Available online at www.sciencedirect.com
ScienceDirect
Journal of Electromyography and Kinesiology 17 (2007) 317–327
Journal of Electromyography and Kinesiology
www.elsevier.com/locate/jelekin

Methodological issues with the interpolated twitch technique

Jonathan P. Folland [a,*], Alun G. Williams [b]

[a] School of Sport and Exercise Sciences, Loughborough University, Loughborough, Leicestershire, UK
[b] Institute for Biophysical and Clinical Research into Human Movement, Manchester Metropolitan University, Cheshire, UK

Received 29 August 2005; received in revised form 31 March 2006; accepted 25 April 2006

Abstract

A number of methodological issues in the use of the interpolated twitch technique were investigated for their effect on true maximum force (TMF) and activation (ACT): timing of control (pre- vs post-contraction) and superimposed twitches (first vs second); type of twitch stimulus (primarily magnitude); and the type of extrapolation utilised. On three occasions subjects performed a series of maximal and sub-maximal contractions of the knee extensors, with electrically evoked twitches delivered before, during and after each contraction. The twitch–voluntary force relationship was concave for all types of twitch stimuli, and extrapolation using this relationship typically calculated TMF 39 N (7%) higher, and ACT 7% lower than linear extrapolation. The timing of the control (2–4%) and superimposed twitches (∼4%) both influenced TMF and ACT. Despite the different twitch stimuli being a range of magnitudes (13–32% maximum voluntary force) they did not affect TMF and ACT. A novel finding was that prior potentiation changed the shape of the twitch–voluntary force relationship. For precise measurement of TMF and ACT it is recommended that: extrapolation is based on the twitch–voluntary force relationship of the experimental model; and post-contraction potentiated twitches be used, as the superimposed twitch on a high level contraction appears to be potentiated.

Keywords: Skeletal muscle; Activation; True maximum force; Potentiation

1. Introduction

The measurement of muscle activation is fundamental to the quantitative assessment of human muscle function for clinical and research purposes. Unfortunately, there is no gold standard for the measurement of muscle activation, although the interpolated twitch technique (ITT) is one method that has been used extensively in this context (Shield and Zhou, 2003). It can facilitate measurement of the central drive to the muscle during a maximum voluntary contraction (MVC), whilst allowing accurate determination of maximum voluntary force.

The ITT typically involves comparing the magnitude of the twitch force evoked at rest with that evoked when superimposed upon an MVC (Gandevia et al., 1998). It is based on the inverse relationship between twitch size and voluntary force i.e. as voluntary force increases twitch force becomes increasingly occluded (Belanger and McComas, 1981). The rationale is that the fraction of the control twitch that remains superimposed on a maximum voluntary contraction indicates the proportion of non-activated muscle. This comparison can be used to extrapolate true maximum force (TMF – the theoretical maximum that could be achieved with full activation), and is implicit in the interpolation of the level of voluntary muscle activation (ACT).

Maximum voluntary force (MVF) depends upon the ACT achieved during a given series of voluntary contractions, and both ACT and MVF are influenced by subjective psychological factors such as motivation, attention, etc. Theoretically, as the estimate of TMF assumes full activation it offers a more consistent and reliable measure of the maximum force capability of a muscle than MVF as it might avoid the influence of these variables. However,

* Corresponding author. Tel.: + 44 1509 226334; fax: + 44 1509 226301.
E-mail address: j.p.folland@lboro.ac.uk (J.P. Folland).

1050-6411/$ - see front matter
doi:10.1016/j.jelekin.2006.04.008

the ITT has rarely been used experimentally to calculate TMF (e.g. De Serres and Enoka, 1998; Folland et al., 2000).

As the technology for measuring and recording force has evolved the ITT has become increasingly sensitive. Recent studies suggest that, on average, well motivated healthy subjects using force feedback achieve ACT values of <100% (i.e. <TMF) during maximum voluntary isometric contractions of major muscle groups (see Gandevia, 2001), indicating incomplete central nervous system recruitment of motor units or sub-maximal firing frequency of individual units. However the observed degree of sub-maximal ACT varies quite widely and one of the explanations for this are alternative choices in the methodology of the ITT. It has also been suggested that the ITT is an insensitive measure of ACT at near maximum contraction intensities (Herbert and Gandevia, 1999), perhaps due to the observed, but unexplained, variation in the magnitude of the superimposed twitch force (Oskouei et al., 2003). Whilst there has been some attention to the methodology of the ITT there remain a number of outstanding issues/variables in the use of this technique, which have either not been considered at all, not been fully addressed or considered in isolation.

1. *Timing of the control twitch:* The phenomenon of post-contraction potentiation has been widely documented, and significantly influences the magnitude of the control twitch (Tc) evoked whilst voluntarily passive. Immediately following a prolonged MVC control twitches can potentiate ~70%, before decaying exponentially, but remain significantly elevated above the pre-contraction unpotentiated twitch for at least 5 min (Hamada et al., 2000). However it is unclear whether pre-contraction (Tc-pre, e.g. Scaglioni et al., 2002) or post-contraction (Tc-post, e.g. Behm et al., 1996) control twitches, which may differ markedly in their degree of potentiation, should be used in the calculation of TMF and ACT. The question of which is the most valid may depend on whether the superimposed twitch is itself potentiated.
2. *Timing of the superimposed twitch:* We have observed anecdotally that the timing of the superimposed twitch upon an MVC may influence the calculation of TMF and ACT, but this has not been previously investigated. Therefore, any difference between two superimposed twitches delivered ~1 s apart (first (Ts-1) vs second (Ts-2)) was assessed.
3. *The form of extrapolation used:* The relationship between twitch force and voluntary force has been traditionally assumed to be a linear reciprocal one, and this is implicit in the calculation of ACT when a simple ratio of the superimposed twitch:control twitch is employed (Allen et al., 1995; Bigland-Ritchie et al., 1986; Chapman et al., 1985; Oskouei et al., 2003; Rice et al., 1992) (Fig. 1). However, there is considerable evidence from a range of models that it is not a linear reciprocal relationship (Allen et al., 1998; Behm et al., 1996; Belanger and McComas, 1981; De Serres and Enoka, 1998; Rutherford et al., 1986) with most of the evidence suggesting a concave curvilinear function. The quantitative effect of the type of extrapolation (linear reciprocal extrapolation (LREx) vs appropriate extrapolation (AppEx) based on the actual relationship) on TMF and ACT has been poorly documented, and could interact with other experimental variables.
4. *The type of superimposed stimulus:* It might be hypothesised that a larger superimposed stimulus might produce a greater signal-to-noise ratio and increase the validity and reliability of the ITT. The use of different types and magnitudes of superimposed stimuli has been addressed in the literature. Behm et al. (1996) found no significant difference between TMF (their predicted MVC) calculated with superimposed stimuli of different magnitudes (single, doublet and quintuplet stimulation). In contrast, larger magnitude pulse trains have been found to be more sensitive for the assessment of central activation failure than single impulses (Miller et al.,

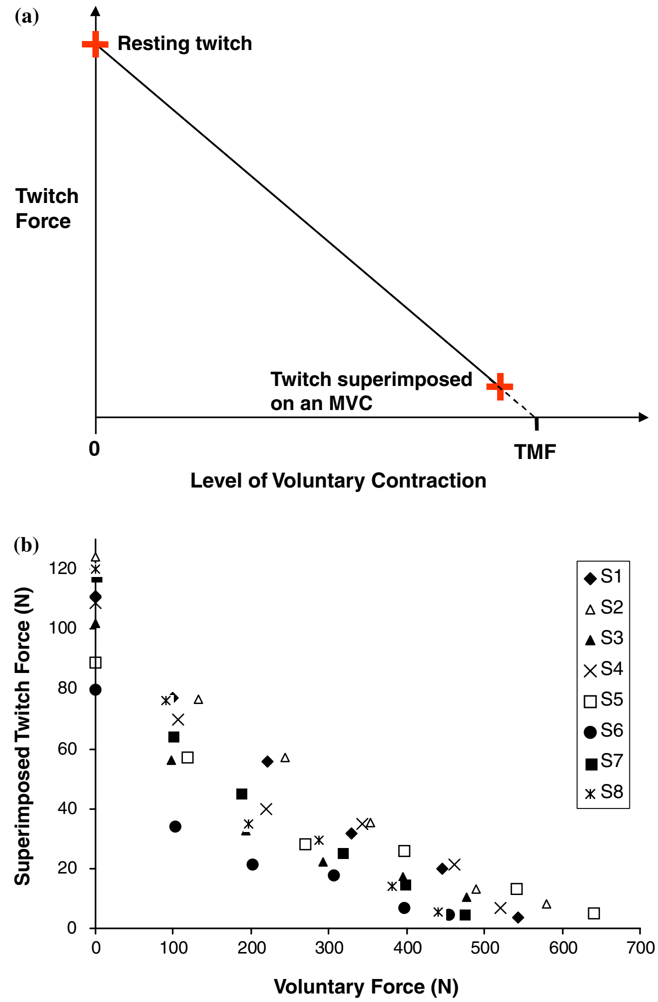

Fig. 1. (a) Assumed simple linear reciprocal relationship between superimposed twitch force and the level of voluntary force and (b) Individual superimposed twitch–voluntary force relationship of eight subjects with the LT stimulus.

1999). In order to examine a range of stimulus magnitudes small twitch (ST), large twitch (LT) and doublet (D) stimuli were compared.

Whilst previous work has examined the influence of some of these variables in isolation, their simultaneous assessment has not been documented, particularly with respect to quantifying their influence on TMF and ACT. The precise calculation of TMF and ACT is important when examining human muscle function *in vivo*, especially for research purposes when it is often used to infer neurological or physiological changes/adaptations. Study 1 examined the timing of the control twitch (Tc-pre vs Tc-post).

The aim of Study 2 was to quantify the influence of four variables (timing of the control twitch, timing of the superimposed twitch, type of twitch stimulus and the type of extrapolation) upon TMF and ACT. To contrast the type of extrapolation (AppEx vs LREx) required describing the twitch–voluntary force relationship for each type of twitch stimulus. The nature of the superimposed twitch (potentiated or unpotentiated) and the twitch–voluntary force relationship were also investigated following prior potentiation of one of the twitch stimuli (LT).

2. Methods

2.1. Experimental model and standard procedures

Isometric measurements were made of net knee extension force in the dominant leg of each subject at a knee joint angle of 1.40 rad (80°, 180° was full extension) with a conventional strength testing chair (described in Parker et al., 1990). Participants sat in the chair with a hip joint angle of 1.57 rad (90°) and tight highly restrictive straps were applied around the waist and shoulders to isolate the knee joint. Net knee extension force was measured with a calibrated U-shaped aluminium strain gauge (Jones and Parker, 1989) with a linear response up to 1000 N. A low noise amplifier was interfaced with an analogue to digital converter (CED micro 1401, CED, Cambridge, UK) and a PC utilising Spike 2 software (CED, Cambridge, UK) in order to sample knee extension force at 1000 Hz. At rest, baseline noise had an amplitude of <0.3 N. Raw data were sampled to the nearest 0.1 N, but for subsequent analysis readings were made to the nearest 0.5 N.

Twitches were evoked by delivery of percutaneous electrical impulses (50 μs duration, square wave pulses), via two carbon rubber electrodes (140 cm^2, Electro-Medical Supplies, Greenham, UK) taped securely on to the anterior surface of the thigh (the anode ~8 cm proximal from the superior border of the patella, and the cathode ~8 cm proximal from the anode). Doublets were elicited by two impulses with an interval of 10 ms. The electrical impulses were delivered with a constant current, variable voltage stimulator (DS7AH, Digitimer Ltd., UK) triggered by the CED micro 1401. In order to assist identification of superimposed twitches the stimulator output was simultaneously recorded on a separate channel.

Subjects were advised not to perform strenuous exercise in the 24 h prior to measurement, and to abstain from stimulants in the 3 h prior to measurement. Measurements on each individual were collected at a consistent time of day. Typically a series of 4 maximum voluntary contractions (MVCs), were used to assess maximum voluntary force (MVF) – the highest force achieved. Participants were instructed to contract as forcefully as possible from the start of each MVC and to try to achieve maximum force for 3 s. Verbal encouragement and biofeedback (an on-line force trace) were given throughout the trials, particularly before and during each MVC. If the subjects were unable to comply with the instructions, data were discarded. The studies were approved by the local Ethics committee.

2.2. Study 1: Timing of the control twitch

The participants were six young, healthy, recreationally active male volunteers (age, 21 ± 1 yr; body mass, 76.7 ± 4.5 kg; stature, 1.78 ± 0.03 m; mean ± SD). On one occasion subjects performed four MVCs to assess MVF. Single twitches were delivered automatically in trains of 10 at 1.25 Hz. The train of twitches commenced with three control twitches (Tc-pre) evoked whilst participants were passive prior to each MVC. Immediately after this, participants initiated an MVC typically with three further twitches superimposed upon it. After the MVC participants were passive as the remaining twitches (Tc-post), at least three, were evoked. In order to minimise potentiation of Tc-pre, and highlight the contrast of Tc-pre vs Tc-post, a 3 min rest interval between MVCs was used. The unpotentiated control twitches were sub-maximal and evoked a force of 10–15% of MVF.

For study 1 the experimental variable considered was the timing of the Tc (Tc-pre vs Tc-post). The magnitude of the superimposed twitches (Ts) and the level of voluntary force (VolF) at which each was evoked were averaged for each MVC and used to calculate a TMF value specific to each MVC using Eq. 1. LREx is implicit in this equation. These values were averaged across the four MVCs for a representative TMF value. This TMF value was used with MVF in order to calculate ACT (Eq. 2), i.e. ACT is expressed as a percentage of TMF. Table 1 lists the abbreviations of measured parameters.

$$\text{TMF} = (1/(1 - \text{Ts}/\text{Tc})) \times \text{VolF}. \quad (1)$$

$$\text{ACT}\ (\%) = \text{MVF}/\text{TMF} \times 100. \quad (2)$$

2.3. Study 2: Investigation of four variables

On four occasions within a 10-day period, 8 healthy, recreationally active, male volunteers (age, 28 ± 5 yr; body mass, 74.9 ± 4.9 kg; stature, 1.77 ± 0.05 m; mean ± SD) attended the laboratory. On each of the first three testing occasions subjects performed identical tasks: a series of

Table 1
Abbreviations of measured parameters

TMF	True maximum force
MVF	Maximum voluntary force
Tc	Control twitch
Tc-pre	Control twitches immediately prior to a contraction
Tc-post	Control twitches immediately after a contraction
Ts	Superimposed twitch
Ts-1	First superimposed twitch
Ts-2	Second superimposed twitch
VolF	Level of voluntary force
LREx	Linear reciprocal extrapolation
AppEx	Appropriate extrapolation
ST	Small twitch
LT	Large twitch
D	Doublet

MVCs and a series of incremental contractions. Subjects were randomly assigned to receive a different type of electrically evoked stimulus of contrasting magnitudes on each of these three measurement occasions (small twitch, ST; large twitch, LT; or a doublet, D). The intention was for the magnitude of the unpotentiated stimuli to be 12%, 20% and 30% MVF for ST, LT and D, respectively. Sub-maximal twitches were used to avoid any confounding influence of maximality of the stimulus. On each occasion subjects were familiarised to progressively larger control twitches of the specified type, by intermittent delivery of twitches until a sufficient force response was observed.

The series of four MVCs were performed with 30 s rest between each. Three twitches were delivered immediately before (Tc-pre), two superimposed during (Ts-1 and Ts-2) and three after (Tc-post) each MVC. Timing of the twitches was controlled manually, as it was felt manual control could provide two superimposed twitches at the highest levels of voluntary force. Typically there was at least 0.5 s between twitches.

The series of incremental contractions involved subjects performing voluntary contractions at 20%, 40%, 60%, 80% and 100% MVF for ~5 s duration, with 7 min rest before each contraction to ensure no prior potentiation. For each contraction the force trace was clearly visible to subjects on a large computer monitor and a marker used to indicate the desired force level. Participants were instructed to increase the force up to the desired level of contraction, and then hold it as steady as possible. Twitches were delivered as follows: three at rest before; three superimposed upon the contraction once the desired level of force was steadily maintained, and three after each contraction.

During the fourth laboratory visit an alternative series of incremental contractions was performed with the LT stimulus. Specifically, an MVC of 2–3 s duration was performed 5 s before each of the contractions at 20%, 40%, 60%, 80% and 100% MVF. The prior MVC ensured potentiation of twitches subsequently superimposed upon each of the incremental contractions. Twitches were delivered as three before and two during the MVC, three between the contractions, as well as three during and three after the incremental contractions.

2.3.1. Twitch magnitudes (Tc-pre, Tc-post, Ts-1, Ts-2)

The magnitude of control twitches was measured by the change in force from immediately prior to the twitch, until its peak and averaged for the three twitches before (Tc-pre) and three twitches after (Tc-post) each voluntary contraction.

The magnitude of each superimposed twitch was measured as the change in force attributable to the twitch. The magnitude of a superimposed twitch is influenced by the level of voluntary force, and any fluctuations during an MVC or other 'steady' contraction. Thus, for each superimposed twitch, the level of voluntary force (VolF) immediately prior to the twitch was also recorded. For sub-maximal contractions, the three superimposed twitches and accompanying voluntary force values were averaged to give mean Ts and VolF values. The two twitches superimposed during each MVC were measured independently and denoted as first (Ts-1) and second (Ts-2) superimposed twitches each with respective VolF values.

2.3.2. Calculation of the superimposed twitch–voluntary force relationship

From the series of incremental contractions, individual data were normalised (Ts to control unpotentiated twitch (Tc-pre), and VolF to MVF achieved in the prior series of MVCs) before being averaged across subjects for each level of contraction. The average normalised twitch–voluntary force relationship was then modelled with a range of linear and curvilinear functions in order to find the best 'fit' to the relationship.

2.3.3. Calculation of TMF

TMF values were calculated for each MVC with each type of twitch stimulus for every combination of the other three experimental variables: type of extrapolation; timing of the control twitch; and timing of the superimposed twitch. For each permutation of the experimental variables four TMF values were generated for each subject (one from each MVC). The within-subject coefficient of variation refers to the variability of these four TMF values, each generated from a different MVC. For comparison of the experimental variables these four values were averaged to give a representative TMF value for each individual, under those specific conditions, before data for alike conditions from all the subjects was pooled.

2.3.3.1. Linear reciprocal extrapolation (LREx). Linear reciprocal extrapolation is given by the reciprocal of the fraction of the control twitch that has been occluded during an MVC, multiplied by VolF (Eq. 1).

2.3.3.2. Appropriate extrapolation (AppEx). Approach to the problem: Different approaches for the use of AppEx with the ITT have been attempted. Some investigators have plotted the relationship (from the incremental series of contractions) for each individual, fitted this relationship with an individual specific function, typically curvilinear, in order to extrapolate up to TMF (De Serres and Enoka, 1998). Other researchers have used a generic function applied to the individual relationship, to find TMF (Scaglioni et al., 2002). The inconsistent nature of the individual relationship, from a single series of incremental contractions (Norregaard et al., 1997) or even from a repeated series of incremental contractions (De Serres and Enoka, 1998), provides little confidence in the description of an individual function for extrapolation up to TMF. It is also heavily reliant upon the validity of the twitch superimposed upon the final contraction of the incremental series – often a weak MVC.

Our approach was to use pooled data to accurately describe the normalised twitch–voluntary force relationship. Then this relationship was used with control and superimposed twitches measured during a particular MVC in order to estimate TMF. As well as the inherent advantages of this method, for the current methodological investigation it provided direct comparison of LREx and AppEx based on the identical source data.

In practice, calculation of TMF with AppEx involved using the function that best described the normalised twitch–voluntary force relationship, scaling this function to the relevant control twitch (Tc-pre or Tc-post) and superimposed twitch (Ts-1 or Ts-2) in order to extrapolate up to TMF. Essentially the function was solved for $Ts = 0$ (the x-axis intercept i.e. TMF), which gives TMF as a percentage of the VolF associated with the Ts, and as the absolute value of VolF was known an absolute TMF was generated.

2.3.4. Calculation of activation

ACT was calculated as the percentage of TMF activated voluntarily, MVF, during the series of MVCs (Eq. 2).

2.4. Statistics

Individual data were averaged for the four MVCs performed with each type of twitch stimulus prior to data being pooled for all eight subjects and expressed as means ± SEM. In order to calculate the influence of the four experimental variables a multivariate repeated measures General Linear Model was completed using SPSS v11.0 (SPSS, Chicago, IL). The two dependent variables were ACT and TMF, with four independent within-subjects factors (type of twitch; timing of the control twitch; timing of the superimposed twitch; and type of extrapolation). If a significant effect was observed a post hoc analysis was performed with Tukey's HSD test.

3. Results

3.1. Study 1: Timing of the control twitch

The average MVF of these subjects was 523 ± 36 N. As expected, Tc-post were clearly potentiated, being on average 46% greater, than Tc-pre (66 ± 2 vs 97 ± 4 N; or 13% vs 19% MVF). Using control twitches from pre-contraction (Tc-pre, unpotentiated) or post-contraction twitches (Tc-post, potentiated) significantly affected the calculation of TMF (Tc-pre, 552 ± 19 N; Tc-post, 528 ± 19 N; $P < 0.01$) and ACT (Tc-pre, 94.9 ± 0.7%; Tc-post, 99.1 ± 0.5%; $P < 0.01$). The four MVCs of each subject had a mean coefficient of variation (CoV) of 3.0%. The CoV for the four TMF values for each subject calculated with Tc-pre and Tc-post were on average 3.1% and 2.8%, respectively.

3.2. Study 2: Investigation of four variables

3.2.1. MVF, control twitches and potentiation

The MVF of these subjects was very consistent across the three measurement occasions with different stimuli (D, 568 ± 13 N; LT, 571 ± 18 N; ST, 575 ± 17 N), and the CoV for MVF of each individual over the three occasions was on average 1.6%. The individual variability of peak MVC force during the series of four attempts on each occasion was 2.9%, 2.1% and 2.7% for the D, LT and ST stimuli, respectively.

The control unpotentiated evoked forces for the different stimuli were on average: ST, 74 N (13 ± 1% MVF); LT, 108 N (19 ± 1% MVF); D, 180 N (32 ± 1% MVF) (Table 2). The initial MVC of each series potentiated the control twitches by 49%, 53% and 13% for ST, LT and D, respectively. This compared with potentiation of 59% for the LT stimulus following two MVCs with just 5 s between them.

With only a 30 s rest between MVCs, Tc-pre increased during the series of 4 MVCs for each type of stimulus, and was thus less consistent than Tc-post (CoV: Tc-pre, 5.5–14.6%; Tc-post, 1.4–3.4%). During the incremental series of contractions, with 7 min rest between each, there was a non-significant decline in Tc-pre from 106.7 to 102.3 N (within-subject CoV: 3.4%).

3.2.2. Superimposed twitch–voluntary force relationship

The shape of the superimposed twitch–voluntary force relationship for each individual was somewhat erratic (Fig. 1(b) – LT stimulus as an example). The pooled normalised relationship for the ST and LT stimuli exhibited a very similar shape, which was clearly curvilinear when the full range of voluntary force was considered (Fig. 2). Initially a quadratic function was used to model the rela-

Table 2
Control twitch magnitude and potentiation during the series of 4 MVCs of Study 2

	ST		LT		D	
	Tc-pre	Tc-post	Tc-pre	Tc-post	Tc-pre	Tc-post
MVC#1 (N)	73	110	108	163	180	205
MVC#4 (N)	102	113	159	173	204	205
$\overline{X}$ (N)	91	112	135	168	195	205
CoV[a] (%)	13.4	3.1	14.6	3.4	5.5	1.4

Mean values shown.
[a] Within-subject coefficient of variation.

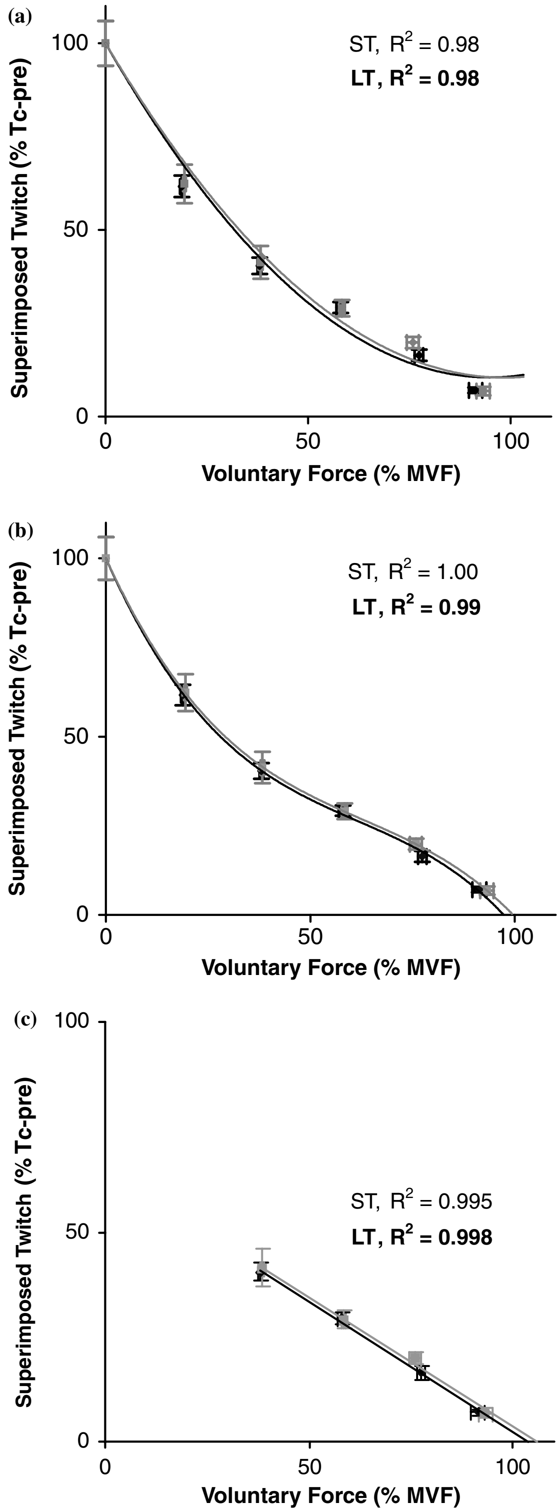

Fig. 2. Superimposed twitch–voluntary force relationship for the LT (black) and ST (grey) stimuli. The relationship was fitted with (a) a quadratic function for all points, (b) a cubic function for all data points (c) a linear function for voluntary forces of 40% MVF and above.

tionship (Fig. 2a), but this did not intercept the x-axis for either the LT or ST data, irrespective of whether the control twitch was included in the curve fitting procedure. As this was both illogical and of no use in extrapolating up to TMF it clearly indicated an inappropriate function. A cubic function was then used to model the relationship for the ST and LT stimuli (Fig. 2b). In both cases this provided a good visual fit to the data, but described a curve which intersected the x-axis at less than 100% MVF, providing an implausible estimate of TMF.

The twitch–voluntary force relationship was clearly curvilinear at low levels of voluntary force; however, when only twitches superimposed at 40% MVF and above were considered a linear function provided a good fit ($R^2 > 0.99$, Fig. 2c) for both the LT and ST relationships, as well as generating feasible TMF values (ST, 105.7% MVF; LT, 103.3% MVF). The superimposed twitch–voluntary force relationship for these two stimuli was described by the following equations (where y is twitch magnitude, and x the level of voluntary force):

$$\text{ST}: y = -0.6179x + 65.30 \quad (3)$$

$$\text{LT}: y = -0.6270x + 64.76 \quad (4)$$

The superimposed twitch–voluntary force relationship for the D stimulus was also clearly not linear (Fig. 3). A quadratic function provided a good visual and statistical fit to the data ($R^2 > 0.99$), in addition to generating feasible values for TMF (104.4% MVF).

$$\text{D}: y = 0.005555x^2 - 1.538x + 100 \quad (5)$$

Fig. 4 illustrates how potentiation affected the twitch–voluntary force relationship for the LT stimulus. Prior potentiation with an MVC significantly increased the magnitude of the Ts at lower levels of voluntary force (20%, 40% and 60% MVF, $P < 0.01$) and hence changed the shape of the twitch–voluntary force relationship. However, at high levels of voluntary force (>70% MVF) the superimposed twitch was of similar magnitude both with and without prior potentiation, and there was convergence of the twitch–voluntary force relationships in this region.

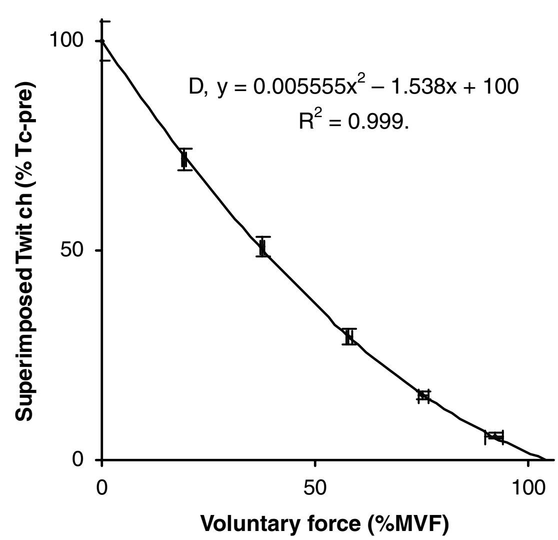

Fig. 3. Superimposed twitch–voluntary force relationship for the D stimulus. The relationship was fitted with a quadratic function.

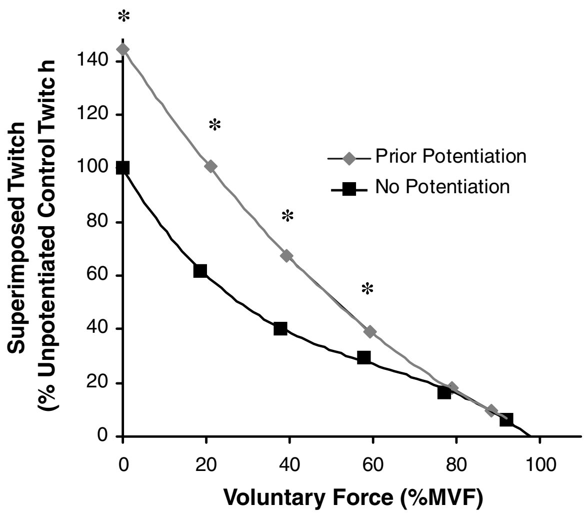

Fig. 4. Potentiation and the twitch–voluntary force relationship for the LT stimulus. The 'prior potentiation' data are fitted with a quadratic function and the 'no potentiation' data with a cubic function [mean, $n = 8$]. *Significant difference between the twitch magnitudes for the two conditions (Paired t-test, $P < 0.05$).

3.2.3. Calculation of TMF and activation

The calculated TMF and ACT values for all combinations of the four experimental variables are shown in Table 3. The range of TMF and ACT values was from 553 to 647 N and 88.3% to 102.4% MVF, respectively. Clearly some of the TMF values presented in Table 3 are implausible, given that it is impossible to achieve >100% ACT (i.e. TMF cannot be <MVF).

The variability of TMF values, calculated with each combination of the experimental variables, are shown in Table 4. Within-subject CoV for TMF was similar for the three types of stimuli (mean, range: D, 2.7%, 2.4–3.3; LT, 2.2%, 1.3–3.3; ST, 2.6%, 2.1–3.2, Table 4). For each type of stimulus, TMF exhibited similar consistency to MVF. It was also notable that the most consistent TMF values were achieved with the ratio of Ts-1:Tc-post for each type of stimulus.

3.2.3.1. Type of extrapolation. AppEx produced significantly higher TMF values than LREx (TMF, data collapsed across the other factors: LREx, 574 ± 15 N vs AppEx, 612 ± 15 N; $P < 0.001$), on average 39 N (6.7%) higher. The type of extrapolation also significantly affected ACT with AppEx producing on average 6.6% lower values (ACT: LREx, 99.6 ± 0.6% vs AppEx, 93.0 ± 0.9%; $P < 0.001$).

3.2.3.2. Timing of the control twitch. Timing of the control twitch significantly influenced the calculation of TMF and ACT, with higher TMF values and lower ACT values generated by Tc-pre compared to Tc-post (data collapsed across the other factors, TMF: Tc-pre, 597 ± 15 N vs Tc-post, 587 ± 15 N; $P < 0.001$. ACT: Tc-pre, 95.5 ± 0.8% vs Tc-post, 97.1 ± 0.7%; $P < 0.001$). The difference in TMF values for Tc-pre vs Tc-post was on average 10 N (1.7%, range: 2–21 N), but varied in magnitude according to the type of twitch stimulus (ST, 11 N; LT, 16 N; D, 3 N) and this was a significant interaction ($P < 0.01$). Similarly the difference in ACT values for Tc-pre vs Tc-post was on average 1.6% and this was significantly influenced by the type of twitch stimulus (ST, 1.7%; LT, 2.5%; D, 0.5%; $P < 0.001$).

3.2.3.3. Type of twitch stimulus. Despite the contrast in the magnitude of the different types of twitch stimuli (13%, 19%, 32% MVF) TMF was not significantly affected by the type of twitch stimulus employed (data collapsed across

Table 3
TMF and ACT calculated with different combinations of the experimental variables

Stimulus (MVF)	Extrapolation	Ts-1:Tc-pre	Ts-2:Tc-pre	Ts-1:Tc-post	Ts-2:Tc-post
ST (MVF: 575 ± 16.6)	LR				
	TMF (N)	591 (17)	569 (17)	583 (16)	562 (16)
	ACT (%)	97.3 (0.8)	101.1 (0.95)	98.6 (0.7)	102.4 (0.8)
	App				
	TMF (N)	631 (19)	608 (17)	617 (17)	595 (16)
	ACT (%)	91.1 (1.2)	94.5 (1.4)	93.2 (1.0)	96.5 (1.1)
LT (MVF: 571 ± 17.0)	LR				
	TMF (N)	596 (18)	570 (18)	584 (18)	559 (18)
	ACT (%)	95.6 (0.6)	100.0 (0.9)	97.6 (0.5)	102.0 (0.8)
	App				
	TMF (N)	647 (20)	617 (18)	626 (19)	598 (17)
	ACT (%)	88.3 (1.1)	92.4 (1.4)	91.2 (0.9)	95.4 (1.1)
D (MVF: 568 ± 14.4)	LR				
	TMF (N)	580 (13)	557 (14)	578 (13)	553 (14)
	ACT (%)	97.8 (0.6)	101.9 (1.2)	98.2 (0.6)	102.3 (1.2)
	App				
	TMF (N)	615 (16)	592 (17)	611 (15)	588 (16)
	ACT (%)	91.3 (1.2)	94.8 (1.2)	91.8 (1.2)	95.4 (1.1)

Type of stimulus (ST vs LT vs D); extrapolation (LR, linear reciprocal vs App, appropriate); timing of the control twitch (Tc-pre vs Tc-post); timing of the superimposed twitch (Ts-1 vs Ts-2).
Mean (SEM).

Table 4
Mean coefficient of variation for TMF calculated from each of four MVCs for each subject, and using different combinations of the experimental variables

Stimulus	Extrapolation	Ts-1:Tc-pre	Ts-2:Tc-pre	Ts-1:Tc-post	Ts-2:Tc-post
ST (MVC, CoV: 2.9%)	LR	2.6	2.7	2.3	2.6
	App	2.9	3.2	2.1	2.7
LT (MVC, CoV: 2.1%)	LR	1.4	1.8	1.3	1.9
	App	3.1	3.3	2.5	2.5
D (MVC, CoV: 2.7%)	LR	2.5	3.3	2.4	3.2
	App	2.6	2.7	2.4	2.6

Type of stimulus (ST vs LT vs D); extrapolation (LR, linear reciprocal vs App, appropriate, based on the superimposed twitch–voluntary force relationship); timing of the control twitch (Tc-pre vs Tc-post); timing of the superimposed twitch (Ts-1 vs Ts-2).
In brackets MVC CoV with each stimulus is shown.

other variables, TMF: ST, 594 ± 15 N vs LT, 600 ± 18 N vs D, 584 ± 14 N; $P = 0.14$). ACT was also not significantly affected by the type of twitch stimulus (ACT: ST, 96.8 ± 1.0% vs LT, 95.3 ± 0.8% vs D, 96.7 ± 0.8%; $P = 0.14$). For specific combinations of the other factors, the type of twitch stimulus had an inconsistent influence upon TMF (ranging from 6 to 33 N, 1.0% to 5.3%) and ACT (0.4–3.0%).

3.2.3.4. Timing of the superimposed twitch. The timing of the superimposed twitch significantly influenced both TMF and ACT (data collapsed across the other factors, TMF: Ts-1, 602 ± 15 N vs Ts-2, 578 ± 15 N; $P < 0.001$; ACT: Ts-1, 94.6 ± 0.7% vs Ts-2, 98.4 ± 0.9%; $P < 0.001$). The effect of the first or second superimposed twitch was on average 23 N (4.0%, range: 21–29 N) upon TMF and, 3.8% (range, 3.3–4.4%) upon ACT.

4. Discussion

The main findings of this study were the significant influence of three of the experimental variables (type of extrapolation, timing of the control twitch and timing of the superimposed twitch) on the calculation of TMF and ACT. The range of possible values for TMF (553–647 N) and ACT (88.3–102.4%) displayed in Table 3 are testament to the influence of these factors. Considering the use of the ITT for research and clinical purposes this range of possibilities appears to be of consequence.

Theoretically, TMF might be expected to exhibit less variation than MVC as it should not be influenced by motivation and ACT, and this would certainly be desirable from a measurement perspective. However, this was not the case and TMF exhibited similar variability to MVC.

4.1. The superimposed twitch–voluntary force relationship and potentiation

In agreement with many authors we found the full range of the superimposed twitch–voluntary force relationship to be clearly non-linear and deviate quite substantially from a simple linear reciprocal relationship for all three types of twitch stimuli (Behm et al., 1996; Belanger and McComas, 1981; Dowling et al., 1994; Scaglioni et al., 2002), but particularly the LT and ST. The 7 min rest prior to each incremental contraction may have minimised potentiation and thus the magnitude of the superimposed twitch at low levels of voluntary force, possibly exaggerating the curvilinear nature of our findings.

The twitch–voluntary force relationship for the D stimulus, the least curvilinear of the three data sets, was well represented by a second order polynomial. Fitting an appropriate curvilinear function to the twitch–voluntary force relationship of the ST and LT stimuli was more problematic, and this is by no means unique in comparison to the literature where different polynomial (Behm et al., 1996), exponential (Scaglioni et al., 2002), and power (De Serres and Enoka, 1998) functions have been applied. We attempted to fit the full range of data with a number of polynomial functions, before finding a linear relationship through the range 40–95% MVF to provide a good and utilitarian fit to the data. Using a very similar quadriceps model to the present experiment, Norregaard et al. (1997) also found a linear twitch–voluntary force relationship in the range 60–100% MVC.

A number of explanations for the non-linearity of the twitch–voluntary force relationship have been put forward (Gandevia, 2001), including an increasing contribution of synergist muscles or co-activation of antagonist muscles at high levels of torque, modification of the motor unit activation pattern (recruitment vs rate coding) throughout the range of voluntary force, collision between central drive and antidromic stimulus current (Yue et al., 2000), and compliance within the biological or measurement system (Loring and Hershenson, 1992). Compliance of any kind could dampen the measurement of twitch force and increase the non-linearity of the relationship, but would tend to cause greater damping at low levels of voluntary force and promote a convex relationship (Belanger and McComas, 1981, 1986), as opposed to the largely concave relationship we have observed. Antidromic collision might also be expected to cause relatively smaller twitches at high levels of voluntary force and thus a convex relationship. Changes in the contribution of synergists or modification of the motor unit activation pattern could conceivably account for the observed relationship, although a consistent pattern across all three stimuli might be expected if this were the case.

The current study found a similar relationship for both single pulse stimuli (LT and ST) despite their different magnitudes and this contrasted with a less curvilinear relationship for the D stimulus. The D stimulus displays markedly less potentiation than the other stimuli, and varying potentiation could account for the curvilinear nature of the ST and LT relationships. Notably, the prior potentiation condition with the LT stimulus (Fig. 4) described a much less curvilinear relationship, similar in shape to the relationship for the D stimulus (Fig. 3). When there is little scope for potentiation a more linear relationship appears to exist, but when there is scope for potentiation a more complex relationship emerges. This suggests potentiation is changing throughout the no potentiation condition for the ST and LT stimuli. Thomas et al. (1990) also suggested that there was a positive correlation between potentiation and the linearity of the twitch–voluntary force relationship.

From the contrast of the prior potentiation and no potentiation conditions in Fig. 4 (LT stimulus only), it seems that in rested unpotentiated muscle the superimposed twitch is unpotentiated at low levels (<40% MVF) and potentiated at high levels of voluntary force (>75% MVF). This is a novel finding that to our knowledge has not been clearly documented before. Following prior potentiation the superimposed twitches were significantly greater at low levels (20–60% MVF) but not high levels (>60% MVF) of voluntary force, compared to the no potentiation condition, making the twitch–voluntary force relationship much less concave/curvilinear. Bulow et al. (1993) found that a twitch superimposed upon a contraction at 20% MVC responded to prior potentiation, but these authors did not consider higher level contractions. Furthermore, in the absence of prior potentiation, the superimposed twitch appears to potentiate according to the level of voluntary contraction in a linear fashion above 40% MVF, and the potentiation appears to occur at the onset of the contraction (within ~1 s).

Evidence from human studies suggests a greater degree of potentiation in type II fibres (Hamada et al., 2000; Vandewoort et al., 1983). This is in agreement with observations in small mammals, where greater potentiation of type II muscle fibres is associated with greater phosphorylation of myosin regulatory light chains, which is regarded as the primary mechanism of potentiation (Grange et al., 1993; Sweeney et al., 1993). According to Henneman's size principle of motor unit recruitment, the type II motor units are increasingly recruited at higher levels of force. An interaction between fibre type and potentiation could explain the increasing potentiation of the superimposed twitch above 40% MVF and the shape of the superimposed twitch–voluntary force relationship (in the no potentiation condition).

4.2. Type of extrapolation

Our finding of the twitch–voluntary force relationship to clearly not be a linear reciprocal, is sufficient to question the use of this extrapolation for the calculation of TMF and ACT. However the present study is the first to specifically quantify this effect, finding AppEx to produce significantly higher TMF (on average 6.4%, 7.6% and 6.1% higher for ST, LT and D) and lower ACT (on average 6.0%, 7.0% and 6.7% lower for ST, LT and D) values than LREx. Hence, use of LREx without evaluating the nature of this relationship seems inappropriate, and in the current study overestimated ACT by 6–7%.

Theoretically it would seem ideal to use the individual twitch–voluntary force relationship to extrapolate up to TMF for each subject. However, the individual relationships (Fig. 1 (b)) demonstrate an erratic and unpredictable shape. This is similar to the individual relationships found by Norregaard et al. (1997) and Behm et al. (1996), and does not inspire confidence in using them to extrapolate up to TMF. It would be beneficial if future studies could improve the reliability of the individual twitch–voluntary force relationship. We employed a different approach applying the pooled normalised relationship to two individual data points (control twitch and superimposed twitch on an MVC) in order to calculate TMF and ACT. This approach clearly does not fully encompass individual differences in the twitch–voluntary force relationship, but did facilitate direct comparison of LREx and AppEx based upon the same source data.

4.3. Timing of the control twitch

From Study 1, the difference in TMF of 24 N and ACT of 4.2% according to the choice of Tc-pre or Tc-post appeared substantial. Given the difference in magnitude between Tc-pre and Tc-post (66.2 vs 96.9 N) this was not surprising – a superimposed twitch of any given magnitude clearly represents a greater fraction of Tc-pre than Tc-post, and automatically leads to calculation of lower ACT and higher TMF values. The 3 min interval between MVCs was used to accentuate the difference in potentiated (Tc-post) and relatively non-potentiated (Tc-pre) control twitches. Study 2 found that even with a standard protocol of 30 s rest between MVCs, and substantial potentiation of Tc-pre, the choice of Tc-pre or Tc-post still influenced TMF and ACT. The interaction effect between the timing of the control twitch and the type of twitch stimulus upon TMF and ACT was not surprising considering that the D exhibits much less potentiation than the single pulse stimuli (ST and LT), and hence is not influenced by the choice of Tc-pre vs Tc-post to the same degree.

Quantifying the effect of using pre or post contraction control twitches does not however address the question of which is the most valid. Our earlier conclusion that for high level voluntary contractions the superimposed twitch is itself potentiated (Fig. 4) suggests that a potentiated control twitch i.e. Tc-post would provide the most valid contrast for the calculation of TMF and ACT. In support of this Bulow et al. (1993) concluded that stable and high potentiation is important for accurate estimation of TMF.

In terms of reliability, a control twitch that exhibits constancy would be preferable. Theoretically an unpotentiated twitch might be expected to be more consistent and reproducible than a highly potentiated twitch. Given the short rest period during the series of MVCs the variability of Tc-pre was entirely expected, explaining why TMF calculated with Tc-post was in general more reliable than with Tc-pre (lower CoV for 11 of 12 comparisons, Table 4). However, even with a 7 min rest between the incremental contractions there was a trend towards a decline in Tc-pre (CoV, 3.44%), similar to that observed in the triceps brachii by Hamada et al. (2000). In contrast Tc-post was more consistent than expected during the series of four MVCs (CoV: 3.1%, 3.37% and 1.4% for ST, LT and D respectively), and hence less variable than Tc-pre. Thus in terms of both reliability and validity the potentiated control twitch (Tc-post) is recommended for the calculation of TMF and ACT.

4.4. Type of twitch stimulus

The different twitch stimuli were of significantly different magnitudes (unpotentiated control twitch: 12.9% (ST), 18.8% (LT) and 31.5% MVF (D)). It was hypothesised that the greater signal-to-noise ratio of a larger superimposed stimulus would increase the validity and reliability of TMF and ACT, and the doublet stimulus has been recommended (Oskouei et al., 2003). Furthermore, the less curvilinear twitch–voluntary force relationship with the D stimulus might be expected to produce more consistent TMF values (steeper intercept with the x-axis). Nevertheless, the type of twitch stimulus did not effect TMF or ACT. In terms of reliability, when data were collapsed across all other factors TMF displayed similar variability for the three stimuli (LT CoV, 2.2%; ST, 2.6%; D, 2.7%). Gandevia (2001) pointed to greater antidromic activation of motoneurons and Renshaw cells as potential confounders with increasing numbers of pulse stimuli, which may counter any improvement in the signal-to-noise ratio.

Previous studies have reported conflicting results with superimposed stimuli of different magnitude and duration for the measurement of ACT. In one of the most thorough investigations to date, Behm et al. (1996) found no effect of single, doublet or quintuplet stimulation on predicted MVC (TMF). Allen et al. (1998) also found different stimuli (single, doublet and quadruple pulses) superimposed upon voluntary contractions above 85% produced evoked responses of similar absolute and relative magnitude. In contrast, Strojnik (1995) and Miller et al. (1999) reported greater torque increments with stimulus trains of >100 ms duration (at 100 Hz) compared to a single twitch. A substantial level of tetanic stimulation may cause discomfort, a significant distraction from production of a genuine maximum voluntary contraction and thus compromise measurement of MVF and ACT.

4.5. Timing of the superimposed twitch

Timing of the superimposed twitch (Ts-1 vs Ts-2) significantly influenced TMF by, on average, 23 N (4%) and ACT by 4%. This is a novel finding that has not been previously documented and appears to be substantial for the precise measurement of TMF and ACT. From the current data it is impossible to comment on the reason for this difference. However, the data produced when using the second superimposed twitch were less plausible – in particular some ACT values exceeded 100% TMF. In terms of reliability, Ts-1 typically produced more reliable TMF values (10 out of 12 comparisons) than Ts-2.

5. Conclusions

In rested unpotentiated muscle, the superimposed twitch appears to be unpotentiated at low levels (<40% MVF) and potentiated at high levels of voluntary force (>75% MVF), and this phenomenon may explain the concave nature of the twitch–voluntary force relationship for single pulse stimuli (ST and LT). In this experiment, the use of AppEx generated TMF values 22–50 N higher and ACT values 5.4–7.6% lower than LREx. When using the ITT for the precise measurement of TMF and ACT it is strongly advised that the twitch–voluntary force relationship is described for the experimental model employed, and that AppEx is utilised.

Post-contraction potentiated twitches are recommended, as this appears more valid given that the superimposed twitch on a high level contraction seems to be potentiated. Short duration stimuli (1–2 pulses) over a range of magnitudes up to ~1/3 MVF produced similar TMF and ACT values, however, the LT stimulus is recommended as it produced more reliable TMF values.

References

Allen GM, Gandevia SC, McKenzie D. Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. Muscle Nerve 1995;18:593–600.

Allen GM, McKenzie DK, Gandevia SC. Twitch interpolation of the elbow flexor muscles at high forces. Muscle Nerve 1998;21:318–28.

Behm DG, St-Pierre DM, Perez D. Muscle inactivation: assessment of interpolated twitch technique. J Appl Physiol 1996;81:2267–73.

Belanger AY, McComas AJ. Extent of motor unit activation during effort. J Appl Physiol 1981;51:1131–5.

Bigland-Ritchie B, Furbush F, Woods JJ. Fatigue of intermittent submaximal contractions: central and peripheral factors. J Appl Physiol 1986;61:421–9.

Bulow PM, Norregaard J, Dannesskiold-Samsoe B, Mehlsen J. Twitch interpolation technique in testing of maximal muscle strength: influence of potentiation, force level, stimulus intensity and preload. Eur J Appl Physiol 1993;67:462–6.

Burke RE. Revisiting the notion of 'motor unit types'. Prog Brain Res 1999;123:167–75.

Chapman SJ, Edwards RHT, Greig C, Rutherford O. Practical application of the twitch interpolation technique for the study of voluntary contraction of the quadriceps muscle in man. J Physiol (Lond) 1985;353:3P.

De Serres SJ, Enoka RM. Older adults can maximally activate the biceps brachii muscle by voluntary command. J Appl Physiol 1998;84:284–91.

Dowling JJ, Konert E, Ljucovic P, Andrews DM. Are humans able to voluntarily elicit maximum force?. Neurosci Lett 1994;179:25–8.

Folland JP, Maas H, Jones DA. The influence of nitric oxide on in-vivo human skeletal muscle properties. Acta Physiol Scand 2000;169:141–8.

Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Phys Rev 2001;81:1725–89.

Gandevia SC, Herbert RD, Leeper JB. Voluntary activation of human elbow flexor muscles during maximal concentric contractions. J Physiol 1998;512:595–602.

Grange RW, Van Denboom R, Houston ME. Physiological significance of myosin phosphorylation in skeletal muscle. Can J Appl Physiol 1993;18:229–42.

Hamada T, Sale DG, MacDougall JD. Post-activation potentiation in endurance-trained male athletes. Med Sci Sports Exerc 2000;32:403–11.

Hamada T, Sale DG, MacDougall JD, Tarnopolsky MA. Postactivation potentiation, fibre type, and twitch contraction time in human knee extensor muscles. J Appl Physiol 2000;88:2131–7.

Henneman E. Relation between size of neurons and their susceptibility to discharge. Science 1957;126:1345–7.

Herbert RD, Gandevia SC. Twitch interpolation in human muscles: mechanisms and implications for measurement of voluntary activation. J Neurophysiol 1999;82:2271–83.

Jones DA, Parker DF. Development of a portable strain gauge to measure human muscle isometric strength. J Physiol 1989;145:11P.

Loring SH, Hershenson MB. Effects of series compliance on twitches superimposed on voluntary contractions. J Appl Physiol 1992;73: 516–521.

Miller M, Downham D, Lexell J. Superimposed single impulse and pulse train electrical stimulation: a quantitative assessment during submaximal isometric knee extension in young healthy men. Muscle Nerve 1999;22:1038–46.

Norregaard J, Lykkegaard JJ, Bulow PM, Danneskiold-Samsoe B. The twitch interpolation technique for estimation of true quadriceps muscle strength. Clin Physiol 1997;17:523–32.

Oskouei MAE, van Mazijk BCF, Schuiling MHC, Herzog W. Variability in the interpolated twitch torque for maximal and sub-maximal contractions. J Appl Physiol 2003;95:1648–55.

Parker DF, Round JM, Sacco P, Jones DA. A cross-sectional survey of upper and lower limb strength in boys and girls during childhood and adolescence. Ann Hum Biol 1990;17:199–211.

Rice CL, Vollmer TL, Bigland-Ritchie B. Neuromuscular responses of patients with multiple sclerosis. Muscle Nerve 1992;15:1123–32.

Rutherford OM, Jones DA, Newham DJ. Clinical and experimental application of the percutaneous twitch superimposition technique for the study of human muscle activation. J Neurol Neurosurg Psychiat 1986;49:1288–91.

Scaglioni G, Ferri A, Minetti AE, Martin A, Van Hoecke J, Capodaglio P, et al.. Plantar flexor activation capacity and H reflex in older adults: adaptations to strength training. J Appl Physiol 2002;92:2292–302.

Shield A, Zhou S. Assessing voluntary muscle activation with the twitch interpolation technique. Sports Med 2003;34:253–67.

Strojnik V. Muscle activation level during voluntary effort. Eur J Appl Physiol 1995;72:144–9.

Sweeney HL, Bowman BF, Stull JT. Myosin light chain phosphorylation in vertebrate striated muscle: regulation and function. Am J Physiol 1993;264:C1085–95.

Thomas CK, Johansson G, Westling G, Bigland-Ritchie B. Twitch properties of human Thenar motor units measured in response to intraneural motor-axon stimulation. J Neurophysiol 1990;64:1339–46.

Vandewoort AA, McComas AJ. A comparison of the contractile properties of the human gastrocnemius and soleus muscles. Eur J Appl Physiol 1983;51:435–40.

Yue GH, Ranganathan VK, Siemionow V, Liu JZ, Sagath V. Evidence of inability to fully activate human limb muscle. Muscle Nerve 2000;26:376–84.

Jonathan Folland was born in Wolverhampton, England, on 6 April 1973. In 1994 he received his B.Sc. degree from Loughborough University, UK, prior to completing a Ph.D. examining resistance training, nutritional and genetic influences on human muscle strength and function at the University of Birmingham, UK, in 2000. He now lectures and researches in exercise physiology in the School of Sport and Exercise Sciences at Loughborough University, UK. His research interests include applied exercise physiology and biomechanics with particular interest in human neuromuscular function in response to acute and chronic exercise interventions and the influence of genetic variation.

Alun Williams was born in Swansea, Wales, on 9 February 1972. He received his M.Sc. in 1996 from the University of Liverpool, UK, and his Ph.D. in 2001 from the University of Birmingham, UK. Most recently, he has worked in the Institute for Biophysical and Clinical Research into Human Movement at Manchester Metropolitan University, UK, teaching and researching Exercise Physiology. His research interests have included some applied exercise physiology, but now mainly focus on genetic, molecular and hormonal influences on muscle function, principally *in vivo*. A particular research interest is the inter-individual variability in human muscle performance and training responses.