 39.2 and 39.1°C in the two subjects tested. Heating had no effect on the duration of the silent period (Fig. 4B).

In heated muscle, the normalized peak relaxation rate of muscle was similar for contractions of 50–100% MVC, and it was significantly faster than in fresh muscle ($F_{1,12} = 64.6; P < 0.001$; Fig. 4D). The average peak relaxation rate of muscle was ~20% faster at the higher temperature (fresh muscle: 9.2 ± 4.3 s$^{-1}$; heated muscle: 11.4 ± 4.3 s$^{-1}$; $P < 0.001$). Again, the peak relaxation rate of muscle appeared to be more sensitive to temperature-induced changes in muscle than the characteristics of the resting twitch evoked by motor nerve stimulation. In heated muscle, the half relaxation time was shorter ($P = 0.048$) but there was only a trend for a faster peak relaxation rate ($P = 0.12$; Table 1).

In heated muscle, the time to peak amplitude also decreased with a decrease in superimposed twitch amplitude ($F_{2,12} = 33.1; P < 0.001$; Fig. 5B) and there was an overall trend for a faster time to peak than in fresh muscle ($F_{1,12} = 4.4; P = 0.08$; Fig. 5B). The time to peak amplitude was significantly faster in heated muscle during the 50% MVC but not during the 75 and 100% MVCs ($F_{2,12} = 5.0; P = 0.026$).

**Experiment 2:** sustained MVC. During the sustained MVC, voluntary torque decreased to 31.6 ± 7.7% of the maximal torque produced by fresh muscle in brief MVCs ($F_{1,83} = 66.9; P < 0.001$; Fig. 6A). The decline in torque (statistically evident at 4 s; $F_{9,53} = 7.4; P < 0.001$) was rapid during the first minute of the contraction but reached a plateau after 1 min 35 s. As a result of the sustained MVC, the amplitude of the estimated resting twitch decreased from 20.2 ± 6.0% MVC in fresh muscle to 7.9 ± 2.9% MVC immediately after the sustained MVC ($P < 0.001$) while the resting twitch evoked by motor nerve stimulation decreased from 9.1 ± 2.2 to 2.3 ± 1.3% MVC ($P < 0.001$).

The decline in voluntary torque was associated with changes in the EMG and torque responses to motor cortex stimulation. In biceps, the size of the MEP (normalized to $M_{\text{max}}$) increased immediately after commencing the sustained MVC and remained unchanged thereafter ($F_{5,29} = 2.9; P = 0.03$; Fig. 7A), whereas the silent period lengthened but reached a plateau after 25 s ($F_{5,29} = 27.2; P < 0.001$; Fig. 7B). The amplitude ($F_{5,28} = 9.9; P < 0.001$; Fig. 6C) and time to peak amplitude ($F_{5,28} = 10.3; P < 0.001$; Fig. 6D) of the superimposed twitch increased and the peak relaxation rate of muscle decreased ($F_{5,28} = 6.2; P < 0.001$; Fig. 7B). The amplitude and time to peak amplitude of the superimposed twitch did not significantly increase further after 25 s. However, there was a trend for an increased time to peak amplitude at 85 s ($P = 0.07$) and 110 s ($P = 0.053$) compared with at 25 s. The decline in the peak relaxation rate of muscle reached a plateau after 55 s.

### Table 1. Characteristics of the resting twitch evoked by motor nerve stimulation in fresh, heated, and fatigued muscle

<table>
<thead>
<tr>
<th></th>
<th>Study 1 (n = 6)</th>
<th>Study 2 (n = 7)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
<td>Fatigued</td>
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<tr>
<td>Peak relaxation rate of resting twitch, s$^{-1}$</td>
<td>12.7±3.8</td>
<td>10.3±1.2</td>
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<tr>
<td>Half relaxation time of resting twitch, ms</td>
<td>70.6±12.1</td>
<td>82.3±11.9</td>
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<tr>
<td>Time to peak amplitude of resting twitch, ms</td>
<td>49.4±7.1</td>
<td>55.5±9.1</td>
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</table>

Data are means ± SD; n, no. of subjects. *Significant difference between fresh and fatigued or fresh and heated muscle, $P < 0.05$.

### Table 2. Reproducibility and variability of parameters that reflect the contractile properties of fresh muscle

<table>
<thead>
<tr>
<th>Stimulus Type</th>
<th>Within-Session CV, %</th>
<th>Between-Session CV, %</th>
<th>Between-Session ICC$_{2,1}$</th>
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<tbody>
<tr>
<td>Peak relaxation rate in silent period</td>
<td>Motor cortex</td>
<td>8.7±3.8</td>
<td>8.2±8.7</td>
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<tr>
<td>Time to peak amplitude of superimposed twitch</td>
<td>Motor cortex</td>
<td>5.3±4.9</td>
<td>5.2±3.4</td>
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<tr>
<td>Peak relaxation rate of resting twitch</td>
<td>Nerve</td>
<td>6.2±2.4</td>
<td>4.8±5.1</td>
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<tr>
<td>Half relaxation time of resting twitch</td>
<td>Nerve</td>
<td>8.4±6.0</td>
<td>5.2±3.4</td>
</tr>
<tr>
<td>Time to peak amplitude of resting twitch</td>
<td>Nerve</td>
<td>11.6±11.0</td>
<td>13.0±9.6</td>
</tr>
</tbody>
</table>

Values are means ± SD. Peak relaxation rate during the silent period evoked by motor cortical stimulation was measured during brief maximal voluntary contractions. The time to peak amplitude of the superimposed twitch represents the average for contractions of 50–100% maximal voluntary contraction. CV, coefficient of variation; ICC, intraclass correlation coefficient.
The use of cortical stimulation has allowed us to make the first direct measurements of the contractile properties of muscle during voluntary contractions in humans. Three aspects of the results will be discussed in detail: first, muscle properties measured during brief voluntary contractions in fresh muscle; second, the effect of muscle fatigue and an elevation in temperature on muscle properties measured during brief voluntary contractions; third, muscle and nervous system mechanisms associated with progressive muscle fatigue induced by a sustained maximal effort.

**Brief Voluntary Contractions in Fresh Muscle**

The cortically evoked silent period follows the synchronized activation of motoneurons seen as the MEP. Events at a spinal level, such as recurrent inhibition, contribute to its initial part. At the same time, intracortical inhibition produced by the stimulus halts voluntary descending drive so that the absence of cortical outflow contributes to the early part of the silent period and is the main mechanism of the latter part. The abrupt cessation of cortical output and motoneuron activity in the silent period means that all of the muscle fibers engaged in the voluntary contraction plus any additional fibers recruited by TMS start to relax within a period of a few milliseconds. Thus relaxation rates reflect properties of the muscle rather than the ability of subjects to withdraw neural drive as in voluntary relaxation. During the cortically evoked silent period, the elbow flexors relaxed and the rate of torque decline was initially slow followed by a rapid decline. The rate of decline seen here is similar to that seen in studies involving rapid calcium removal from single isolated myofibrils (46) and muscle fibers relaxing from maximum tetanic force (14, 29). The time course of relaxation following rapid calcium removal has a slow phase followed by a fast, nearly exponential phase (for review, see Refs. 38, 59). In single muscle fibers, sarcomeres remain isometric in the slow relaxation phase, whereas the fast-relaxation phase appears to be initiated by a sudden elongation of some sarcomeres. The sudden elongation should accelerate the decline in tension by promoting cross-bridge detachment (Refs. 46, 59; for review, see Ref. 38). The peak relaxation rate of muscle occurred at approximately 90 ms after the cortical stimulus while the silent period duration was about 122 ms. Therefore, the duration of the silent period was sufficient to allow for measurement of the peak relaxation rate of muscle fibers in contractions of 50–100% MVC. Although a longer silent period can be evoked by higher stimulus intensities, this will also increase the activation of other muscles by TMS. In the present study, peak relaxation rate was not likely to be affected by activation of antagonist muscles because the MEP in triceps was very small. However, activation of antagonists by TMS may be problematic in other muscle groups.

To validate the technique for measurement of peak relaxation rate of muscle during voluntary contractions, we contrasted the results to data obtained from the resting twitch evoked by traditional motor nerve stimulation, and we calculated reproducibility and variability in fresh muscle. The average peak relaxation rate measured during the cortically evoked silent period was similar to the peak relaxation rate of the resting twitch evoked by motor cortex stimulation. One might
have expected a slower relaxation rate in the silent period because of the extra calcium present after repetitive activation. During voluntary contractions, the peak relaxation rate was slightly faster during 75% MVCs than in the 50 and 100% MVCs. This is difficult to explain. However, it may reflect the balance of motor units repetitively activated by voluntary drive vs. those only recruited by TMS as voluntary contraction strength increases.

The relaxation properties of fresh muscle were faster in study 1 than in study 2, both for relaxation measured at rest and during a voluntary contraction. A possible explanation for this is a different proportion of men and women in each study. Study 1 had equal numbers of men and women, whereas study 2 had more women \((n = 5)\) than men \((n = 2)\). Adult men have a significantly faster peak relaxation rate of muscle than age-matched women \((28)\). This is consistent with men possessing more fast-twitch muscle fibers in elbow flexor muscles than women and is supported by the finding that men have greater rates of anaerobic glycolysis \((42)\).

The peak relaxation rate in the cortically evoked silent period and the time to peak amplitude of the superimposed twitch were reproducible across sessions with minimal variability. The reproducibility and variability were similar or better than resting twitch parameters derived from motor nerve stimulation. Thus, with one cortical stimulus, we can reliably assess the contractile properties of muscle, as well as aspects of neural drive during voluntary contractions. This is particularly important when investigating muscle fatigue, a condition that has both a central and peripheral origin (for review, see Ref. 16, 18).

**Brief voluntary contractions in fatigued and heated muscle.** The technique based on motor cortical stimulation can reveal changes in the contractile properties of muscle that one would expect with isometric fatigue and an elevation in temperature. In addition, muscle properties measured during voluntary contractions were more sensitive to an altered muscle state than twitch parameters measured at rest.

In mammalian and amphibian muscle, the conduction velocity of action potentials along muscle fibers is faster at higher temperatures and the resting muscle membrane potential becomes more hyperpolarized due to a temperature-related change in sodium flux or a change in transverse membrane resistance \((10, 36, 37)\). The sensitivity of actomyosin to calcium is also decreased at higher temperatures \((24)\). As a result of these temperature-related changes, muscle relaxation is faster in heated muscles \((e.g., \text{Refs. 11, 12, 40, 57})\). In the second study, the peak relaxation rate of muscle, measured during the cortically evoked silent period, increased by 20% with a 2–3°C elevation in muscle temperature. This increase in peak relaxation rate is comparable to the decrease in half relaxation time measured in isolated rat lower limb muscles \((47)\). In our study, we also observed a decrease in the half relaxation time of the motor nerve resting twitch, but the peak relaxation rate of the resting twitch was not significantly faster.

Fatigue also changes contractile properties. During fatigue, particularly under experimental conditions in which there is restricted or no blood supply, twitch contraction and relaxation rates are slowed due to reduced calcium uptake in the sarcoplasmic reticulum \((e.g., \text{Ref. 22; for review, see Refs. 1, 9})\). Accordingly, when fatigue reduced maximal voluntary force by 40%, we observed a 20% decrease in the peak relaxation rate of fatigued muscle measured during a brief voluntary contraction. In contrast, there was only a trend for a slower peak relaxation rate and half relaxation time of the resting twitch evoked by motor nerve stimulation. Fatigue is also accompanied by a decline in force output from muscle due to impaired excitation-contraction coupling \((\text{Refs. 1, 58; for review, see Ref. 16})\). The estimated resting twitch evoked by the cortical stimulus at rest \((\text{see METHODS})\) was reduced, but there was only a trend for a reduction in the resting twitch evoked by motor nerve stimulation. Thus muscle contractile properties measured with TMS during voluntary contractions were more sensitive to altered muscle state than properties of the resting twitch evoked by motor nerve stimulation. Because the contractile properties measured with TMS were from all of the elbow flexors whereas the twitch was from only biceps and brachialis, it is not clear whether the greater sensitivity is intrinsic to the tests or depends on the muscles tested. Biceps and brachialis only contribute ~50% of the total elbow flexion torque \((3)\).

**Sustained MVC**

Once the reproducibility and sensitivity of the new technique were established, we applied it to study the onset and progression of the central and peripheral mechanisms associated with muscle fatigue.

Changes within the central nervous system occur during sustained MVCs. There is an impairment of voluntary activation that occurs at or above the level of the motor cortical output \((\text{Refs. 19, 52, 54; for review see Ref. 18})\), and motor unit firing rates and voluntary EMG are reduced \((e.g., \text{Refs. 7, 6, 23, 32, 60})\). An increasing amplitude of the superimposed twitch evoked by TMS indicates that voluntary output from the motor cortex becomes suboptimal to drive the motor units to generate maximal force from the muscle. At the same time, the size of the motor evoked potential increases \((19, 34, 50, 51)\), partly due to an increase in motor cortical excitability \((51, 52)\), and the silent period lengthens, suggesting an increase in intracortical inhibition \((e.g., \text{Refs. 19, 34, 50})\). However, under some conditions, the changes in EMG responses to TMS can be dissociated from impairment of voluntary activation and this suggests that they are not causally related. In the present study, we show that these central nervous system changes, which occur with a sustained fatiguing MVC, can be studied at the same time as peripheral changes within the muscle.

In the first minute of the sustained MVC, voluntary torque declined rapidly. Contraction time also lengthened and the peak relaxation rate of muscle fibers slowed. The lengthening of the contraction time is likely due to an increase in the amplitude of the cortically evoked superimposed twitch as well as processes occurring within the muscle, such as reduced calcium ion concentration for activation of the calcium regulatory system and contractile apparatus. The slowed peak relaxation rate of muscle fibers is due to reduced calcium pump activity in the sarcoplasmic reticulum caused mainly by a reduced pH (for review see Ref. 48).

In the second minute of the sustained contraction, voluntary torque continued to decline. There was also a trend for continued slowing of contraction time. The time to peak amplitude of the superimposed twitch tended to increase further despite no further changes in superimposed twitch amplitude or peak.
relaxation rate of muscle fibers. This suggests that the continued slowing was due to processes occurring in the muscle and that these processes differ from those that contribute to slowed muscle relaxation. This is consistent with findings in animal muscle preparations, where different processes are known to govern muscle contraction and relaxation (48, 31).

The ongoing decline in voluntary torque is due to reduced force output from muscle fibers as well as to the decline in voluntary activation represented by the increase in the superimposed twitch. With fatigue, the size of the estimated resting twitch and the resting twitch evoked by motor nerve stimulation are reduced. This suggests a reduction in the ability of muscle fibers to produce force. No technique is currently available to measure the time course for changes in the force-generating capacity of muscle fibers during sustained contractions. However, a model exists for estimation of the decline in force-generating capacity during sustained maximal efforts (44). The model is based on measurement of the amplitude of the motor nerve superimposed twitch, the time course for the decline in voluntary force, and the ratio between the motor nerve resting twitch evoked immediately before and after a sustained contraction. Establishing this time course with certainty could improve understanding of the contribution of different mechanisms to fatigue in voluntary contractions. For example, if the time course for the decline in the force-generating capacity of muscle is similar to the time course for the decline in voluntary force it would also suggest a continued decline in voluntary activation, even though the size of the superimposed twitch appears to plateau after 25 s. Voluntary activation would continue to decline because the constant superimposed twitch would represent an increasing proportion of the total muscle force.

TMS allows us to measure the contractile properties of muscle during voluntary contractions. These measures are reliable and appear more sensitive to change during muscle fatigue and muscle heating than similar measures of a twitch in relaxed muscle. However, care must be taken to minimize antagonist contributions to the evoked forces and this may restrict the muscle groups that can be examined with this method. Measurement of the contractile properties of muscle during voluntary contractions means that central and peripheral changes that accompany muscle fatigue can now be investigated simultaneously.

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