Voluntary activation of the knee extensors measured using transcranial magnetic stimulation

Goodall, S.[1], Romer, L.M.[1] and Ross, E.Z.[1, 2]

[1]Centre for Sports Medicine and Human Performance, Brunel University, Uxbridge, UK
[2] Chelsea School Research Centre, University of Brighton, Eastbourne, UK

Running Head: Measurement of cortical drive to a lower limb muscle group

Corresponding Author:

Dr Emma Ross
Chelsea School
University of Brighton
Hilbrow
Denton Road
Eastbourne
BN20 7SP

Email: E.Z.Ross@brighton.ac.uk
Tel+44 (0)1273643723
Fax: +44 (0)1273643704

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Abstract

The aim of this study was to determine the applicability and reliability of a transcranial magnetic stimulation twitch interpolation technique for measuring voluntary activation of a lower-limb muscle group. Cortical voluntary activation of the knee extensors was determined in nine healthy men at two separate visits by measuring superimposed twitch torques evoked by transcranial magnetic stimulation during isometric knee extensions of varying intensity. Superimposed twitch amplitude decreased linearly with increasing voluntary torque between 50 and 100% of mean maximal torque, allowing estimation of resting twitch amplitude and subsequent calculation of voluntary activation. There were no systematic differences for maximal voluntary activation within-day (mean ± SD 90.9 ± 6.2 vs. 90.7 ± 5.9%; $P = 0.98$) or between-days (90.8 ± 6.0 vs. 91.2 ± 5.7%; $P = 0.92$). Systematic bias and random error components of the 95% limits of agreement were 0.23 and 9.3% within-day, vs. –0.38 and 7.5% between-days. Voluntary activation was also determined immediately after a 2 min maximal voluntary isometric contraction; in four of these subjects voluntary activation was determined 30 min after the sustained contraction. Immediately after the sustained isometric contraction, maximal voluntary activation was reduced from 91.2 ± 5.7 to 74.2 ± 12.0% ($P < 0.001$), indicating supraspinal fatigue. After 30 min, voluntary activation had recovered to 85.4 ± 8.8% ($P = 0.39$ vs. baseline). These results demonstrate that TMS enables reliable measurement of maximal voluntary activation and assessment of supraspinal fatigue of the knee extensors.

Keywords: TMS, quadriceps, fatigue.

Introduction

Voluntary activation describes the level of neural drive to a muscle during contraction and is most commonly estimated using twitch interpolation (Merton, 1954). This method involves the application of a single supramaximal stimulus to the motor nerve during a maximal voluntary contraction (MVC). Voluntary activation is deemed to be less than maximal or incomplete if the supramaximal stimulus delivered during an MVC can evoke extra force from the muscle under investigation. Conversely, if the supramaximal stimulus fails to evoke any extra force then activation is considered to be complete (Allen *et al*. 1995; Herbert & Gandevia, 1999). To quantify voluntary activation, the size of the superimposed twitch evoked during a contraction is compared with the force produced by the same stimulus delivered to the resting potentiated muscle. The site of neural drive impairment responsible for incomplete voluntary activation, when assessed by motor nerve stimulation, can be identified as at or above the site of stimulation of the motor axons (Gandevia, 2001).

To further localise the site of impaired neural drive, transcranial magnetic stimulation (TMS) has been used to quantify voluntary activation (Todd *et al*. 2003; Lee *et al*. 2008; Sidhu *et al*. 2009a). The presence of a superimposed twitch produced by TMS during an MVC suggests that the drive from the motor cortex is sub-optimal. Thus, the impairment of voluntary drive can be located at or above the level of motor cortical output (Todd *et al*. 2004). However, when using TMS to assess voluntary activation it is inappropriate to normalise the superimposed twitch force (SIT) evoked during a voluntary contraction to that evoked at rest, as performed in the more conventional twitch interpolation technique. This is because motor cortical and motoneuronal excitability increase with activity, and the same magnetic stimulus would evoke less cortical output (and therefore recruit fewer motor units) at rest than during voluntary activity (Lee *et al*. 2008). Todd *et al*. (2003) devised a method to overcome the problem of different levels of background excitability at rest compared to during activity, whereby the resting motor cortical output that would be evoked by TMS if background

excitability were maintained during rest can be estimated. The 'estimated' resting twitch (ERT) is then placed in the conventional formula to establish voluntary activation (voluntary activation (%) = (1 – SIT/ERT) x 100). The ERT is estimated via a linear extrapolation of the regression between the SIT produced by cortical stimulation superimposed onto submaximal voluntary contractions and MVCs (Todd *et al*. 2004; Lee *et al*. 2008). Between contraction intensities of 50 and 100% MVC, the SIT has been shown to decrease linearly in fresh and fatigued elbow flexor muscles (Todd *et al*. 2003, 2004) and, more recently, in the wrist extensors (Lee *et al*. 2008). However, study of the applicability of this technique has been confined to the upper limb, and to date, the feasibility and reliability of this method of assessing voluntary activation of lower limb muscle groups is limited.

It is important that certain criteria are met when applying the TMS twitch interpolation technique to a new muscle (Taylor *et al*. 2006). First, the muscle under investigation must have strong excitatory connections from the motor cortex to achieve a near maximal excitatory response with a minimal response in antagonist muscle groups. Second, the ability of the agonist muscle to produce force must be optimised relative to the antagonist group. Finally, when a muscle is under voluntary contraction the response elicited by a TMS stimulus should be greater than that evoked when the same muscle is at rest. The knee extensors are a muscle group that meet these criteria. In particular, a large motor evoked potential (MEP) can be elicited in the vastus lateralis through stimulation of the motor cortex while responses in the biceps femoris are absent (Tremblay *et al*. 2001), and this response is exaggerated during a contraction (Urbach & Awiszus, 2000; Tremblay *et al*. 2001). Recent evidence has shown that voluntary activation can be reliably assessed in fresh and fatigued knee extensors (Sidhu *et al*. 2009a; 2009b), although only responses from the rectus femoris have been studied.

Therefore, the aim of the present study was to investigate whether the method devised by Todd & colleagues (2003) can reliably predict voluntary activation of the knee extensors, specifically the responses from the vastus lateralis. Furthermore, in response to a sustained isometric contraction of the knee extensors we assessed the ability of the technique to identify supraspinal fatigue, defined as a reduction of output from the motor cortex (Taylor *et al*. 2006). The knee extensors play a key role in ambulatory, functional and sporting activities (Maffiuletti *et al*. 2008). From both a research and clinical perspective there is a need for establishing the feasibility and reliability of techniques such as twitch interpolation with TMS in lower limb muscles.

Methods

Subjects

Nine healthy recreationally-active men volunteered to participate in the study (mean ± SD age 23 ± 7 y, stature 1.79 ± 0.05 m and body mass 80 ± 9 kg). Subjects gave written informed consent prior to testing and approval for all experimental procedures was obtained from the institutional ethics committee. The study was conducted according to the Declaration of Helsinki.

Experimental Design

At two separate visits to the laboratory, torque and EMG responses to cortical stimulation were measured while subjects activated their knee extensors. Voluntary activation was calculated by estimating the size of the resting twitch evoked by TMS, using the linear relationship that exists between contraction intensity and superimposed twitch amplitude. At the first visit to the laboratory, the twitch interpolation method was performed before and after 30 min of rest for the subsequent determination of within-day reliability. At the second visit to the laboratory (19 ± 10 d), the baseline measurements were repeated for the

determination of between-day reliability. In addition, cortical voluntary activation was measured up to 30 min after a 2 min isometric MVC of the knee extensors (Place *et al*. 2007).

Torque and EMG Recordings

Knee extensor force during voluntary and evoked contractions was measured using a calibrated load cell (Model ABA Ergo Meter, Globus Italia, Codogne, Italy), which was connected to a noncompliant strap attached around the subject's right leg just superior to the malleoli of the ankle joint. The load cell was fixed to a custom-built chair and adjusted to a height that was in the direct line of applied force for each subject. Torque measurements were later determined as the product of force and shank length. Subjects lay semi-recumbent on the chair with the right knee at 1.57 rads (90°) of flexion and arms folded across the chest. This position of knee flexion optimises knee extensor torque during isometric contractions while minimising the torque produced by the antagonists (Narici *et al.* 1988).

Electromyographic activity was recorded with pairs of surface electrodes (Kendall H59P, Tyco Healthcare Group, Mansfield, USA) spaced 2 cm apart over the vastus lateralis and biceps femoris. The positions of the EMG electrodes were marked with indelible ink and recorded on acetate in relation to anatomical landmarks to ensure they were placed in the same location at both visits. All of the signals were amplified (gain 1000; 1902, Cambridge Electronic Design, Cambridge, UK), then band-pass filtered (EMG only: 20-2000 Hz), digitised (4 kHz; micro 1401, Cambridge Electronic Design), and finally acquired and later analysed (Spike 2 v5.03, Cambridge Electronic Design).

Motor Nerve Stimulation

Peripheral stimulation of the right femoral nerve was administered using a magnetic stimulator (Magstim 200, The Magstim Company Ltd., Whitland, UK) and a double 70-mm coil (maximum output 2.2 Tesla). The site of stimulation that produced the largest quadriceps twitch torque (Q_{tw}) and M-wave amplitude (M_{max}) was located by positioning the coil-head

high in the femoral triangle lateral to the femoral artery. All peripheral stimulations were performed with the stimulator at 100% of its maximal possible intensity. To determine whether nerve stimulation was supramaximal, two single twitches were delivered to the femoral nerve at 50, 60, 70, 80, 85, 90, 95 and 100% of the maximal power output of the stimulator. Plateaus were evident in Q_{tw} and vastus lateralis M_{max} indicating maximum depolarization of the femoral nerve (see online repository).

Transcranial Magnetic Stimulation

Motor evoked potentials (MEPs) were elicited in the right vastus lateralis using TMS. Single (1 Hz) magnetic stimuli (1 ms duration) were applied over the contralateral motor cortex using a magnetic stimulator (Magstim 200) with a double 110-mm cone coil (maximum output 1.4 Tesla), which induced a postero-anterior intracranial current. The optimal coil position for eliciting a large MEP in the vastus lateralis and a minimal MEP in the antagonist muscle (biceps femoris) was determined at each visit and marked on the scalp with indelible ink. The junction of the double cone coil was measured in relation to the vertex to ensure reproducibility of the stimulation conditions for that individual throughout the entire experimental protocol (1.2 ± 0.6 cm lateral to the vertex). The resting motor threshold for the quadriceps was then identified by constructing a stimulus-response curve for each subject. The threshold was established by decreasing stimulator output from 80% by 5% increments until the MEP response was below 0.05 mV in more than one-half of eight stimuli. The resting motor threshold was apparent at 58 ± 8% of maximum stimulator output. TMS was subsequently delivered at 130% of motor threshold during all of the experimental procedures (75 ± 11% maximum stimulator output); this stimulation intensity elicited a large MEP in the vastus lateralis, with an area between 80 and 100% M_{max} during extension contractions ≥50% MVC and only a small MEP in the biceps femoris (Fig. 1).

Protocol

Visit One (Trials 1 & 2)

Six single transcranial stimuli were delivered over the motor cortex to elicit responses in the relaxed vastus lateralis. Resting MEP amplitude was calculated as the average of the six responses. To determine voluntary activation with cortical stimulation, single transcranial stimuli were delivered during six different levels of voluntary contraction. Target torques were displayed as visual feedback on a computer screen based on the mean maximal torque response from 5 MVC manoeuvres, each sustained for 3 s. In addition to the target torques, one MVC was performed such that one set comprised six contractions (10, 25, 50, 75, 80 and 100% mean maximal torque), the order of which was randomised. Each set was performed four times with 15 s between each contraction and 45 s between each set, taking a total time of 8.5 min. Participants were instructed to increase torque to the desired level of contraction and hold it as steady as possible before a single motor cortical stimulus was delivered. After the four sets had been completed, another five MVC manoeuvres were performed with peripheral stimulations delivered before, during and after. Mean maximal torque and potentiated quadriceps twitch torque ($Q_{tw,pot}$) were evaluated after each MVC to ensure that the brief sets of submaximal contractions were not causing peripheral fatigue (Kufel *et al*. 2002). In addition, to determine quadriceps voluntary activation with peripheral stimulation the torque increment obtained via supramaximal stimulus of the femoral nerve during an MVC was compared to the $Q_{tw,pot}$ (Merton, 1954). To assess within-day reliability, the measurements were repeated during trial 2 after 30 min of rest.

Visit Two (Trials 3 & 4)

The protocol in trial 3 was identical to that in trials 1 and 2 to enable between-day reliability to be assessed. In trial 4, voluntary activation determined by TMS twitch interpolation was assessed in the fatigued knee extensor muscles. A 2 min isometric MVC of the quadriceps was performed to induce fatigue (Place *et al*. 2007), defined as an exercise-induced decrease

in maximal force production (Bigland-Ritchie *et al.* 1978; Gandevia, 2001). During the sustained isometric MVC, maximal torque decreased by 72 ± 8% from baseline (236 ± 56 vs. 64 ± 23 Nm, $P < 0.001$). Strong verbal encouragement and visual online feedback were used to motivate subjects. Immediately after the sustained contraction, cortical voluntary activation was determined as outlined in trials 1 and 2. Four of the subjects were also tested 30 min after the fatiguing contraction to assess the recovery profile of cortical voluntary drive.

Data Analyses

The areas of MEP and M_{max} evoked by TMS and motor nerve stimuli respectively were measured between cursors placed to encompass all phases of evoked potentials (Sidhu *et al.* 2009b). Voluntary activation was quantified by measurement of the torque responses to single pulse motor cortical stimulation. The resting twitch for each subject was derived from extrapolating the linear regression between the SIT and voluntary torque over two torque ranges: 1) 25-100% and 2) 50-100% mean maximal torque. The *y*-intercept was taken as the estimated amplitude of the resting twitch; therefore, each set of contractions yielded an estimated resting twitch. The level of voluntary drive was then quantified using the following equation: voluntary activation (%) = (1 – SIT/ERT) x 100.

Statistical Analyses

Repeated measures ANOVA was used to compare SIT, MEP, ERT amplitudes and voluntary activation between-trials (1, 2 & 3). To determine the extent to which the repeated measures varied, within- and between-day reliability for each variable was assessed by obtaining 95% limits of agreement according to Bland & Altman (1986). Examining the direction and magnitude of the scatter around the zero line on these Bland-Altman plots provides an approximate indication of the systematic bias and random error, respectively. To make comparisons with previous literature we also calculated the intraclass correlation coefficient ($ICC_{2,1}$), with trial as the independent variable; and the coefficient of variation (CV),

determined using the typical error of measurement between trials 1, 2 and 3 for maximal cortical voluntary activation. Paired samples *t*-tests were used to determine whether group mean differences occurred before vs. after the fatigue protocol for each of the variables. The level of statistical significance was set at 0.05 and data are expressed as group means ± SD. Statistical analyses were performed using SPSS version 15.0 for Windows.

Results

Motor Evoked Potentials (MEPs)

The largest MEP area was evoked during a contraction at 50% mean maximal torque (mean area across trials: 91 ± 24% of M_{max}). With increasing contraction strength MEP area decreased (75% mean maximal torque average area: 81 ± 16% and 100% MVC average area: 74 ± 21% of M_{max}; Fig.1). MEP areas did not differ significantly at any of the contraction strengths within- or between-days, immediately after the sustained contraction or after 30 min of recovery ($P > 0.05$).

The largest peak-to-peak MEP amplitude was evoked during a contraction at 50% mean maximal torque. With further increasing contraction intensity MEP amplitudes decreased (Fig. 2). The MEP evoked during each of the contraction strengths did not change in amplitude within- or between-days ($P > 0.05$). MEP amplitude was significantly decreased at rest ($P = 0.01$) but not during any contraction intensity immediately after or 30 min after the sustained contraction ($P > 0.05$).

Superimposed Twitch Responses to TMS

The amplitude of the SIT decreased linearly between 50 and 100% mean maximal torque (Fig. 3), demonstrating a strong linear relationship within- and between-days (trial 1 $r^2 = 0.96 \pm 0.05$; trial 2 $r^2 = 0.97 \pm 0.03$; trial 3 $r^2 = 0.98 \pm 0.03$). The amplitude of the SIT after the fatiguing contraction also decreased linearly between 50 and 100% mean maximal torque ($r^2 =$

0.88 ± 0.10 [immediately post], 0.98 ± 0.02 [30 min post]). There were no systematic differences in the SIT amplitude evoked during any of the contraction strengths within- or between-days ($P > 0.05$); however, immediately after the fatiguing contraction the SIT evoked during an MVC increased significantly ($P < 0.001$). In the 4 subjects tested 30 min after the sustained contraction, the SIT amplitude returned to baseline levels ($P = 0.82$).

Estimated Resting Twitch

The ERT differed significantly within-day when data were obtained from 25 to 100% mean maximal torque (68 ± 17 vs. 55 ± 15 Nm; $P = 0.01$) but not when data were used from 50 to 100% mean maximal torque (77 ± 23 vs. 66 ± 18 Nm; $P = 0.05$). No differences were apparent between-days over either of the contraction ranges ($P > 0.05$; Fig. 3). When determined from data between 25 and 100% mean maximal torque, the ERT was significantly reduced below baseline values immediately after the 2 min sustained contraction ($P < 0.001$). The ERT remained lower than baseline values 30 min after the sustained contraction, but the decrease was non-significant ($P = 0.21$). Similarly, when ERT was derived from data between 50 and 100% mean maximal torque there was a tendency for a reduction below baseline (immediately post, $P = 0.07$; 30 min post, $P = 0.20$).

Voluntary Activation Measured with TMS

As the intensity of voluntary contraction increased, voluntary activation increased linearly in all subjects (Fig. 4). When using data between 50 and 100% mean maximal torque, there were no systematic differences in maximal voluntary activation either within-day (90.9 ± 6.2 vs. 90.7 ± 5.9%, $P = 0.98$) or between-days (90.8 ± 6.0 vs. 91.2 ± 5.7%, $P = 0.92$). Immediately after the sustained contraction, voluntary activation during a maximal effort decreased significantly by 17 ± 12% (91.2 ± 5.7 vs. 74.2 ± 12.0%, $P < 0.001$; Fig. 5). After 30 min, voluntary activation had recovered to 85.4 ± 8.8% ($P = 0.39$ vs. baseline).

Motor Nerve Stimulation

There were no differences in the baseline $Q_{tw,pot}$ during the reliability protocols (within- and between-day, trials 1, 2 and 3). The $Q_{tw,pot}$ evoked 39 ± 5% of mean maximal torque. In addition, the ERT derived from linear extrapolation of the TMS responses was 88 ± 25% of the $Q_{tw,pot}$ (77 ± 23 vs. 88 ± 12 Nm). After the 2 min MVC, $Q_{tw,pot}$ was reduced below pre-fatigue baseline values (57 ± 6 vs. 77 ± 9 Nm, $P < 0.01$).

Mean Maximal Torque

Group mean values for maximal torque were not different before vs. after the TMS protocol for trial 1 (229 ± 51 vs. 232 ± 52 Nm, $P = 0.52$), trial 2 (230 ± 49 vs. 231 ± 58 Nm, $P = 0.79$) or trial 3 (228 ± 63 vs. 230 ± 65 Nm, $P = 0.65$). Mean maximal torque was significantly reduced following the fatiguing contraction (230 ± 65 vs. 155 ± 39 Nm, $P < 0.01$) but not after 30 min ($P = 0.27$). Peripherally-determined voluntary activation was not different following trial 1 (90 ± 4%), trial 2 (89 ± 4%) or trial 3 (89 ± 4%).

Reliability

Individual subject differences were plotted against individual means for maximal voluntary activation and are presented in Fig. 6. Within-day, maximal voluntary activation showed minimal systematic bias (0.23%) and a random error component of ±9.3%. Between-day, maximal voluntary activation also showed minimal bias (–0.38%) with random error of ±7.5%. Additional reliability data are summarised in the online repository.

Discussion

The main finding was that TMS can be used to reliably estimate voluntary activation of the knee extensors, both within- and between-days. The method has previously been shown to be valid and reliable in measuring voluntary activation of upper limb muscle groups (Todd *et al*. 2003, 2004; Lee *et al*. 2008). Until now, however, the responses from a lower limb muscle are

limited. In addition, our results show that twitch interpolation using TMS is a technique sensitive enough to detect changes in cortical drive following a fatiguing protocol.

Twitch Interpolation and Voluntary Activation

The SIT evoked from the quadriceps muscle in response to TMS decreased linearly with increasing voluntary contraction. This linear relationship has previously been demonstrated in the elbow flexors (Todd *et al*. 2003) and wrist extensors (Lee *et al*. 2008). The robust nature of this relationship is important, since it allows us to reliably estimate the size of the resting twitch by extrapolation of data collected from a series of submaximal contractions. To assess voluntary activation using cortical stimulation, the size of the twitch superimposed onto voluntary contraction of the knee extensors was compared with the amplitude of the estimated resting twitch.

At contraction intensities of 50 to 100% of maximal effort voluntary activation increased linearly (Fig. 4). At 100% of maximal effort, however, voluntary activation was incomplete (~90%). Similar findings have been reported for the knee extensors (Sidhu *et al*. 2009a) and other muscle groups (Todd *et al*. 2003; Lee *et al*. 2008). The decrement in voluntary activation implies that during a maximal effort some motoneurons and corticospinal cells that activate the knee extensors cannot be recruited voluntarily or be driven sufficiently to produce maximal force. When determined with peripheral nerve stimulation, quadriceps voluntary activation was also ~90%. Although some studies have reported voluntary activation values >95% for the quadriceps (Amann *et al*. 2006, 2007; Katayama *et al*. 2007; Szubski *et al*. 2007), others have reported values which are similar to those in the present study (for example, Bulow *et al*. 1995; Millet *et al*. 2002; Romer *et al*. 2006, 2007).

Motor Evoked Potentials (MEPs)

In the present study the largest MEP was observed during a contraction of 50% mean maximal torque (Fig. 2) suggesting that at this intensity most motoneurones were activated by

the motor cortical stimulus. With increasing intensity (>50% mean maximal torque) MEP amplitude and area plateaued, a finding that has been previously observed for the elbow flexors (Todd *et al*. 2003, 2004), wrist extensors (Lee *et al*. 2008) and more recently another muscle within the knee extensors (Sidhu *et al*. 2009b). The similar MEP amplitudes evoked during contractions of 50-100% MVC suggest that the cortical stimulus activates a comparable proportion of motoneurones during all these contraction intensities. However, this is not the case for stimulations delivered during lower intensity contractions (<50% mean maximal torque). This finding provides a strong physiological rationale for using 50, 75 and 100% mean maximal torque as the submaximal contraction intensities from which to extrapolate the ERT. The plateau in MEP amplitude at higher forces is the result of a decline in motoneuronal output in response to the stimulus, arising from the inability of some motoneurons to fire in response to the excitatory input (Todd *et al*. 2003). The plateau in MEP area may be due to the inability of the cortical stimulus to excite the firing motoneurone when it arrives at the beginning of its recovery cycle (Matthews, 1999). The higher motoneurone firing rates required to produce strong contractions result in increased refractoriness associated to an after-hyperpolarisation trajectory (Todd *et al*., 2003, 2004; Sidhu *et al*., 2009a). A further important finding is the consistently low MEP obtained from the biceps femoris (Fig. 1), suggesting that inadvertent antagonist activation did not influence measurement of voluntary activation (Lee *et al*. 2008).

Reliability

Within-day maximal voluntary activation showed minimal systematic bias (0.23%) and a random error component of ±9.3%. These values mean that if a subject's maximal voluntary activation was 81.5% in Trial 1 (the lowest value observed for the group), it is possible that the same subject could obtain a result as low as 72.2% or as high as 90.8% in Trial 2. Between-day, maximal voluntary activation also showed minimal bias (–0.38%) with a random error of ±7.5% (Fig. 6). Although we are unable to compare directly our limits of

agreement with the reliability statistics reported in previous studies, our reliability coefficients for maximal voluntary activation were similar to those reported within-day for the knee extensors (CV of 3.7% in the present study vs. 3.1%; Sidhu *et al*. 2009a) and elbow flexors (CV of 3.7% in the present study vs. 3.7%; Todd *et al*. 2004) and between-day for the wrist extensors ($ICC_{2,1}$ of 0.94 in the present study vs. 0.95; Lee *et al*. 2008).

Fatigue

When the knee extensors were fatigued, a linear relationship was still evident between increasing voluntary strength and SIT torque. Therefore, extrapolation to identify the ERT amplitude is justified (Todd *et al*. 2003). The ERT amplitude was decreased following fatigue and consequently voluntary activation was significantly decreased (Fig. 5), indicating that supraspinal fatigue was present (Taylor & Gandevia, 2008). In comparison to the pre-fatigue state, the SIT amplitude was significantly increased during a maximal effort, indicating that motor cortical output was not maximal and was insufficient to drive the motoneurones maximally (Taylor *et al*. 2006). The 72% loss of torque during the 2-min isometric contraction is similar to the 77% reduction reported by Place *et al*. (2007) when implementing the same method to induce fatigue of the quadriceps.

Because the linear relationship between voluntary torque and cortical activation was still evident with fatigue, it was possible to determine the contribution of supraspinal fatigue during the fatiguing protocol. A comparison with the actual torque loss gives an estimate of the proportion of the total torque loss attributable to supraspinal mechanisms (Smith *et al*. 2007). Using this approach, we determined that mean maximal torque decreased by 31% (~99% to 68%) whereas cortical voluntary activation decreased by 17% (91% to 74%; Fig 5). Assuming that voluntary activation had remained at 91%, then mean maximal torque would have only dropped to 80% rather than 68% of control values. Thus, the remainder of the fall to 68% was due to reduced cortical voluntary activation in response to supraspinal fatigue, which accounted for 38% of the 31% reduction in mean maximal torque from the beginning

to the end of the fatiguing protocol. When assessed 30 min following the fatiguing contraction, voluntary activation had returned to values similar to those attained pre-fatigue. That the decrease in voluntary activation was reversed by a period of recovery indicates that the sustained contraction induced fatigue (Allen *et al*. 2008; Taylor & Gandevia, 2008). In addition to a decrease in voluntary activation, indicating supraspinal fatigue, peripheral fatigue was also present, as evidenced by a significant decrease in the amplitude of the resting twitch after the sustained contraction (~26% for motor nerve stimulation, ~25% for cortical stimulation). Therefore, the exercise-induced reduction in maximal volitional force was due to both a reduction in output from the motor cortex and peripheral factors, such as impairment in excitation-contraction coupling (Bigland-Ritchie *et al.* 1978).

Methodological Considerations

A potential concern when deriving voluntary activation is that the relationship between the SIT amplitude and voluntary torque is nonlinear. Kooistra *et al*. (2007), for example, suggested that since the relationship between SIT and voluntary torque is curvilinear with peripheral electrical stimulation, the ERT, and subsequently voluntary activation, may be overestimated using the extrapolation technique. In fact, when using TMS to derive ERT a curvilinear relationship is also observed, and at lower submaximal forces (<25%) the SIT evoked does not maintain the linear response (Lee *et al*. 2008). A linear relationship is expected if the TMS pulse activates the same number of motoneurons at different contraction strengths, and this has been shown to occur at contraction strengths above 50% MVC (Todd *et al*. 2003; Lee *et al*. 2008). The TMS pulse is less effective at activating motoneurons at lower force levels because cortical and spinal excitability are reduced (Todd *et al*. 2003). For linear extrapolation to be valid it is important that the stimulation activates most of the motoneurons (evoking a large MEP in relation to M_{max}; Fig. 1), which is achieved at high force levels. If the relationship at these high force levels is linear (Fig. 3) then it is appropriate to regress back to the *y*-axis and determine the estimated resting twitch amplitude (Todd *et al*. 2003; Lee *et al*. 2008; Sidhu *et al*. 2009a).

The severity of supraspinal fatigue may have been underestimated in the present study due to the time it took to complete the brief sets of test contractions. The 4 sets of contractions, including superimposed stimuli at 6 different contraction intensities, took 8.5 min to complete. That voluntary activation and MEP amplitudes were decreased provides evidence that fatigue was still apparent despite this prolonged testing procedure. It has previously been shown that central fatigue is still evident some time after cessation of this type of isometric exercise, as demonstrated by a decrease in MVC and depressed MEP area of the elbow flexors up to 8 min after a 2 min sustained contraction (Todd *et al*. 2005). However, a rationale can be established from our data to reliably use only three contraction intensities (50, 75 and 100% mean maximal torque) with the aim of estimating the resting twitch, thereby reducing the time necessary to carry out the testing protocol in future studies.

It is also important to consider how potentiation may have affected our results. The sets of submaximal contractions were administered in a randomised order, not always preceded by an MVC. Previous work has highlighted the need to fully potentiate the quadriceps muscle before delivering a peripheral stimulation (Bulow *et al*. 1993). However, the lack of significant difference for the SIT and consistent calculation of voluntary activation within- and between-days suggests that potentiation did not erroneously affect our results.

Conclusion

Using the procedures described in the present study, TMS provided reliable estimates of maximal voluntary activation of the knee extensors and enabled the assessment of supraspinal fatigue. The technique may be useful for quantifying cortical motor drive following fatigue and rehabilitation interventions. The technique may also be useful for monitoring muscle function, movement disorders and disease progression (Zwarts *et al*. 2008). Finally, the addition of the knee extensors to the small number of muscle groups in which this technique

has been previously validated provides empirical evidence that the technique may be applicable to a range of human muscle groups.

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Figure Legends

Figure 1. Group mean ± SD (n = 9 subjects) MEP areas evoked from the vastus lateralis (open symbols) and biceps femoris (closed symbols) by cortical stimulation at varying contraction intensities during trial 1 (circles), trial 2 (squares) and trial 3 (triangles). Trials 1 and 2 were separated by 30 min, and trial 3 was carried out after 19 ± 10 days. When compared with the area of the maximal M wave (M_{max}) evoked by peripheral stimulation of the femoral nerve during MVC (% control), the vastus lateralis MEP area grew rapidly until 50% mean maximal torque and decreased thereafter.

Figure 2. Raw vastus lateralis EMG data from a single subject showing the MEPs in response to TMS at different intensities of mean maximal torque and the maximal M-wave evoked by femoral nerve stimulation (M_{max}) (dashed lines indicate the delivery of stimulation). For all subjects, the largest MEP was evoked at 50% mean maximal torque; thereafter, MEP amplitude did not increase further.

Figure 3. Group mean ± SD (n = 9 subjects) amplitude of superimposed twitches (SIT) produced by TMS during contractions of increasing intensity across 3 trials separated by 30 min (Trials 1 & 2) and 19 ± 10 days (Trial 3). All torques are plotted as percentages of control mean maximal torque.

Figure 4. Group mean ± SD (n = 12 subjects) voluntary activation levels within- and between-days with the *y*-intercept determined from data between 50 and 100% MMT. 30 min elapsed between Trial 1 and Trial 2; 19 ± 10 d elapsed between Trial 2 and Trial 3. Dashed line is the line of identity.

Figure 5. Group mean ± SD (n = 9 subjects) voluntary activation levels before and immediately after a 2 min MVC with the *y*-intercept determined from data between 50 and 100% mean maximal torque. All torques are plotted as percentages of the MVC of the unfatigued muscle although with fatigue, contraction targets were set in relation to the fatigued muscle maximal voluntary torque. Dashed line is the line of identity.

Figure 6. Within-day (panel A) and between-day (panel B) Bland-Altman plots for maximal voluntary activation (VA) assessed using transcranial magnetic stimulation. The solid lines show the mean difference between the measures (systematic bias) and the dashed lines are the random error components.