Methodological issues with the assessment of voluntary activation using TMS in the knee extensors

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ABSTRACT

The assessment of voluntary activation of the knee extensors using transcranial magnetic stimulation (VA_{TMS}) is routinely performed to assess the supraspinal function. Yet methodological scrutiny of the technique, whether used at rest or more crucially following exercise, is scarce. The aim of the present study was to examine face validity and reliability of VA_{TMS} and its two main determinants (superimposed twitch during a maximal voluntary contraction [$SIT_{100\%}$] and estimated resting twitch [ERT]) at rest and following intermittent isometric fatiguing exercise. Responsiveness of VA_{TMS} to the exercise intervention was also measured. The findings indicated issues regarding the accuracy of ERT and suggested a three-point relationship should not to be used to determine ERT. Reliabilities for VA_{TMS}, $SIT_{100\%}$ and ERT were acceptable at rest but much weaker post-exercise (especially for $SIT_{100\%}$). Despite statistically significant changes in the main neuromuscular variables following the intermittent isometric fatiguing exercise ($P<0.05$), when post-exercise reliability was considered, the exercise effect on VA_{TMS} was smaller than the smallest detectable change in 18 of the 20 individual tests performed, and for the whole sample for one of the two visits. Consequently, these changes were not deemed detectable. Finally, neuromuscular fatigue was present following the neuromuscular assessment (NMA) at rest, and recovery was evidenced during the post-exercise NMA questioning the face validity of this routinely used protocol.

Words: 217

INTRODUCTION

The generation of muscle force during a voluntary contraction is initiated by the motor cortex driving motor neurons that activate motor units. The level of neural drive from the primary motor cortex (M1) to the force-generating muscles, i.e. voluntary activation (VA; see review Gandevia, 2001), can reach 90-95% during maximal voluntary contractions (MVC) of non-fatigued healthy muscles (Todd et al., 2003; Lee et al., 2008; Sidhu et al., 2009a;b). Exercise may reduce VA (Todd et al., 2003; Goodall et al., 2009; Sidhu et al., 2009a), a phenomenon defined as central fatigue (see review Gandevia, 2001).

Major advances in the design of neuromuscular assessment protocols (NMA) to study VA have been made since the interpolated twitch technique was first proposed (Merton, 1954). To quantify VA, a single supramaximal stimulation of an alpha-motoneuron can be performed during an isometric voluntary contraction. The presence of an evoked superimposed twitch (SIT), the amplitude of which is normalized to a twitch elicited by the same supra-maximal stimulation in the potentiated but relaxed muscle (i.e. Resting Twitch; RT), may be interpreted as sub-optimal VA (Merton, 1954). In complement to this peripheral stimulation, magnetic stimulation of the first neuron of the corticospinal tract provides further information regarding the site of neural drive impairment, i.e. supraspinal mechanisms (see review Gandevia, 2001). The presence of a superimposed twitch from transcranial magnetic stimulation (TMS) of the M1 region evidences submaximal motor output from the motor cortex (Gandevia et al., 1996;Todd et al., 2003;Lee et al., 2008;Sidhu et al., 2009a;b)

In their original work on the elbow flexors, Todd et al. (2003) recognised the challenges associated with the measure of VA from transcranial magnetic stimulation of the motor cortex (VA_{TMS}) due to the inappropriateness of the cortically evoked resting twitch to normalise the superimposed twitch (Ugawa et al., 1995;Di Lazzaro et al., 1998), mirroring the original method based on supramaximal stimulation of the alpha-motoneuron (Todd et al., 2003). A method for estimating the resting motoneural output evoked by cortical stimulation, based on a linear extrapolation of the relationship between cortically evoked super-imposed twitch (SIT) and voluntary

force (> 50% MVC) was proposed, tested and validated for the elbow flexors (Todd et al., 2003; 2004;Todd et al., 2007). This estimated resting twitch (ERT in Equation 1) is then used for computation of VA_{TMS}. Since then, this technique has been validated in the knee extensors (Sidhu et al., 2009a; Goodall et al., 2009), plantar flexors (Green et al., 2014), back extensors (Lagan et al., 2008) and wrist extensors (Lee et al., 2008).

Equation 1: $\mathbf{VA_{TMS}}\ (\%) = \left(\mathbf{1} - \frac{\mathbf{SIT}}{\mathbf{ERT}}\right) \boldsymbol{x}\ \mathbf{100}$

In exercise physiology, a significant loss in VA_{TMS} following physical exercise has a clear and accepted qualitative meaning - supraspinal fatigue is present (Søgaard et al., 2006;Taylor et al., 2006). For the 'interpretability' (Mokkink et al., 2010) of a reduction in VA_{TMS} as evidence of supraspinal fatigue, its measure must be highly *(1)* reliable (i.e. free from measurement error - also called 'absolute reliability' or 'agreement'; Terwee et al., 2007) and *(2)* responsive (i.e. ability to detect change over time in the construct being measured; Terwee et al., 2007). This interpretability also requires for the measurement to hold strong *(3)* face validity (i.e. adequate reflection of the construct to be measured), both pre- and post-exercise (Mokkink et al., 2010). Because the reliability of both ERT and SIT threatens the evaluative properties of VA_{TMS} (Equation 1), minimal measurement errors for these variables should also be sought.

A three-contraction NMA (100%, 75% and 50% MVC), repeated three times, is today the gold standard protocol used in the measurement of supraspinal fatigue following cycle (Sidhu et al., 2009b; Girard et al., 2013; Jubeau et al., 2014; Thomas et al., 2015; Thomas et al., 2016) or knee-extension exercise (Goodall et al., 2010; Gruet et al., 2014; Périard et al., 2014). This method seems to provide good measures of absolute reliability for VA_{TMS} in the resting muscle, with coefficients of variation (CV) < 3% (Goodall et al., 2009; Thomas et al., 2015; Thomas et al., 2016; Goodall et al., 2017). Absolute reliabilities in a fatigued state have been reported in a single study with indications that reproducibility is much weaker compared to rest (ERT: 8-9%, VA_{TMS}: 5-18%; Goodall et al., 2017). Poor reliability in a fatigued state could mean that the technique of VA_{TMS} may not be accurate in calculating the degree of

supraspinal fatigue experienced by exercise performers. Intraclass Correlation Coefficients (ICC) indicates good relative reliability for VA_{TMS} of the resting knee extensors (r = 0.85-0.95 in Sidhu et al., 2009; 0.94 in Goodall et al., 2009; 0.90 in Goodall et al., 2017; 0.98 in Thomas et al., 2015; 0.90 in Thomas et al., 2016) and this finding is of value for those interested in the diagnosis of corticospinal drive impairments at rest (Sidhu et al., 2009a). But it is a high absolute reliability that is critical when interpreting VA_{TMS} changes post-intervention so that a true change can be detected (Schambra et al., 2015;Beaulieu et al., 2017). Currently there is only one study reporting reliability of SIT scores (Goodall et al., 2009).

The calculation of the ERT assumes a linear relationship between SIT and voluntary torque. Whilst the exact number of data points used to estimate this relationship is often not explicitly stated, in the literature there appears to have been a shift from the inclusion of multiple (Sidhu et al., 2009a;b: 5-28 points), to a minimum of three points with scarce evidence regarding the goodness-of-fit of the linear model. Finally, face validity of any NMA protocol may be threatened by a possible NMA-induced fatigue effect or, when the NMA is performed after the completion of a fatiguing exercise, confounded by a potential recovery effect. Goodall et al. (2009) reported a recovery of SIT during their NMA protocol. MVC, potentiated twitch force, and VA_{TMS} (Gruet et al., 2014) have been shown to recover within a few minutes in the knee extensors (see review Carroll et al., 2017). This threat to the face validity of what is today the gold standard protocol for the measure of VA_{TMS} has not been scrutinised any further.

Therefore, the present investigation is a scrutiny of the three-contraction protocol (100%, 75% and 50% MVC) routinely used to assess supraspinal fatigue following exercise in the knee extensors. The present study was designed to (1) test the reproducibility of previously published findings (Goodall et al., 2009; Sidhu et al., 2009a; Thomas et al., 2015; Thomas et al., 2016; Goodall et al., 2017) by quantifying the absolute reliability of VA_{TMS} in the resting knee extensors, with the addition of the reliability of the two main VA_{TMS} determinants (i.e. $SIT_{100\%}$ and ERT; Equation 1) alongside an examination of the relationship between SIT amplitude and voluntary torque; (2) to quantify absolute and relative reliability for SIT, ERT and cortical VA_{TMS} in the fatigued knee extensors; (3) to ascertain whether the main measurement

outcomes hold face validity in a fresh muscle (pre-exercise) by testing for a fatigue effect, and in a fatigued muscle (post-exercise) by testing for a recovery effect; (4) to test the responsiveness of the main measurement outcomes following a fatiguing exercise. We hypothesized that: (1) Pre-exercise, absolute and relative reliability for VA_{TMS} and ERT would be good (CV ≤ 5%, ICC > 0.85), in accordance with previous findings. There is no published evidence concerning the reliability of the SIT, but because VA_{TMS} has good reliability at rest, we expected similar values for both ERT and SIT; (2) Lower absolute and relative reliability of all NMA variables in the fatigued muscles, in accordance with previous findings (Goodall et al., 2017); (3) No development of fatigue throughout the NMA assessment in a fresh muscle but a significant muscular recovery for MVC and potentiated twitch force while the NMA protocol is taking place post-exercise.

METHODS

Ethical approval

All experimental procedures were conducted in accordance with the *Declaration of Helsinki* with approval granted by the institute's research ethics committee. Written informed consent was provided by all volunteers prior to participation.

Participants

Ten healthy, recreationally active males (mean ± SD; age: 24 ± 5 years) volunteered to participate in the present investigation. Prior to enrolment, participants were informed of the purpose of the investigation and completed a health-screening questionnaire, ensuring each was free of contraindications to TMS (Rossi et al., 2011). Participants were not taking prescribed medication and reported no history of cardiovascular, neurological or musculoskeletal disorders. Over the duration of the investigation, participants were instructed to refrain from the consumption of both caffeine and alcohol, and the performance of strenuous exercise in the 24 hours preceding each visit.

Experimental set-up

Isometric contractions of the right knee extensors were performed on a multi-joint isokinetic dynamometer (CON-TREX® MJ, CMV AG, Dubendorf, Switzerland). The reliability of this system in the assessment of KE function has previously been reported (Maffiuletti et al., 2007). Participants sat on the high-backed dynamometer with hip and knee angles set at approximately 85º and 90º, respectively (0º = full extension). Extraneous movements of the upper body were minimized through straps fastened across both the chest and pelvis, and a cushioned restraint placed across the active mid-thigh. Participants' head motion was constrained through a cervical neck brace attached to the back of the dynamometer. A shin-pad attached to the lever arm of the dynamometer was secured to the participant's leg approximately 3-4 cm proximal to the lateral malleolus. The centre of the rotational axis of the dynamometer was aligned to the axis of the knee joint (lateral femoral epicondyle) before the start of each trial. During KE contractions, participants were instructed to place their arms across their chest, griping the contralateral shoulder strap.

Torque and Electromyography (EMG)

Isometric torque was digitized (4 kHz) and analysed using LabChart v7.0 software (ADInstruments, Oxfordshire, UK). Surface EMG activity was recorded from the right *vastus lateralis* (VL) and *bicep femoris* (BF) with pairs of self-adhesive electrodes (Kendall™ H59P, Coviden, Massachusettes, USA). Electrode pairs were positioned intersecting the muscle belly based on SENIAM guidelines (Hermens et al., 2000) and adjusted to optimise the electrically-evoked responses. The reference electrode was placed on the electrical neutral ipsilateral patella. The skin-electrode interface was prepared by shaving the recording area, lightly abrading and cleansing with a 70% (v/v %) isopropyl alcohol wipe to minimize electrical resistance. The site of electrode placement was recorded in relation to set anatomical landmarks and photographs taken to standardise electrode orientation across repeated measures. EMG signals were amplified (gain x1000) (PowerLab 26T; ADInstruments), digital band-pass filtered (20-2000 Hz), digitized (4 kHz), recorded and later analysed off-line (LabChart v7.0).

Stimulation techniques

Torque and EMG responses to TMS over the primary motor cortex and electrical femoral nerve stimulation were used to characterise VA_{TMS} and peripheral neuromuscular function of the KE, respectively.

Femoral nerve stimulation: Single percutaneous electrical stimuli (duration: 200 μs) were delivered to the right femoral nerve via a pair of square (5 x 5 cm) self-adhesive neuro-stimulation electrodes (Valutrode CF5050; Axelgaard Manufacting Co., Ltd., California, USA), attached to a high-voltage (maximal voltage: 400 V) constant-current stimulator (Model DS7AH, Digitimer Ltd., Hertfordshire, UK). The cathode was placed high in the femoral triangle with the anode positioned midway between the ipsilateral greater trochanter and iliac crest (Sidhu et al., 2009a). Precise location of cathode placement was determined through systematic adjustments of the electrode until the greatest twitch torque (Q_{tw}) and VL muscle compound action potential (M-wave) amplitude was elicited for a particular sub-maximal current (~70 – 90 mA) (Johnson et al., 2015). This position was recorded and marked with indelible ink for replication between each trial. Optimal stimulation intensity was defined as the intensity at which a plateau in both Q_{tw} and VL M-wave was exhibited. Optimal stimulation intensity was determined through progressive increments in stimulator current (+20 mA) from 10 mA, with two stimuli delivered at each intensity. Stimulation intensity was increased by a further 30% in order to ensure full spatial recruitment of KE motor units. This process was repeated before each trial, with a small difference observed between sessions (147 ± 41 mA; 132 ± 39 mA; $t_{(9)} = 2.45$, P=0.04).

TMS: Single magnetic, monophasic stimuli (duration: 1 ms) were manually delivered over contralateral (left) primary motor cortex, powered by a magnetic stimulator (maximum output of 1.4 T) (Magstim^{200}, The Magstim Company Ltd., Whitland, UK), using a concave (110 mm) double-cone coil. Orientation of the coil was positioned so as to induce a posterior-anterior intracranial current flow within the cortex. Optimal coil position (1-2 cm left of vertex) was defined as the site at which the largest motor evoked potential (MEP) was evoked in the VL during a weak

contraction (20% MVC) of the KE at 70% maximal stimulator output, with minimal concurrent activation of the antagonist BF. This site was marked directly onto the scalp with indelible ink. KE MEP response plateaus with increasing stimulator output, but antagonist excitability increases with higher intensities which may reduce the size of the superimposed twitch (Temesi et al., 2014) resulting in the possible overestimation of VA (Bachasson et al., 2016;Todd et al., 2016). As such, stimulator output intensity during the assessment of VA_{TMS} was selected based on the largest SIT evoked during a brief (~6 s) contraction at 50% MVC (Thomas et al., 2016). Stimulator output intensity was increased step-wise in 5% increments from 50% of maximal stimulator output until a plateau was reached, with two stimuli delivered at each intensity during a single contraction, then averaged. Each contraction was separated by 15 s rest. The determination of stimulator intensity was conducted prior to each trial, with no difference in mean stimulator output observed throughout the experimental period (66 ± 8%; 65 ± 8%; t_9 = 1.41, P=0.19). The stimulator output activated a similar proportion of the KE motoneurone pool across sessions, as evidenced by the comparable MEP/M_{max} ratio during KE MVCs (no between-session difference, $F_{(1,8)}$=0.56, P=0.48; no exercise effect, $F_{(1,8)}$=0.01, P=0.90; significant difference between the three levels of contractions, $F_{(2,16)}$=6.08, P=0.01; Figure 1). Moreover, this intensity simultaneously evoked small absolute MEP responses in the antagonist BF (between-session difference, $F_{(1,9)}$=9.82, P=0.01; no exercise effect, $F_{(1,9)}$=1.94, P=0.19; significant difference between the three levels of contractions, $F_{(2,18)}$=7.67, P=0.01, but with no difference in the pairwise comparisons (P>0.05); Figure 1).

Figure 1. here please

Experimental design

The reliability and accuracy of VA_{TMS} was compared across two experimental sessions, both at rest and after the induction of neuromuscular fatigue. Participants visited the laboratories on three separate occasions, with a minimum of 48 hours separating each session (mean experimental duration: 6 ± 4 days). Individual participant trials were conducted at the same time of day (± 2 hours) to account for diurnal variations in maximal torque generation and corticospinal excitability (Tamm et al., 2009). During the preliminary session, participants were thoroughly

familiarised with the performance of MVCs and the procedures used within the assessment of VA_{TMS} and peripheral neuromuscular function, before performing a fatiguing single-joint exercise task (*see Fatiguing exercise*). The subsequent trials represented the basis of the main experimental investigation. Each trial commenced with a standardised isometric KE contraction warm-up (Froyd et al., 2013), followed by the performance of 3-4 MVCs (each separated by 2 minutes) until coefficient of variation (CV) across the final three contractions was <5% (Girard and Racinais, 2014). Participants rested while seated for 5 minutes before the experimental trial commenced. Strong verbal encouragement was provided throughout all voluntary contractions, with visual feedback of torque provided on a monitor positioned approximately 1.5 m directly in front of the dynamometer.

Neuromuscular assessment (NMA) protocol: The NMA protocol began with the performance of three brief (3-5 s) MVCs. Percutaneous electrical stimulation of the femoral nerve was applied both on the plateau in voluntary torque during the final contraction and 1-2s after contraction ended on the relaxed muscle; this was performed in order to record a fully potentiated twitch force (POT, Kufel et al., 2002). The greatest voluntary torque recorded during the three brief maximal contractions was used to set visual guidelines for the individual submaximal torque levels. Three sets of contractions followed, each consisting of voluntary contractions at 100%, 75% and 50% MVC, performed in descending order, with a single TMS pulse superimposed onto each contraction. Each MVC was followed by percutaneous stimulation of the femoral nerve. Rest periods of 25 s preceded each MVC, with 15 s preceding each sub-maximal contraction (50% and 75% MVC). Upon completing the three sets of contractions, a final MVC with resting femoral stimulation was performed in order to assess recovery of neuromuscular fatigue across the assessment of VA_{TMS}. In total, the number of contractions performed during the assessment of VA_{TMS} was 13 and the NMA protocol lasted 279 s (Figure 2). The NMA was repeated, after a small delay (10 s), upon completing the single-joint fatiguing exercise to characterise the development of neuromuscular fatigue.

Fatiguing exercise: Devised by Gruet et al. (2014), a fatiguing isometric exercise reported to induce rapid reductions in VA_{TMS} was adopted. The exercise task consisted of a sustained isometric contraction at 50% MVC for 15 s, followed

immediately by 5 s of maximal effort (MVC). This sequence was subsequently repeated following 10 s of rest. During the familiarisation session, the sequence of contractions was performed until task failure, defined as the point at which voluntary torque fell below 50% MVC for >2 s (Gruet et al., 2014). During the experimental trials, participants performed only the number of successfully completed contractions completed during the familiarisation session, in order to standardise time in contraction between trials (mean: 165 s ± 38 s [range: 120–240 s]).

Figure 2. here please

Data analysis

For all voluntary contractions conducted during the VA_{TMS} assessment protocols, torque was recorded as the greatest 500 ms average, prior to stimulation. Mechanical (i.e. SIT, POT) and EMG responses (i.e. MEP and M-wave) were analysed for peak-to-peak amplitude over discreet time-windows (800 ms) following each stimulation.

Agonist MEP responses were normalised to the electrically evoked EMG response during the maximal contraction (M_{max}) preceding the VA_{TMS} assessment sets. It has previously been reported that M_{max} is unaffected by increases in voluntary force from 40% to 100% MVC (Bachasson et al., 2016), removing the necessity for M_{max} at each voluntary torque level. Absolute antagonist MEP amplitude was assessed at each torque level. All torque and EMG variables were averaged across sets for each voluntary torque level. To investigate the magnitude of the fatigue effect, indices of peripheral and central neuromuscular function were compared before and after the performance of the single-joint exercise.

Fatigue index (%) during the single-joint exercise task was quantified as the change in maximal voluntary torque from the first to the last contraction of the task. Maximal voluntary torque recorded during the fatiguing exercise was recorded as the greatest 4 s average during the last 5 s of each contraction sequence.

Two methods were used to model the linear regression between SIT amplitude and voluntary torque (Todd et al., 2003; 2004): (1) all 9 data points over the three contraction levels were included in the linear regression (Todd et al., 2004;Lee et al.,

2008;Sidhu et al., 2009b); (2) an average of the three values for each level of contraction was computed, providing three data points for the linear regression VA_{TMS} was then calculated using Equation 1.

Statistical analysis

Data are reported as mean ± SD for parametric sets unless otherwise stated. Normal Gaussian distribution set was verified for each data using the Shapiro-Wilk test. Two- and three-way ANOVAs with repeated measures were performed on the main neuromuscular variables to assess effects for fatiguing exercise (2 levels; pre- *vs* post-exercise), NMA protocol (2 levels; pre- *vs* post-NMA), and session (2 levels: Session 1 *vs* 2) depending on the research question. The compound symmetry, or sphericity, was checked using Mauchly's test. When the assumption of sphericity was not met, the significance of F-ratios was adjusted according to the Greenhouse–Geisser procedure. Least-squares linear regressions were performed to determine ERT as the *y*-intercept of the linear SIT-VC relationship. Coefficients of determination (r^2) and standard error (SE) associated with slope and *y*-intercept estimates were calculated to examine the goodness-of-fit of the models. Relationships between two variables were explored using Pearson's product-moment correlation. Paired sample *t*-tests were used to test for a between-session difference in ERT, $SIT_{100\%}$, and VA_{TMS}. All statistical procedures were performed using SPSS (version 22, Chicago, USA) with the null hypothesis rejected at an alpha level of 0.05. Effect sizes are presented as partial eta squared (η_p^2) for main and interaction effects and Cohen's d_{av} for pairwise comparisons.

Absolute reliability was assessed through calculation of Typical Error of Measurement (TEM = SD of individual differences / $\sqrt{2}$) sometimes named 'Standard Error of Measurement' (Hopkins, 2000). Systematic biases and random errors were assessed from Bland and Altman plots (Atkinson and Nevill, 1998;Hopkins, 2000). Heteroscedasticity was examined by plotting absolute differences against individual means with subsequent calculation of Pearson correlation coefficient following prior check for normal Gaussian distributions (heteroscedasticity correlation coefficient, HCC). HCC was used to assess the significance of the relationships. If

heteroscedasticity was detected or the differences not normally distributed, the data were logarithmically transformed. In a second step, heteroscedasticity and normal Gaussian distribution were tested from the log-transformed data. The 95% absolute or ratio limits of agreement were calculated accordingly. Relative reliability was quantified through calculation of Intraclass Correlation Coefficient (two-way random effect; A,1; McGraw and Wong, 1996). Due to the ceiling effect associated with the measure of cortical VA, ICC was not calculated for this variable (Clark et al., 2007).

The smallest detectable change or the minimum chance for a change likely to be 'real' ($P<0.05$) for one individual was also calculated for each key variable (SDC_{ind} = 1.96 x $\sqrt{2}$ x SEM; Terwee et al., 2007). To be noted, SDC is the same as the 95% limit of agreement from the Bland and Altman plot. Sample's SDC values were derived from SDC_{ind} (Terwee et al., 2010). Responsiveness of the key measures of neuromuscular fatigue was ascertained for each participant and for the sample of participants when an individual pre- to post-intervention difference (Δ change) and the mean change in the individual differences (Δ change in the mean) were greater than SDC_{ind} and $SDC_{sample,}$ respectively (Table 3).

RESULTS

Descriptive data and statistical analysis for the fatiguing exercise are presented in Appendix 1. Absolute torque values for the sets of three voluntary contractions (VC) and three SITs used to calculate ERT are presented in Table 1. There was no significant difference between the two sessions for each variable (VC: $F_{(1,9)}=0.30$, $P=0.59$, $\eta_p^2=0.03$; and SIT $F_{(1,9)}=0.03$, $P=0.86$ $\eta_p^2=0.004$;).

Table 1. here please

Reliability of neuromuscular assessment

Absolute and relative reliabilities for all variables pre-and post-exercise are presented in Table 2. Data for 100% of MVC and POT is included for further information. For each variable, the between-session difference was not significant (Table 2; $P>0.05$).

Table 2. here please

Relationship between the SIT and voluntary torque

There was a significant decrease in SIT as the level of voluntary contraction increased ($F_{(2,18)}$=55.9, P<0.01, η_p^2=0.93). The relationship between SIT and VC torque amplitudes was analyzed using linear regressions (Figure 3). Only 16 of the 120 three-point relationships (session 1 and 2*;* within-NMA set 1, 2, and 3; n=10) were statistically linear (P<0.05, r^2 of 1); the remaining 104 relationships were not (P>0.05, r^2=0.89 ± 0.13). Because so few of these relationships were linear, these data were not analyzed further.

The nine-point linear regression was significant for each individual NMA carried out pre-exercise (P<0.05). The relationship post-exercise was not linear for one participant (r^2 = 0.33; P=0.11). Removal of one identified outlier in their data set (a SIT at 50%MVC; >1.96 SD from casewise diagnostic) led to a significant relationship (r^2=0.61; P=0.02), with an 8-point regression used for ERT determination as a consequence. All other individual 9-point regressions were significantly linear (P<0.05). The two-way ANOVA with repeated measures found no significant difference in the models goodness-of-fit (r^2=0.91 ± 0.03 pre-exercise, session 1; r^2=0.88 ± 0.05 pre-exercise, session 2; r^2=0.82 ± 0.12 post-exercise, session 1; r^2 = 0.80 ± 0.10 post-exercise, session 2; $F_{(1,9)}$<0.1; P=0.98, η_p^2<0.01) and standard error in the ERT estimates (3.23 ± 1.10 N.m pre-exercise, session 1; 3.72 ± 1.42 N.m pre-exercise, session 2; 2.38 ± 0.92 N.m post-exercise, session 1; 2.20 ± 1.09 N.m post-exercise, session 2; $F_{(1,9)}$=0.19; P=0.68, η_p^2=0.02) between the two sessions but with a significantly weaker r^2 ($F_{(1,9)}$=12.5; P=0.006, η_p^2=0.58) and smaller SE-ERT ($F_{(1,9)}$=10.8; P=0.009, η_p^2=0.54) post-exercise. No significant difference was depicted for the SE associated with estimation of the slope of the relationship (P>0.05).

Face validity of the neuromuscular assessment

To examine whether there was a fatiguing effect of the NMA pre-exercise, or a recovery between NMA sets post-exercise, MVC and POT were recorded immediately before and after each neuromuscular assessment (Figure 4). A three-way ANOVA (session x NMA x exercise) did not find a significant between-session difference (P>0.05; η_p^2=0.12 for POT and η_p^2=0.009 for MVC) or session-factored

interaction effect (P>0.05; exercise *x* session: η_p^2=0.06 for POT and η_p^2=0.16 for MVC; NMA *x* session: η_p^2=0.09 for POT and η_p^2=0.006 for MVC). The sets of data from the two sessions were therefore pooled together for further investigation of a possible effect of the NMA protocol (*n*=20). Interaction effects (MVC: $F_{(1,19)}$=32.4, P<0.001, η_p^2=0.63; POT: $F_{(1,19)}$=5.60, P=0.026, η_p^2 =0.235) showed that the NMA reduced MVC and POT pre-exercise (MVC: -12.5 ± 18.2 N.m, P=0.006; POT: -2.90 ± 2.88 N.m, P<0.001). Only MVC significantly recovered during the NMA performed post-exercise (15.1 ± 15.6 N.m, P<0.001). POT was not statistically different despite a clear trend (6.9 ± 3.9 N.m, P=0.06), with visual inspection of Figure 4 indicating POT recovered in all but one participant.

Exercise significantly reduced MVC torque (~27%; $F_{(1,9)}$ = 63.6, P<0.001, η_p^2=0.88) and POT (~39%; $F_{(1,9)}$ = 87.2, P<0.001, η_p^2=0.91; Table 2). When normalized to MVC, SIT did not change significantly following exercise ($F_{(1,19)}$ = 1.74, P=0.20, η_p^2=0.24, Table 1). However, there was a significant change in absolute SIT scores (in N.m) ($F_{(1,9)}$ = 41.3, P<0.01, η_p^2=0.82; Table 1), with larger decreases at lower % MVCs ($F_{(2,18)}$ = 67.7, P<0.01, η_p^2=0.88; Table 1). These changes led to significant decreases in both slope ($F_{(1,9)}$ = 18.2, P<0.01, η_p^2=0.67) and *y*-intercept (e.g. ERT; ~46%; $F_{(1,9)}$ = 72.9, P<0.001, η_p^2=0.89; Table 2) of the linear relationship between SIT and VC following exercise (Figure 2). VA_{TMS} decreased significantly as a consequence (~13%; $F_{(1,9)}$ = 40.7, P<0.001, η_p^2=0.82; Table 2).

Figure 4. here please

The responsiveness of the NMA to fatiguing exercise, examined using calculation of smallest detectable change (Terwee et al., 2007), is displayed in Table 3.

Table 3. here please

DISCUSSION

The present study examined the reliability and validity of the three-contraction neuromuscular assessment protocol routinely used to measure VA_{TMS} of the knee

extensors. Absolute and relative reliability, face validity, and responsiveness to a fatiguing exercise for the determinants of VA_{TMS} were measured. As hypothesized, whilst the NMA had acceptable reliability pre-fatiguing exercise, it was less reliable after. The relationship between SIT and voluntary torque, used to calculate ERT, was only linear when nine points were used in the model. The NMA itself induced fatigue pre-exercise, and there was recovery of neuromuscular performance during the NMA post-exercise. These results suggest that the calculation of VA_{TMS} using the established three-contraction protocol may be problematic. To our knowledge, this is the first study quantifying absolute and relative reliability of these three variables at pre-and post-fatiguing exercise. An intermittent isometric fatiguing exercise reported to induce neuromuscular fatigue in the knee extensors (Gruet et al., 2014) was used in the present study. Performance in the task was reliable and reduced peak torque (*see supplementary materials*). The decrements in both MVC and POT were greater than the pre- and post-exercise TEM and their respective SDC obtained in the present study for both measures (Table 2 and 3) and therefore display detectable change.

The present findings regarding TEM (in % of the mean) for VA_{TMS} (2.5% and 11.9% in the fresh and fatigued muscle fibers recruited with TMS, respectively; Table 2) are consistent with the between-session coefficients of variation reported in the literature (< 3% at rest; Goodall et al., 2009; Goodall et al., 2017; Thomas et al., 2015; Thomas et al., 2016; 5-18% post-exercise; Goodall et al., 2017) and suggest that changes in VA_{TMS} measured in a fresh state are likely to be detected (Table 2 and 3). Some caution is warranted however, considering the very poor reliability of $SIT_{100\%}$, one of VA_{TMS} constituents (Table 2), and a lack of sensitivity in VA_{TMS} in response to a change in $SIT_{100\%}$ (as previously reported in Goodall et al., 2009). This may be due to the fact that both determinants of VA_{TMS}, i.e. $SIT_{100\%}$ and ERT (Equation 1), share putative mechanisms and can therefore be affected by the same covariates. Examples would be peripheral fatigue (Contessa et al., 2016) or co-activation of the knee flexors with TMS (*technical challenge 1,* Todd et al., 2016). When $SIT_{100\%}$ and ERT are affected in similar proportions, VA_{TMS} as a ratio remains the same (Equation 1). Furthermore, because of the orders magnitude of the SITs compared to the voluntary contractions (about a fifth), a large change in $SIT_{100\%}$ (increase caused by a sub-maximal MVC for example) will have an inherently small impact (decrease) on the

539 extrapolated ERT and computed VA_{TMS} (Equation 1). This may explain the better
540 reliability of ERT alongside VA_{TMS} despite weak reliability in $SIT_{100\%}$.
541
542 Absolute reliability of $SIT_{100\%}$ has only been reported once (pre-exercise with similar
543 findings; Goodall et al., 2009) yet has a critical influence of VA_{TMS} estimation
544 (Equation 1). This intra-individual variability in the present study could be partially
545 due to variability in recruitment of the antagonists (MEP responses in the antagonist
546 BF were session-dependent in our study; Figure 1), and / or the NMA protocol
547 implemented. The present protocol was proposed in the original NMA protocol
548 (Goodall et al., 2009; Sidhu et al., 2009a) and is still in use today (Thomas et al.,
549 2015; Thomas et al., 2016; Goodall et al., 2017). In the present study, mean torque
550 developed voluntary while evoking $SIT_{100\%}$ through TMS was sub-maximal (96 ± 2%
551 and 98 ± 3% of the pre-determined MVC for pre- and post-exercise, respectively; the
552 former was significantly different to 100%, $P<0.05$) and could be a result of
553 antagonist co-activation (Todd et al., 2016; Figure 1). To our knowledge, there is no
554 report of such data to compare our results with. Recent publications show that some
555 research groups have modified the NMA protocol to measure $SIT_{100\%}$ during a 'true'
556 MVC (Gruet et al., 2014; Bachasson et al., 2016) in order to strengthen both face
557 validity of the measure and internal validity of the experiment. This however remains
558 speculative with an inherent effect of human behavior on any voluntary contraction
559 (Peacock et al., 1981; Tok et al., 2013), and with no evidence of better consistency or
560 higher reliability in both MVC scores when evoking $SIT_{100\%}$, and $SIT_{100\%}$ itself, when
561 using the modified NMA protocol. The poor reliability of $SIT_{100\%}$ in the present study
562 (Table 3) is worrisome considering its direct threat to VA_{TMS} validity itself.
563
564 Based on post-exercise reliability, analysis of VA_{TMS} change following the exercise
565 intervention shows that the detection of a detectable reduction for a given participant
566 was unsuccessful in 18 of the 20 measures (reductions < 27.1%), and was also
567 unsuccessful for one of the two visits when considering the change in the sample's
568 mean (<10.5%). This is despite a large decrement in VA_{TMS} following the intermittent
569 fatiguing exercise (-13 ± 10%). The present lack of responsiveness calls into question
570 the interpretation of similar changes following the same intermittent fatiguing
571 exercise (Gruet et al., 2014).
572

Research methodologies for the modeling of the linear relationship and the goodness-of-fit of the model between SIT and VC can be particularly unclear (Todd et al., 2016). In the present study, 85% (104 out of 120) of the three-point relationships were not significantly linear, thus despite 63% of them (65 / 104) exceeding the arbitrary level of r^2 acceptability as *per* literature (*i.e.* > 0.90; Hunter et al., 2006). To our knowledge, the significance of three-point relationship has never been reported for the knee extensors as the sole report of r^2 is routinely accepted as a sufficient indicator of the goodness of fit of the model in the research field (Goodall et al., 2009; Sidhu et al., 2009b; Gruet et al., 2014; Thomas et al., 2015; Bachasson et al., 2016; Thomas et al., 2016). Some ERT calculations have been based on the performance of only one set of three contractions in some published work (i.e. 50, 75, and 100%MVC; Sidhu et al., 2009b; Goodall et al., 2009; Gruet et al., 2014). Others have used averages over the three sets of contractions to model the SIT - VC relationship (Goodall et al., 2009; Thomas et al., 2015; Thomas et al., 2016). While there may be a temptation to model a three-point relationship for computation of ERT, especially following a fatiguing exercise when recovery is a threat to face validity, one must be aware that in addition to the lack of significance of such relationship, standard errors associated with the *y*-intercept of the relationship (i.e. SE-ERT) is likely to be ~20% of the ERT mean, whether at rest or post-exercise, yielding to extremely poor accuracy in the estimates (95% CI of ± 247% of the mean). This is concerning considering most studies investigating VA_{TMS} of the knee extensors have used a three-point relationship so that accuracy of ERT estimates, and detection of a real / true effect of their intervention is questionable; intervention-induced ERT change would lie within inaccuracy range.

In the present study, nine points (eight in one occasion) were also entered in the model, with no difference in the goodness-of-fit of the data between the two visits, and a better fit of the linear model pre- compared to post-exercise. The use of 8-9 points therefore allowed for more accuracy in the estimation of ERT with only 3 of the 20 trials having a post-exercise ERT value inside the 95% CI for the pre- exercise estimate. Based on these findings, a ‘true’ effect of the exercise was therefore detectable in 85% of the individual cases (as opposed to 7.5% of the cases for the three-point relationship). The 85% chance of detecting a ‘real’ change for a given participant is explained by the very large decrement in ERT following the fatiguing

exercise in the present study (-46%). These changes are great enough to be deemed of true value (>SDC; Table 3). Issue with accuracy in ERT estimates put aside (i.e. three-point relationship), some other interventions have shown to reduce ERT significantly, but to smaller extent (10 and 20 minutes of moderate intensity cycling, -27% and 37% respectively, O'Leary et al., 2016; 6 sustained MVCs in females, -27%, Hunter et al., 2006 ; 120 minutes of simulated soccer, -20%, Goodall et al., 2017) The size of the effect is within our SDC range for a given sample (Table 2 and 3) so that the meaningfulness of these changes is questionable.

There are limitations associated with a NMA protocol: The present study was designed to ascertain whether the main measurement outcomes hold face validity in a fresh muscle (pre-exercise) by testing for a fatigue effect, and in a fatigued muscle (post-exercise) by testing for a recovery effect. Interestingly, both mean MVC and POT were significantly reduced following the pre-exercise NMA protocol, indicating a development of neuromuscular and peripheral fatigue throughout the nine-contraction protocol. Longer time periods between contractions could be implemented in the future. The data also showed a rapid recovery of MVC force throughout the post-exercise NMA (Figure 4). The use of 25 and 15 s between maximal and submaximal contractions – these are shorter time periods compared to the original protocol (45 s and 15 s) of Goodall et al. (2009) - still provided a window for recovery to occur (Gruet et al. 2014; Mira et al. 2018). A shorter NMA protocol should be considered when purposing the measure of VA_{TMS} following exercise.

The present study assessed VA_{TMS} using guidelines set from the maximum of three MVCs (Table 1). Three to six MVCs have previously been used to set guidelines for subsequent sub-maximal contractions (Goodall et al. 2009, 2017; Thomas et al. 2017; Brownstein et al. 2017). From the present data (Table 1), it is evident that the use of three MVCs during the NMA induces a degree of neuromuscular fatigue. Therefore, the pre-exercise NMA may not have been performed in a truly non-fatigued muscle. Although the present pre-exercise VA_{TMS} values are comparable to those reported using NMA with fewer MVCs (e.g. Bachasson et al., 2016), it is possible that pre-exercise VA_{TMS} may have been underestimated as a consequence. Conversely, it is also possible that post-exercise VA_{TMS} may have been affected by the sets of three MVCs used to set guidelines for subsequent sub-maximal contractions. Interestingly

in this instance, the fatigue-inducing effect may have offset the recovery effect. A less strenuous NMA protocol should nonetheless be considered.

CONCLUSION

The present study exposes the weaknesses of a three-contraction protocol for estimation of VA_{TMS} in the knee extensors. Despite acceptable levels of absolute reliability at rest, our results demonstrate a need to consider post-exercise reliability when investigating exercise-induced central fatigue. When doing so, VA_{TMS} does not respond to a fatiguing exercise protocol. Extrapolation of ERT from three-point linear leads to extremely poor accuracy, a nine-point modeling improves estimate accuracy considerably. However, the face validity of the nine-contraction protocol is threatened by the development of neuromuscular fatigue when performed at rest, and by recovery when performed at the end of a fatiguing exercise. A compromise between a three- and a nine-contraction protocol should be considered.

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826 **Tables**

827

Table 1. Mean ± SD torque (N.m) during the neuromuscular assessment, pre and-post fatiguing exercise, across the two trials

	Trial	Pre-NMA					NMA											
		MVCs					100% of MVC			75% of MVC			50% of MVC			$SIT_{100\%}$		
		1st	2nd	3rd	Max	End MVC	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 1	Set 2	Set 3
Pre Exercise	**1**	240.4 ±60.2	233.5 ±58.8	225.7 ±56.8	243.0 ±61.9	231.0 ±61.0	233.7 ±57.3	234.0 ±58.4	232.9 ±61.1	178.2 ±45.3	178.5 ±44.5	178.6 ±45.9	119.8 ±30.7	121.0 ±31.3	119.1 ±29.5	2.1 ±1.9	2.3 ±1.8	1.8 ±1.1
	2	236.4 ±50.3	229.0 ±52.9	221.95 ±43.4	237.4 ±51.6	224.4 ±47.9	231.7 ±49.9	229.0 ±49.4	224.9 ±47.9	174.2 ±39.7	175.7 ±38.2	174.9 ±40.7	115.9 ±24.9	116.0 ±26.0	117.7 ±25.9	1.8 ±1.3	1.6 ±1.1	1.8 ±1.5
Post Exercise	**1**	162.4 ±47.7	164.1 ±52.8	166.5 ±47.5	171.4 ±51.0	184.7 ±54.4	169.1 ±50.5	168.0 ±50.5	169.7 ±49.7	125.5 ±37.4	124.1 ±36.3	125.2 ±36.8	86.2 ±25.5	86.9 ±24.0	84.5 ±25.6	2.4 ±1.9	3.2 ±2.2	2.5 ±1.6
	2	167.3 ±31.9	161.1 ±31.2	162.5 ±31.9	171.5 ±30.6	188.4 ±37.0	169.6 ±31.2	165.5 ±30.0	163.3 ±31.8	127.9 ±22.2	125.4 ±22.5	127.5 ±24.9	85.7 ±13.6	83.0 ±15.3	84.1 ±15.4	3.5 ±2.4	3.7 ±2.2	3.1 ±1.8

MVC; maximum voluntary contraction, NMA; neuromuscular assessment; $SIT_{100\%}$; superimposed twitch during 100% contraction

Table 2. Descriptive statistics and reliability data for VA_{TMS} and constituent variables determined pre- and post-exercise (n=10)

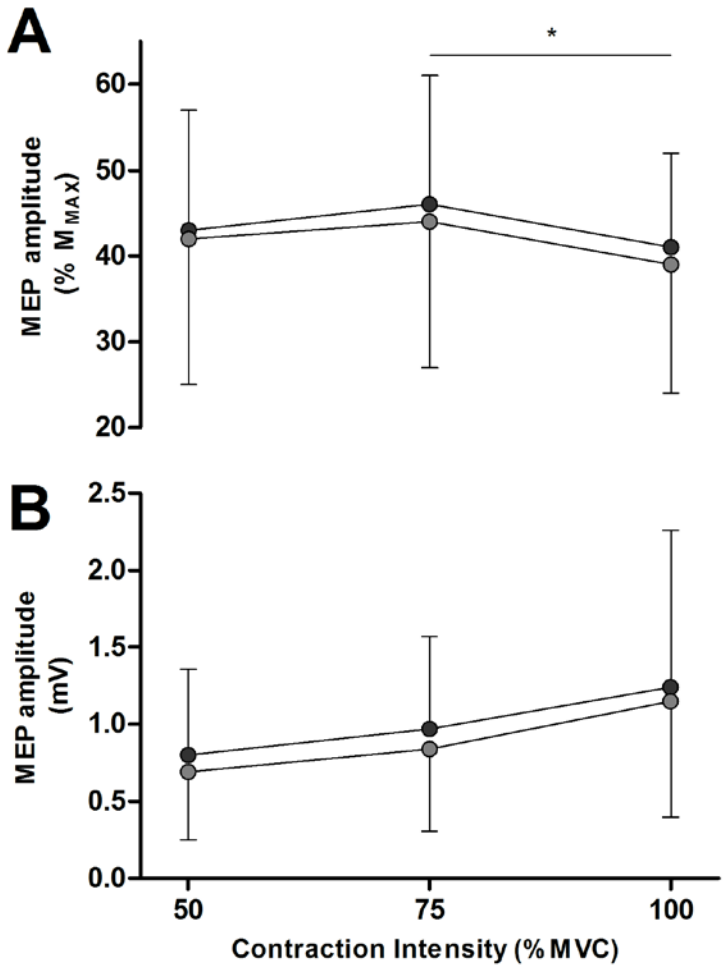
	Trial 1 Mean ± SD (Range)	**Trial 2 Mean ± SD (Range)**	**TEM (%of the mean)**	**Bias**	**SDCind (%of the mean)**	**$ICC_{2,1}$** (95%CI)
Pre-exercise						
ERT	35.1 ± 9.7 N.m (18.5–46.1)	35.5 ± 6.9 N.m (21.9–44.1)	4.7 N.m (13.4%)	0.4 (HO)	13.1 N.m (37.0%)	.71* (.16 - .92)
$SIT_{100\%}$	2.1 ± 1.0 N.m (0.9–3.4)	1.7 ± 1.1 N.m (0.5–4.2)	0.9 N.m (45.9%)	-0.4 (HO)	2.4 N.m (38.4%)	.34 *n.s* (-.32 - .78)
VA_{TMS}	94.1 ± 2.4% (89.8–97.0)	94.8 ± 3.8% (87.7–98.9)	2.3% (2.5%)	0.7 (HO)	6.5% (6.9%)	*n.a.*
100% MVC	234 ± 59 N.m (124 – 300)	229 ± 49 N.m (141 – 288)	11 N.m (4.6 %)	-5 (HO)	30 N.m (12.8%)	.96* (86 - .99)
POT	56.8 ± 9.9 N.m (41.5 – 76.1)	57.0 ± 7.3 N.m (47.9 – 70.7)	4.0 N.m (7.1)	0.1 (HO)	11.2 N.m (6.2 %)	.80* (37 - .95)
Post-exercise						
ERT	19.5 ± 6.0 N.m (8.7–26.7)	19.0 ± 9.2 N.m (7.9–37.7	4.4 N.m (23.1%)	0.5 (HO)	12.3 N.m (64.0%)	.69* (.13 - .91)
$SIT_{100\%}$	Median: 2.2 N.m (1.1–7.0)	3.4 ± 1.9 N.m (0.6–6.4	1.7 N.m (54.6%)	-0.04	4.6 N.m (151.3%)	.14 *n.s* (-.50 – .68)
VA_{TMS}	85.8 ± 6.9% (71.4–95.7)	78.3 ± 12.3% (63.2–98.4	9.8% (11.9%)	*n.a.*	27.1% (33.1%)	*n.a.*
100% MVC	169 ± 50 N.m (99 – 240)	166 ± 31 N.m (112 – 219	19 N.m (11.2%)	-2.9 (HO)	52 N.m (31%)	.81* (40-.95)
POT	37.3 ± 10.7 N.m (23.5 – 63.4)	37.9± 6.7 N.m (31.0 – 54.7)	4.8 N.m (12.8%)	0.7 (HO)	13.3 N.m (35.4%)	.73* (.21 – .92)

(H0) Homoscledasticity verified ($P<0.05$); *Significantly correlated ($P<0.05$); *n.c* no significant between session-difference ($P<0.05$); *n.a.* for non applicable (no homoscledasticity on raw untransformed or log transformed data for calculation of Bias ± 95% LA; ceiling effect for ICC)

830 Table 3: Responsiveness of key measures of neuromuscular fatigue to a fatiguing exercise

		Individual detectable change from pre-exercise	**Individual detectable change from post-exercise**	**Sample's detectable change**	
		Δchange > SDC_{ind}		**Δchange in the means > SDC_{sample}***	
	Quality			**Session 1**	**Session 2**
MVC (N.m)	Neuromuscular fatigue	18/20 occurrences i.e. 90% of cases	13/20 occurrences i.e. 65% of cases	Yes	Yes
POT (N.m)	Peripheral fatigue	18/20 occurrences i.e. 90% of cases	15/20 occurrences i.e. 75% of cases	Yes	Yes
$SIT_{100\%}$	Critical determinant of VA_{TMS}	0/20 occurrences i.e. 0% of cases	0/20 occurrences i.e. 0% of cases	No	No
ERT (N.m)	Critical determinant of VA_{TMS}	16/20 occurrences i.e. 80% of cases	12/20 occurrences i.e. 60% of cases	Yes	Yes
VA_{TMS}	Supra-spinal fatigue	15/20 occurrences i.e. 75% of cases	2/20 occurrences i.e. 10% of cases	No	Yes

831 **Δchange for change from pre- to post-exercise; * $SDC_{sample} = SDC_{ind} / \sqrt{n}$**



832

833 *Figure 1: MEP amplitude across contraction intensities for the VA_{TMS} protocol. Panel A: Agonist (VL) MEP*
834 *amplitude normalized to M_{MAX}. Panel B: Non-normalised antagonist (BF) MEP amplitude. * = P<0.05*
835 *significantly different between time points.*

836

Figures

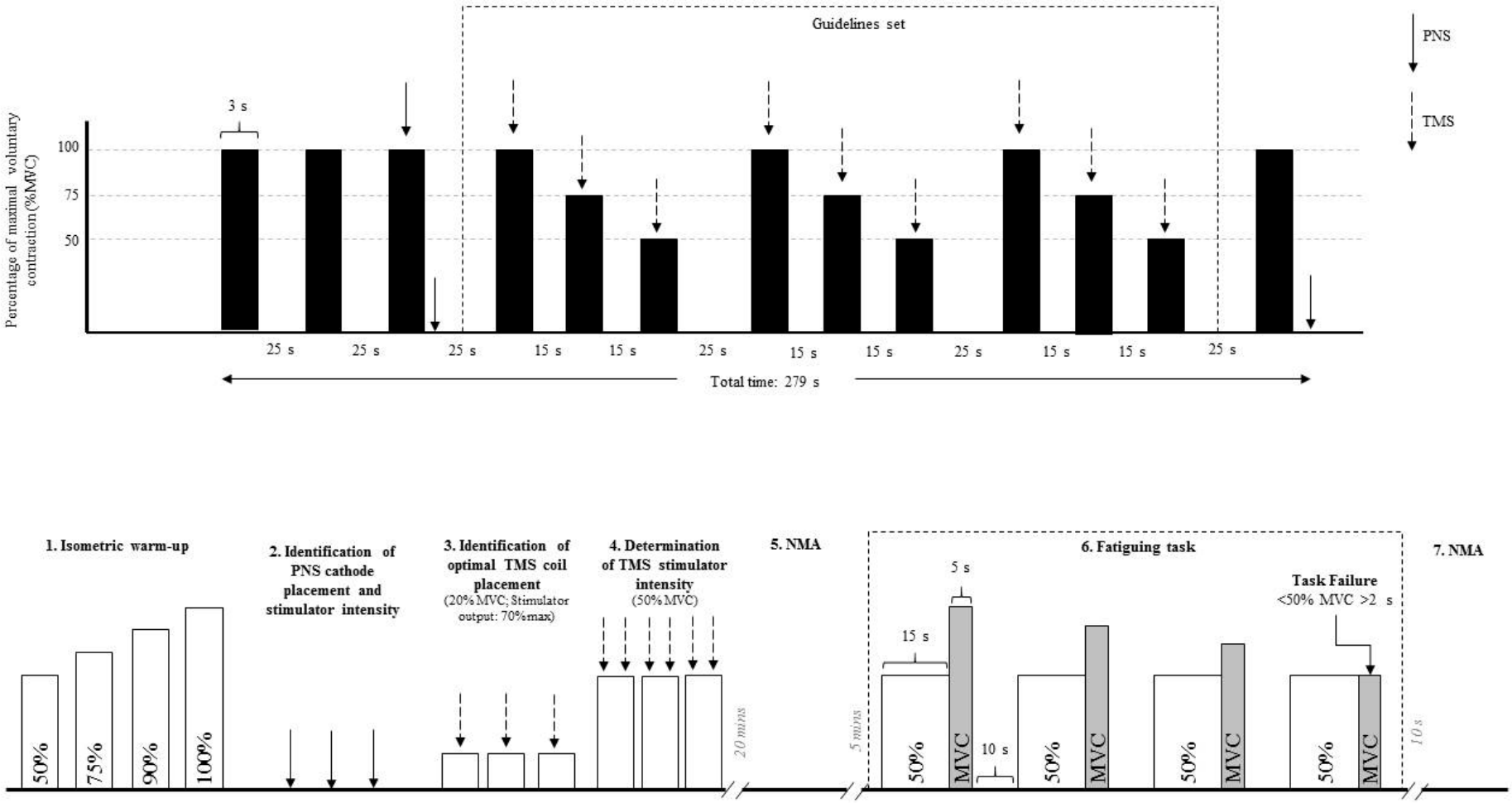
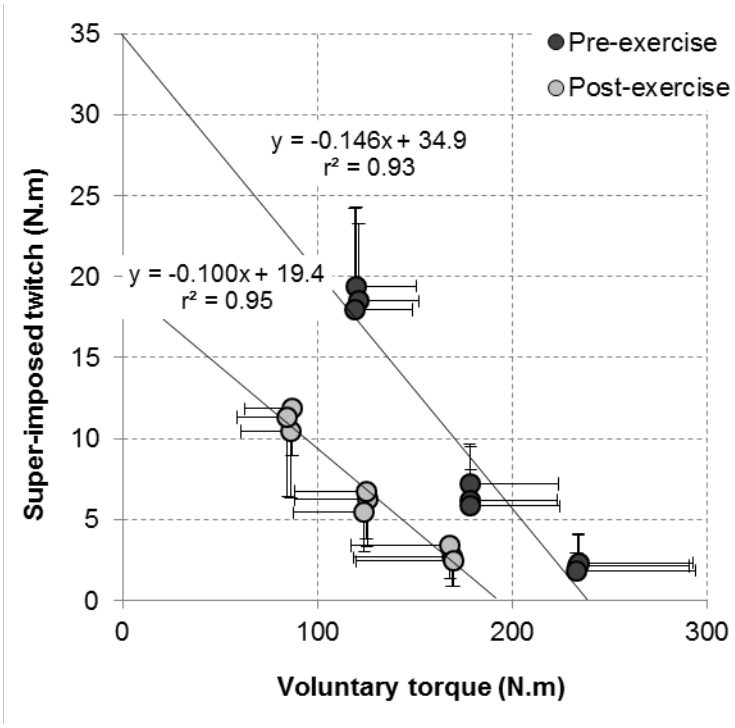
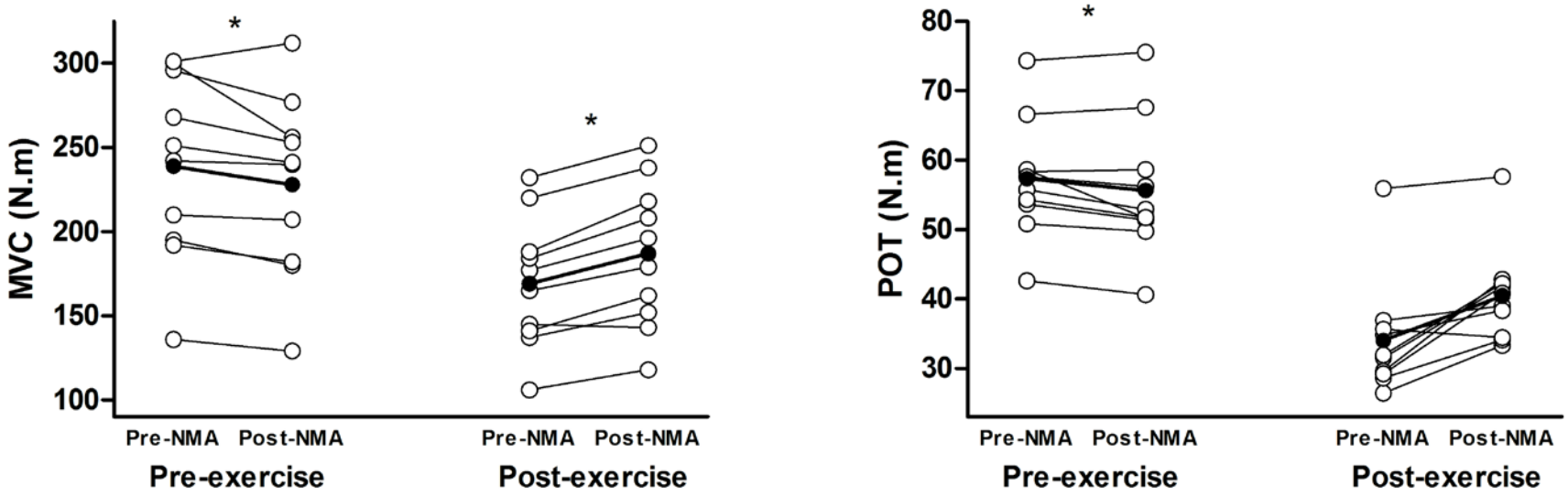


Figure 2: Schematic of the protocol



840

841 *Figure 3: Linear regression between voluntary torque and TMS-evoked super-imposed twitch in the fresh and*
842 *fatigued knee extensors*



843

844 *Figure 4: MVC and POT pre and post the neuromuscular assessment (NMA) protocol carried out before and after the fatiguing exercise. Individual data points are*
845 *represented with unfilled circles, group mean data is represented by the filled circles.*

Supplementary Material

Exercise task performance

The fatiguing task lasted 164 ± 36 s with a ~67% decrease in the MVC torque ($F_{(1,9)}$ = 83.8, P<0.001, η_p^2=0.90) from the first (Session 1: 200 ± 53 N.m; Session 2: 204 ± 40 N.m) to the last repetition (Session 1: 130 ± 33 N.m; Session 2: 138 ± 20 N.m). This was not significantly difference between the two sessions ($F_{(1,9)}$ = 1.00, P=0.34, η_p^2=0.10). There was no between-session difference in the average of the MVCs over the fatiguing task (Session 1: 166 ± 41 N.m; Session 2: 170 ± 28 N.m; $t_{(9)}$=-1.08; P=0.31) and the level of contraction maintained throughout the sections at targeted 50% MVC (Session 1: 150 ± 24 N.m; Session 2: 157 ± 28 N.m; $t_{(9)}$=-0.66; P=0.53). The high $ICC_{2,1}$ (averaged MVC scores: *r*=0.85, P=0.001; 50% of MVC: *r*=0.89, P<0.001) and low typical error between the two sets of data (averaged MVCs: 8.4% of the mean; 50% of MVC: 10.1% of the mean) evidence strong absolute and relative reliabilities of the fatiguing task between session 1 and 2.